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# GASTRIC ACID SECRETION IN THE LIZARD
# IONIC REQUIREMENTS AND EFFECTS OF INHIBITORS

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## SUMMARY

1. The effects of ion substitution and various inhibitors on the transmucosal potential, short circuit current, mucosal resistance and acid secretion of the lizard gastric mucosa, incubated in an Ussing chamber, have been determined.

2. Ion substitution experiments indicate that the serosal potential step consists of a combined $Cl^-$ and $K^+$ diffusion potential, and that the mucosal potential step is $Na^+$ dependent and behaves primarily as a $Na^+$ diffusion potential.

3. Experiments with ouabain indicate that the major ($Na^+$, $K^+$)-ATPase activity responsible for maintenance of cation gradients is located on the serosal side of the mucosal cells, and that this pump activity is non-electrogenic.

4. Experiments with amiloride indicate that a passive sodium influx on the mucosal side is essential for the maintenance of the transmucosal potential and short circuit current.

5. Acid secretion requires the presence of sodium and chloride on the serosal side and the maintenance of a high intracellular potassium level through the ($Na^+$, $K^+$)-ATPase system.

6. The effects of acetazolamide and thiocyanate are compatible with an involvement of carbonic anhydrase and anion-dependent ATPase in acid secretion.

7. Upon initiation of acid secretion the serosal membrane permeability for chloride increases and that for potassium decreases.

## INTRODUCTION

In a study of the role of the ($Na^+$, $K^+$)-activated ATPase system in gastric secretion we have previously reported the occurrence and properties of the enzyme system in the lizard gastric mucosa [1]. A direct involvement of the enzyme in the maintenance of the cellular cation gradients has been concluded from the equivalent

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exchange of $Na^+$ and $K^+$ without change in cellular water content and extracellular space upon treatment of the tissue with ouabain.

These findings raise the question of how the transmucosal potential is related to the ion gradients across the cell membrane, how the acid secretion process is related to this potential difference and, further, how the acid secretion rate and the transmucosal potential depend on the different ions.

In order to further elucidate this role we have investigated the nature of the transmucosal potential by ion substitution experiments in the Ussing chamber. The results indicate that this potential consists of a serosal potential step, which is a combined $K^+$ and $Cl^-$ diffusion potential, and a mucosal potential step, which is $Na^+$-dependent. The presence of $Na^+$ and $Cl^-$ on the serosal side is essential for acid secretion, but there is no simple relation between transmucosal potential and acid secretion.

In addition, we report more detailed studies on the effects of ouabain and other inhibitors on gastric acid secretion and transmucosal potential generation by the lizard gastric mucosa aimed at further elucidation of the role of the $(Na^+, K^+)$-activated ATPase system in gastric secretion.

## MATERIALS AND METHODS

*Lacerta viridis* animals from Italy are fed with insect larvae and maintained at 25 °C. The animals are killed by decapitation and the entire stomach is excised. The mucosa is dissected off from the adjacent muscular layer.

*Ussing chamber.* Potential differences and short circuit currents are measured following Ussing and Zerahn [2]. In view of the small size of the lizard gastric mucosa we have used modified chambers with a surface area of only 0.785 $cm^2$ (Fig. 1). The serosal side of the gastric mucosa always faces the bottom chamber. The serosal bathing medium (22 ml) is aerated and kept at either 20 or 30 °C in a small receptacle, and is circulated by a peristaltic pump. The upper chamber which contains only 2 ml medium, has both electrodes introduced through holes in the cover, while

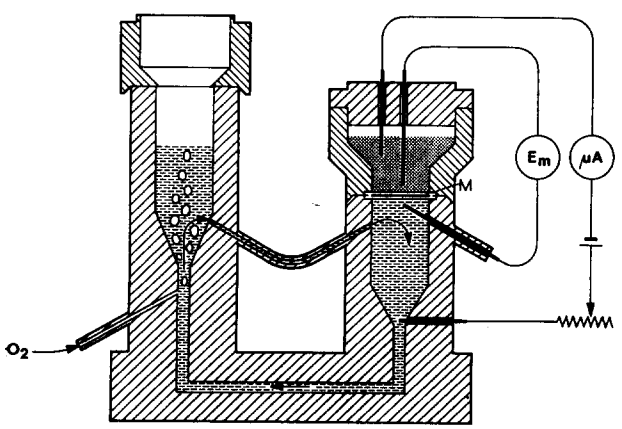


Fig. 1. Modified Ussing chamber used for simultaneous measurements of acid secretion and electrical parameters. M is the gastric mucosa.

TABLE I

BATHING MEDIA USED IN ION-SUBSTITUTION EXPERIMENTS

The final pH of media B, D, F and K after gassing with 100 % oxygen is 7.0–7.4, and of media A, C, E, G and H after gassing with 95 % $O_2$/5 % $CO_2$, 7.4. All concentrations are expressed in mmol/l.

| | A normal serosal | B normal mucosal | C $Cl^-$ free serosal | D $Cl^-$ free mucosal | E $Na^+$ free serosal | F $Na^+$ free mucosal | G $K^+$ free serosal | H high $K^+$ serosal | K high $K^+$ mucosal |
|---|---|---|---|---|---|---|---|---|---|
| NaCl | 145 | 170 | — | — | — | — | 145 | — | — |
| $NaHCO_3$ | 25 | — | 25 | — | — | — | 25 | 25 | — |
| $Na_2SO_4$ | — | — | 72.5 | 85 | — | — | — | — | — |
| Choline chloride | — | — | — | — | 170 | 170 | — | — | — |
| KCl | — | 3 | — | — | — | 3 | — | 145 | 170 |
| $K_2SO_4$ | — | — | — | 1.5 | — | — | — | — | — |
| $KH_2PO_4$ | 3 | 0.09 | 3 | 0.09 | 0.32 | 0.09 | — | 3 | 0.09 |
| $K_2HPO_4$ | — | 0.39 | — | 0.39 | 1.34 | 0.39 | — | — | 0.39 |
| $NaH_2PO_4$ | — | — | — | — | — | — | 3 | — | — |
| $CaCl_2$ | 0.45 | 0.45 | — | — | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| Calcium glucuronate | 0.45 | 0.45 | 0.90 | 0.90 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| $MgSO_4$ | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Glucose | 26 | 26 | 26 | 26 | 26 | 26 | 26 | 26 | 26 |
| Sucrose | — | — | 72.5 | 85 | — | — | — | — | — |

a third hole permits complete removal of the mucosal bathing medium within a few seconds. The mucosal bathing medium, previously brought to the desired temperature, is placed in the upper chamber and stirred by a stream of oxygen.

*Bathing media.* The compositions of the media are given in Table I. Since the plasma $Na^+$ level of the animals is 171 mM (S.E. = 2; $n = 8$), the $Na^+$ level of the media has been made 170 mM. Sucrose is added to the $Na_2SO_4$-containing media in order to maintain constant osmolarity.

Complete substitution of an ion is obtained by twice replacing the entire incubation medium 10 min apart, whereupon the electrical measurements are made. The initial conditions are re-established after each substitution step, and correction for small changes in the parameters is achieved by linear interpolation between the values of the control media. All values are the means of 4–6 experiments.

Partial changes in ionic composition are obtained by replacing a fraction of the bathing medium by another medium (medium C, E or H replacing medium A). Corrections for tissue deterioration are made again by interpolation between control values at the beginning and the end of the experiment.

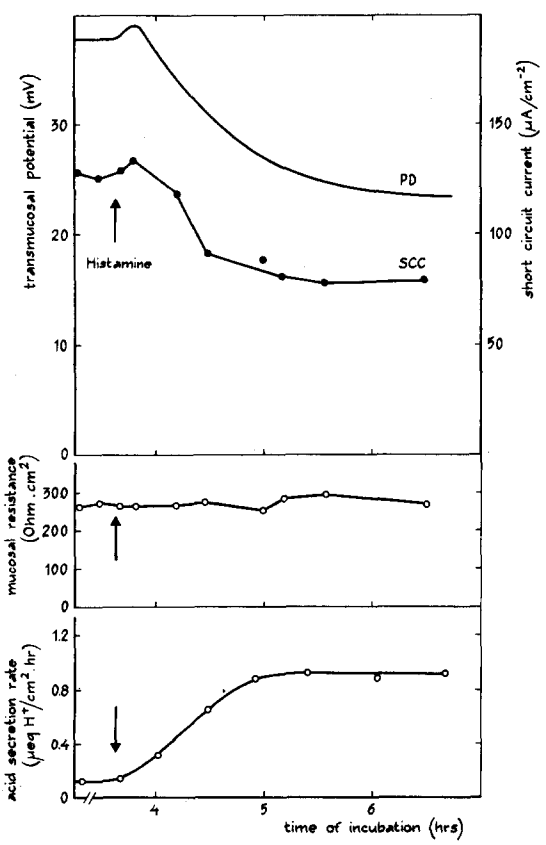


Fig. 2. Effect of histamine ($10^{-4}$ M) added on the serosal side, on transmucosal potential, short circuit current, mucosal resistance and acid secretion rate (20 °C). The potential was continuously recorded.

*Stability of electric parameters and secretion.* When the lizard gastric mucosa is stripped off and mounted in an Ussing chamber, a transmucosal potential can be measured ranging from 20 to 60 mV, the mucosal side being negative with respect to the serosal side. The potential is low in winter, high in spring. The transmucosal potential, or in short circuited preparations the short circuit current, as well as the mucosal resistance are stable for 6 to 15 h under these conditions. Mean values before secretion with standard errors for 12 experiments are: PD $42.0 \pm 0.9$ mV; s.c.c. $179 \pm 4.8$ $\mu A/cm^2$; mucosal resistance $180 \pm 5.2$ $\Omega \cdot cm^2$.

When acid secretion is evoked by histamine ($10^{-4}$ M), transmucosal potential and short circuit current increase transiently and thereupon decrease to relatively stable values after 1–1½ h (Fig. 2). These stable values are used as the 100 % reference values in expressing the effects of ionic substitution on transmucosal potential and on short circuit in the stimulated gastric mucosa. The acid secretion rate commences immediately after stimulation, generally reaching a maximal, constant value after 1–1½ h, and varies between 2 and 6 $\mu$equiv. $H^+/cm^2/$ h (mean: 3.11 $\mu$equiv/$cm^2$) at 30 °C and between 0.5 and 1.0 $\mu$equiv. $H^+/cm^2/$ h at 20 °C. The mucosal resistance varies little upon histamine stimulation.

Potassium and sodium determinations are carried out by flame photometry and chloride is determined coulometrically [3] in the media after each replacement step.

*Determination of the acid secretion rate.* The mucosal media are removed at regular time intervals and are titrated with 4 mM NaOH from a syringe microburet (65 $\mu$l delivery per mm micrometer screw displacement) to the original pH of the solution.

*Determination of the tissue sodium content.* After gentle blotting on filter paper impregnated with the bathing medium, the wet weight of the tissue samples is determined. They are then dried for 15 h at 110 °C and reweighed. The dried samples are extracted for 48 h in 4 ml 0.1 M $HNO_3$. An aliquot is diluted in twice-distilled water, and the $Na^+$ concentration is determined flame photometrically against standards containing $HNO_3$ in the same concentration as in the samples.

*Materials.* Ouabain was obtained from Sigma Chemical Co. (St. Louis, U.S.A.), amiloride from Sharp & Dohme (Rahway, N. J., U.S.A.), acetazolamide from Lederle Co. (Wayne, N. J., U.S.A.) and sodium thiocyanate from Merck AG (Darmstadt, Germany). All other reagents were of the highest obtainable purity.

## RESULTS

### *Effects of ion substitution on electric parameters*

The effects of sodium and chloride substitution are summarized in Table II. Substituting chloride on the serosal side strongly lowers the transmucosal PD as well as the short-circuited current. A comparable decrease is observed, when sodium or chloride is replaced on both sides by choline or sulfate, respectively. The most dramatic change is seen when serosal chloride replacement is combined with mucosal sodium replacement. The signs of potential and current are then reversed and the electrical resistance increases by 60 %.

Upon gradual replacement of serosal chloride by sulfate at constant sodium and potassium concentrations, the transmucosal PD varies linearly with the logarithm of the serosal chloride concentration in the range of 10–100 mM chloride. In the resting mucosa, an average depolarisation of 11.1 mV is induced by a 10-fold change

TABLE II

EFFECTS OF ION SUBSTITUTION ON TRANSMUCOSAL POTENTIAL (PD), SHORT CIRCUITED CURRENT (S.C.C.), ELECTRIC RESISTANCE (R) AND ACID SECRETION RATE (ACID)

Averages of relative values are expressed as percentages of control values (NaCl-NaCl) for 6 experiments.

| Bathing medium | | PD | S.C.C. | R | Acid |
|---|---|---|---|---|---|
| Mucosal | Serosal | | | | |
| NaCl | NaCl | 100 | 100 | 100 | 100 |
| NaCl | Choline chloride | 76 | 76 | 107 | 49 |
| NaCl | $Na_2SO_4$ | 37 | 39 | 102 | 0–10 |
| $Na_2SO_4$ | NaCl | 118 | 103 | 104 | |
| Choline chloride | NaCl | 96 | 93 | 100 | |
| Choline chloride | Choline chloride | 23 | 18 | 126 | |
| $Na_2SO_4$ | Choline chloride | 96 | 75 | 137 | |
| $Na_2SO_4$ | $Na_2SO_4$ | 41 | 38 | 117 | |
| Choline chloride | $Na_2SO_4$ | −73 | −23 | 160 | |

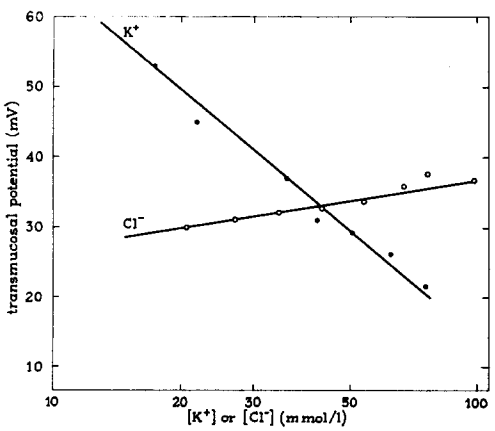


Fig. 3. Relation between transmucosal potential and logarithm of the serosal potassium (●—●) and chloride concentrations (○—○) in the resting mucosa (30 °C). Results for one potassium and one chloride experiment out of a total of four experiments each are presented.

in serosal chloride concentration (Fig. 3): in the secreting mucosa this value is 27.7 mV (Fig. 4).

The relation between transmucosal PD and serosal potassium concentration is determined by gradual replacement of serosal NaCl by KCl (medium H, Table I), since lowering of the serosal sodium concentration from 170 to 20 mM by choline substitution does not significantly change potential and resistance. A linear relation between potential and serosal potassium level is observed with an average decrease of 49.4 mV at a 10-fold increase of potassium level in the resting mucosa (Fig. 3) and of 28.5 mV in the secreting mucosa (Fig. 4). On the other hand, potassium substitution on the mucosal side causes only a minor (2–3 mV) and transient increase in potential.

The sum of the potential changes obtained at 10-fold changes in serosal concentrations of potassium and chloride amounts to 60.5 mV in the non-secreting

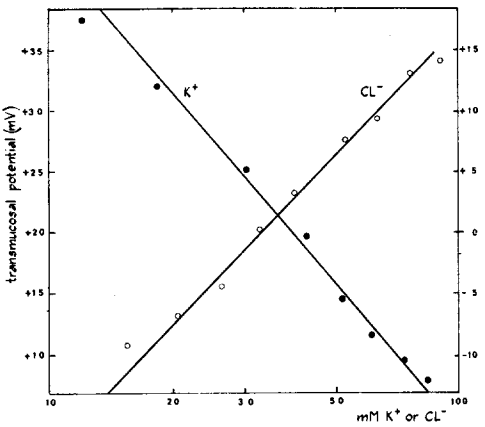


Fig. 4. Relation between transmucosal potential and logarithm of the serosal potassium (● — ●) and chloride concentrations (○ — ○) in the secreting mucosa. Potential differences indicated at the left refer to the potassium experiment, those at the right to the chloride experiment (30 °C). Results for one potassium and one chloride experiment out of a total of four experiments each are presented.

and 56.2 mV in the secreting mucosa, which is about what is to be expected on the basis of the Nernst equation at 30 °C. This suggests that there exists a serosal potential step which can be described as a combined diffusion potential for potassium and chloride. Transport numbers, representing the contribution of each ion to the membrane conductance, can then be calculated by means of the equation:

$$\frac{F}{RT}\,dE = \frac{t}{z}\,d(\ln a),$$

where $F$ is the Faraday constant (96 500 Coulombs), $R$ the gas constant, $T$ the absolute temperature, $t$ the transport number, $z$ the valency of the ion and $a$ the activity of the ion in solution [4, 5]. The results in Table III indicate that the contribution of potassium to the serosal membrane conductance is 84 % in the resting and 48 % in the secreting gastric mucosa, while the contribution of chloride is 19 and 46 %, respectively, in these two cases. The sum of the two transport numbers is close to 1.0, suggesting that there is no significant contribution of other ions to the serosal membrane conductance. The finding that the mucosal resistance is decreased with increasing serosal potassium concentration (Fig. 5), but is not affected by lowering the serosal chloride concentration (Table II), does not necessarily invalidate these calculations. It merely indicates that potassium and chloride ions follow different routes during permeation.

TABLE III

TRANSPORT NUMBERS* IN RESTING AND SECRETING LIZARD GASTRIC MUCOSA

| | $t_{K+}$ | $t_{Cl-}$ | sum |
|---|---|---|---|
| Resting mucosa | 0.84 | 0.19 | 1.03 |
| Secreting mucosa | 0.48 | 0.46 | 0.94 |

* Calculated for the serosal membrane by means of the equation of Ciani and Conti [5].

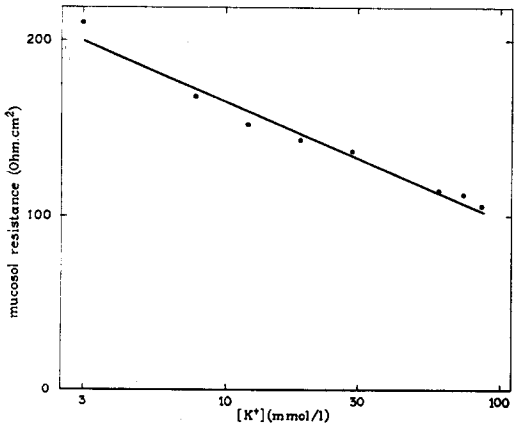


Fig. 5. Relation between mucosal resistance and serosal potassium concentration in the secreting mucosa (30 °C).

*Effects of ion substitution on acid secretion*

The effects of ion substitution on acid secretion are shown in Table II and Fig. 6. The acid secretion is sodium dependent. Replacing sodium chloride in the serosal bathing medium by choline chloride immediately decreases the acid secretion rate. The residual activity varies from 2 to 75 % (average 49 %) after 30 min and from 18 to 40 % (average 24 %) after 90 min. There is substantial (up to 76 %) recovery upon replacement of choline by sodium. Partial substitution of sodium by choline on the serosal side, or complete substitution in the mucosal bathing medium leaves the acid secretion unaffected.

The acid secretion rate never reaches a zero value after sodium substitution, in contrast to what is found after chloride replacement. One possible explanation

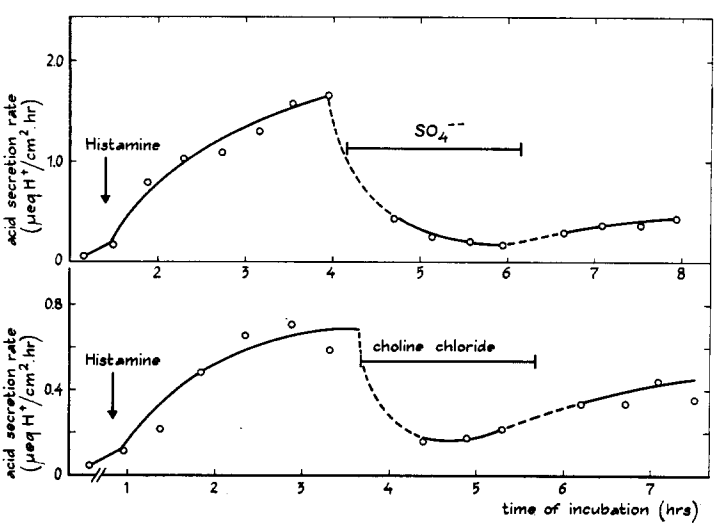


Fig. 6. Effect on acid secretion rate of complete substitution of serosal chloride by sulfate and of serosal sodium by choline (20 °C).

may be that the residual secretion rate is related to the sodium remaining in the tissue. Hence, we have determined the sodium content of histamine-stimulated gastric mucosa after choline treatment (Table IV). During incubation in the choline chloride medium the tissue sodium level decreases in 2 h to 20 % (23 mM) and in 4 h to 8 %

TABLE IV

SODIUM REMAINING IN THE GASTRIC MUCOSA AFTER CHOLINE CHLORIDE TREATMENT

Incubation was preceded by 90 min preincubation in 170 mM $Na^+$ medium, histamine being added after 45 min. The incubation medium is replaced after 2 h. Averages are given with standard errors of the mean.

| Incubation conditions | Sodium content (mmol/l tissue water) | Number of experiments |
|---|---|---|
| 170 mM $Na^+$ | | |
| 2 h | 119±4.0 | 24 |
| $Na^+$-free | | |
| 2 h | 23±3.0 | 12 |
| 4 h | 10±1.6 | 12 |

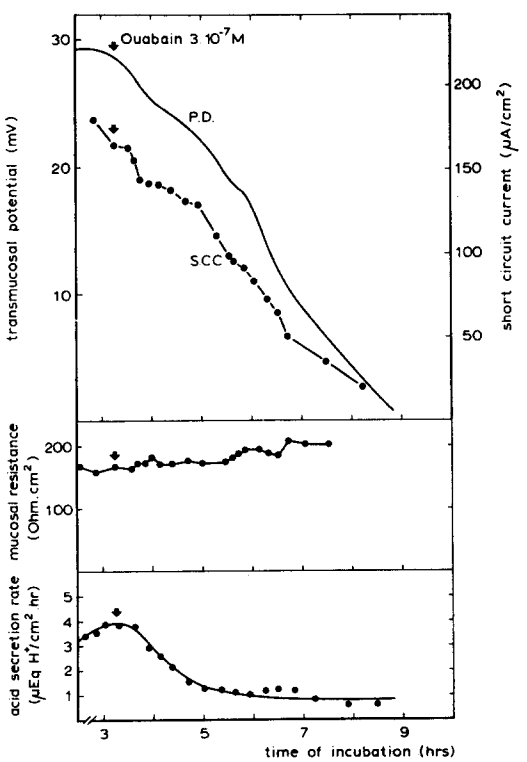


Fig. 7. Effects of serosal application of $3 \cdot 10^{-7}$ M ouabain on the electric parameters and the acid secretion rate (30 °C).

(10 mM) of the initial value. Since in the Ussing chamber experiments the tissue is exposed for less than 2 h to the choline chloride medium, we may assume that more than 23 mM sodium remains in the tissue. This may indeed explain the residual acid secretion.

Complete substitution of chloride by sulfate on the serosal side results in nearly complete inhibition of the acid secretion rate (Fig. 6). There is a rapid decrease to 26–54 % within the first 20 min after substitution. Maximal inhibition is found after 90–120 min, when the residual secretion rate amounts to only 0–10 %. When sulfate is then replaced again by chloride, only partial restoration (20–30 %) of the acid secretion rate is observed. When exposure by sulfate is short (30 min), the inhibition is reversible up to 75 %. On the other hand, complete substitution of chloride by sulfate on the mucosal side leaves the acid secretion unaffected.

No change in acid secretion rate is observed following the stepwise increase of the serosal potassium concentration.

*Effects of ouabain on electric parameters and acid secretion*

Serosal application of ouabain at concentrations of $3 \cdot 10^{-7}$ M or higher leads to complete inhibition of the transmucosal potential and the short circuit current and nearly complete inhibition of the acid secretion rate (Fig. 7). Ouabain concen-

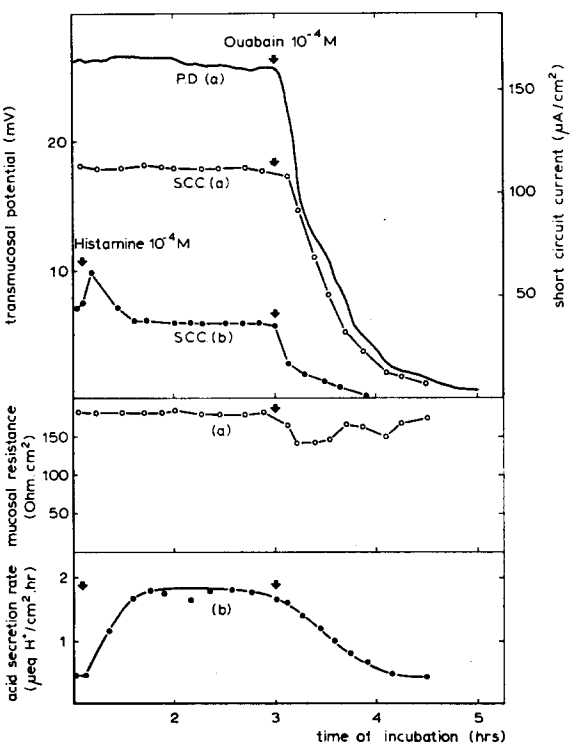


Fig. 8. Effects of serosal application of $10^{-4}$ M ouabain on the electric parameters of resting (a) and secreting (b) mucosa and on the acid secretion rate (30 °C). The secreting mucosa is kept short circuited during the entire experiment.

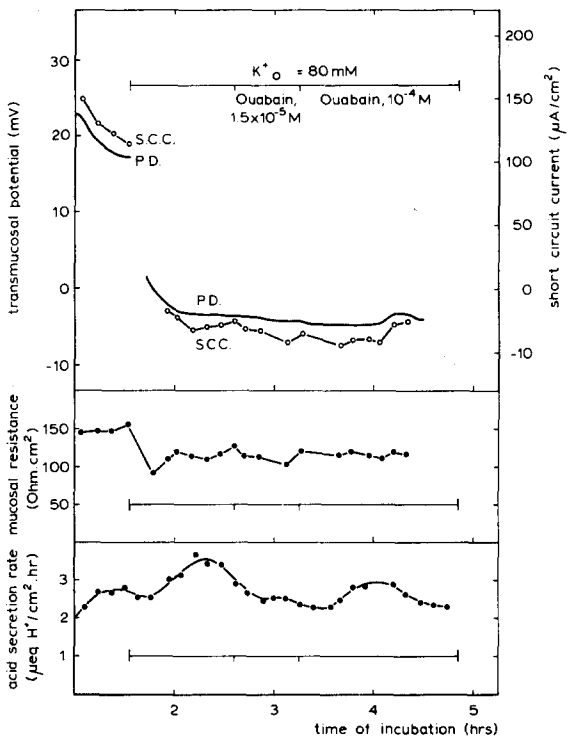


Fig. 9. Effects of serosal application of 80 mM $K^+$ and ouabain ($1.5 \cdot 10^{-5}$ M and $10^{-4}$ M) on electric parameters and acid secretion rate of the secreting mucosa (30 °C).

trations of $10^{-7}$ M or less do not significantly change these parameters. The rate of inhibition is slow and varies for the different parameters. At $3 \cdot 10^{-7}$ M ouabain the acid secretion is completely abolished after 2 h, while at that time the transmucosal potential and the short circuit current have decreased by only 35 %. The mucosal resistance remains constant. During the next three hours the transmucosal potential and the short circuit current are completely abolished. At higher ouabain concentrations ($10^{-4}$ M) the effects are more rapid, but it still takes 60 min to reduce all three parameters to zero (Fig. 8).

When potassium leakage from the cells upon treatment with ouabain is prevented by replacement of the normal serosal bathing medium by a medium with 80 mM $K^+$ (medium C, Table I), even high ouabain concentrations ($10^{-5}$–$10^{-4}$ M) on the mucosal side do not significantly affect either the acid secretion rate, or the transmucosal potential and short circuit current (Fig. 9).

Mucosal application of ouabain leads to quite different effects compared to those of serosal application. Inhibition of the transmucosal potential and short circuit current is only observed at very high ouabain concentrations. With $10^{-4}$ M ouabain no change of the transmucosal potential and short circuit current is observed. Addition of $5 \cdot 10^{-4}$ M ouabain begins to lower the transmucosal potential and short circuit content only after about one hour, and nearly complete inhibition is obtained after 7 h (Fig. 10). These effects can be speeded up (30 min instead of 1 h) or can be

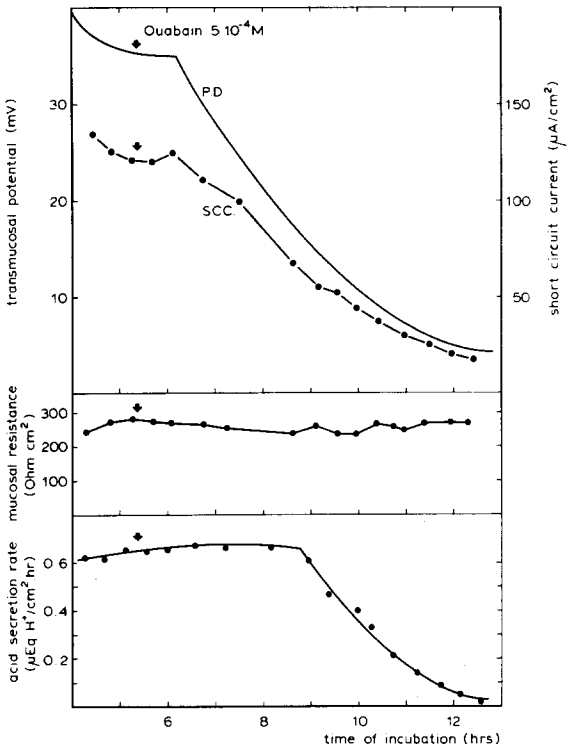


Fig. 10. Effects of mucosal application of $5 \cdot 10^{-4}$ M ouabain on electric parameters and acid secretion rate of the secreting mucosa (20 °C).

achieved at lower ouabain concentation ($10^{-4}$ M), when simultaneously a transmucosal osmotic gradient is applied through reduction of the mucosal sodium chloride concentration from 170 (medium B) to 100 mM (medium D). Another noteworthy point is that the acid secretion rate remains constant for up to 3 h after mucosal application of $5 \cdot 10^{-4}$ M ouabain, and then reaches its minimal value at about the same time as the electric parameters. These findings for mucosally applied ouabain strongly suggest that the ouabain-sensitive cation pump system is located on the serosal side of the mucosa.

*Effects of other drugs*

Amiloride, an inhibitor of passive sodium diffusion, decreases transmucosal PD and short circuit current and slightly increases mucosal resistance when added to the mucosal side of the resting mucosa in concentrations of $10^{-9}$–$10^{-6}$ M (Fig. 11), without affecting the basal acid secretion. At $10^{-6}$ M the average maximal decrease in potential is 34 % and in current 39 %. These effects are completely reversible. No effects of amiloride (up to $10^{-6}$ M) are observed, when it is applied serosally to the resting mucosa or on either side of the secreting mucosa. The absence of any effects in the secreting mucosa may be due to the drug being kept away from its receptor sites by the volume flow during secretion.

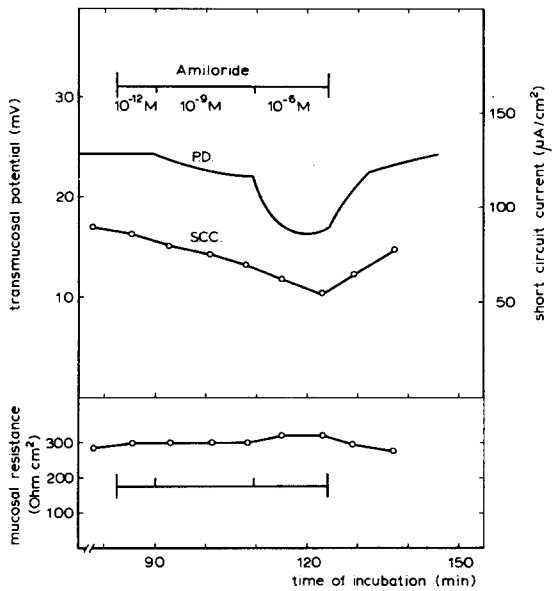


Fig. 11. Effects of mucosal application of amiloride on the electric parameters of the resting mucosa (30 °C).

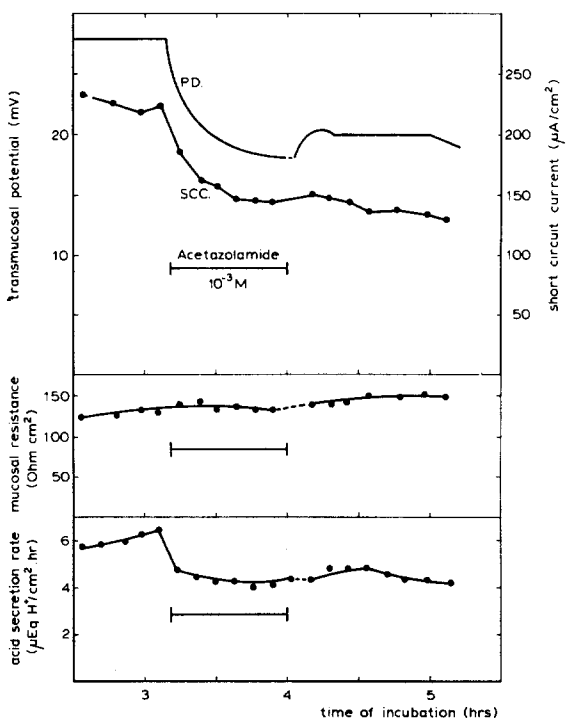


Fig. 12. Effects of $10^{-3}$ M acetazolamide on electric parameters and acid secretion rate of the secreting mucosa (30 °C).

Acetazolamide, an inhibitor of carbonic anhydrase, has also been used. Addition of $10^{-5}$ or $10^{-4}$ M acetazolamide to the serosal bicarbonate containing medium A has no effect on the transmucosal potential and short circuit current in the secreting mucosa, but at $10^{-3}$ M there is a rapid decrease of about 25 % in these two parameters without any change in mucosal resistance (Fig. 12). These effects are only slightly reversible. The acid secretion rate is slightly more sensitive to this substance, with $10^{-4}$ M acetazolamide decreasing it by 23.7 % ($P < 0.01$), and the effect is faster, beginning already in the first 8-min sample after acetazolamide administration and reaching maximal lowering in the second 8-min interval. With a bicarbonate-free medium (medium F) on the serosal side, the effect of acetazolamide is slightly more pronounced ($10^{-4}$ M : 37 %). Washing out the drug does not restore the original acid secretion rate, either when the gastric mucosa is washed with the normal bathing medium A (25 mM $HCO_3^-$), or when a bicarbonate-enriched medium (medium E: 50 mM $HCO_3^-$) is used.

Sodium thiocyanate, a well-known inhibitor of gastric secretion, has also been studied. Addition of 6 or 12 mM NaSCN to the serosal side of the secreting mucosa causes a decrease (36 and 53 %, respectively) in the acid secretion rate (Fig. 13). This inhibitory effect is nearly completely reversible. There is no clear change in the transmucosal potential, short circuit current and mucosal resistance in the presence of NaSCN.

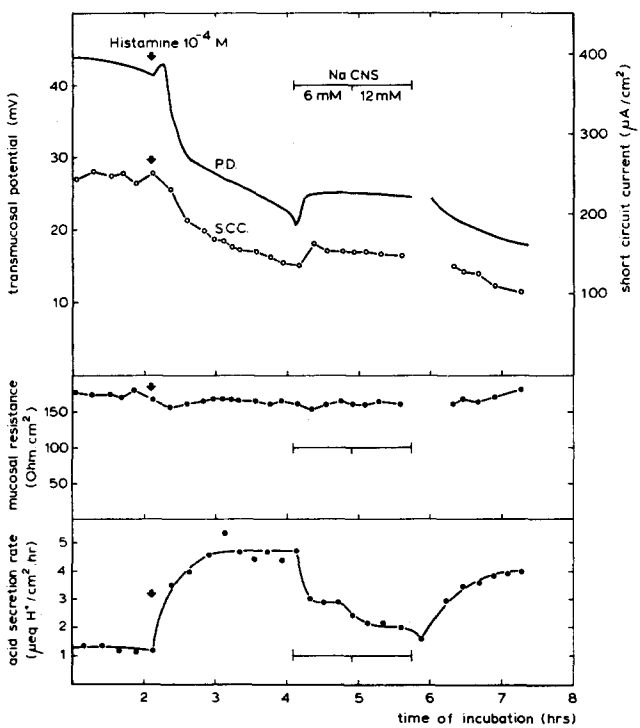


Fig. 13. Effects of serosal application of sodium thiocyanate (6 and 12 mM) on electric parameters and acid secretion rate of the secreting mucosa (30 °C).

## DISCUSSION

The isolated lizard gastric mucosa is an excellent preparation for studying the gastric secretion process in vitro, as previously indicated by Dandrifosse et al. [6]. The transmucosal potential, the mucosal resistance and the acid secretion rate are stable for several hours, when the stripped mucosa is incubated in an Ussing chamber with isotonic media. The resting gastric mucosa can be stimulated in vitro by histamine. In vitro secretion rates (2–6 $\mu$equiv. $H^+/cm^2$ per h) and transmucosal potentials (20–60 mV) are comparable to or higher than those observed in amphibia, suggesting that these parameters and hence the entire secretion mechanism are not greatly affected by isolation and dissection.

The transmucosal potential can very probably be considered to be composed of two membrane potentials, a serosal and a mucosal potential step, since both sides react differently upon ion substitution. The nature of these two potential steps has been elucidated to a considerable extent by the ion substitution experiments.

Stepwise changes of the serosal potassium and chloride concentrations cause changes in transmucosal potential, which are linear with the logarithm of the concentrations over a wide range. The slopes of the linear parts of the curves indicate that the serosal potential step represents a combined potassium chloride diffusion potential. Calculations of the transport numbers for chloride and potassium show that in the resting mucosa 19 % and 84 %, and in the secreting mucosa 46 % and 48 %, respectively, of the membrane conductance are attributable to these ions. On the other hand, the serosal sodium concentration has little effect on the serosal potential step: there is no change upon reduction from 170 to 20 mM and only 24 % reduction upon complete removal of sodium. This suggests that sodium diffusion does not contribute directly to this potential step.

These findings agree with the results for frog and toad, where by means of ion substitution experiments in the Ussing chamber the serosal potential step in the gastric mucosa is also found to be dependent on potassium and chloride [7]. A similar result was obtained for mudpuppy [8] by means of micropuncture potential measurements. In the secreting bullfrog gastric mucosa Forte et al. [9] have measured a potential difference of 54 mV for a tenfold concentration change of the serosal chloride (substitution by isothionate), suggesting that here chloride determines the entire serosal membrane potential.

An additional argument for a contribution of potassium to the serosal potential step in our preparation follows from the observation that the potassium ion conductance varies linearly with the logarithm of the potassium concentration. Such a logarithmic relation indicates the occurrence of negatively charged pores for potassium diffusion [10]. The absence of resistance dependency for chloride in the face of a potential dependency for this anion indicates that chloride ions must diffuse through different pores.

The nature of the mucosal potential step appears to be quite different. The slight effects of mucosal substitution of potassium and chloride appear to rule out that this potential step represents a diffusion potential for either of these ions. Replacement of mucosal sodium by choline has little effect, when the serosal side is bathed with sodium chloride-containing medium. When, however, both sides are bathed with sodium-free media, the transmucosal potential is reduced to one third of its original

value. Application of sodium-free medium on the mucosal side and chloride-free medium on the serosal side results in complete reversal of the transmucosal potential. This substantial potential change, accompanied by a 60 % increase in transmucosal resistance suggests the contribution of an extracellular shunt pathway as described by Sachs et al. [8]. The occurrence of such a shunt can easily mask the actual potential changes, when its resistivity is low. Nevertheless, qualitatively these observations, and also the behaviour towards amiloride, favour the existence of a sodium-dependent mucosal potential step. Under in vivo conditions, however, this step may be negligible, since then both membranes are bathed by sodium chloride-containing fluids.

The asymmetric behaviour of the gastric mucosa is also demonstrated by the ouabain experiments. Ouabain affects the transmucosal potential more rapidly and at 1000-fold lower concentration, when it is added on the serosal rather than on the mucosal side. In addition, on mucosal application there is a time lag of more than 1 h. These findings indicate that an ouabain-sensitive ($Na^+$, $K^+$)-ATPase system, located on the serosal side of the mucosa, must play a major role in the generation of the transmucosal potential. The slow, gradual course of the ouabain effects strongly suggests that no ouabain-sensitive electrogenic sodium pump is involved, which is in agreement with the indications for a combined potassium chloride diffusion potential on the serosal side. This leads to the tentative conclusion that ouabain affects the transmucosal potential indirectly through abolition of the cation gradients across the membranes of the mucosal cells. This is further indicated by the fact that replacement of the normal serosal bathing medium (3 mM $K^+$) by a medium with 80 mM $K^+$ (equal to the intracellular potassium concentration) abolishes the effect of ouabain on the transmucosal potential, even when applied in high concentrations ($10^{-5}$–$10^{-4}$) on the serosal side. Under these conditions the leakage of potassium from the cells, which normally results from ouabain treatment [1], is prevented.

Our observation that the rate of the ouabain effect is strongly dependent on the serosal ouabain concentration ($t_{\frac{1}{2}} = 10$ min at $10^{-4}$ M, $t_{\frac{1}{2}} = 3$ h at $3 \cdot 10^{-7}$ M for the transmucosal potential) also supports this conclusion. At $10^{-4}$ M ouabain the ($Na^+$, $K^+$)-ATPase system is inhibited for 100 %, and at $3 \cdot 10^{-7}$ M for 57 % [1]. An estimate of the minimal time required for abolition of the cation gradients upon complete inhibition of the enzyme system can be obtained from our previously reported data [1]. Under these conditions 43 mmol $K^+$ is lost per l cell water, which at an inulin space of 34 % and a tissue water content of 81.5 % is equal to 125 mmol/kg dry wt. The ($Na^+$, $K^+$)-ATPase activity is 250 mmol/h per kg dry wt, which can pump 750 mmol $K^+$/h per kg dry wt. Assuming that the cation loss upon complete inhibition of the enzyme matches the pump capacity, this cation loss would occur in minimally 125/750 h or 10 min. This suggests that abolition of the cation gradients by $10^{-4}$ M ouabain can occur in approximately the time required for removal of the transmucosal potential. Incomplete inhibition of the cation pump system as with $3 \cdot 10^{-7}$ M ouabain would considerably extend the time required for abolition of the cation gradients, thus explaining the slower effect on the potential.

The acid secretion in the lizard is chloride-dependent. A drastic decrease (90–100 %) is found upon complete replacement of serosal chloride by sulfate. In this respect there is a difference with amphibia, where adequate acid secretion remains if chloride is replaced by sulfate [11]. One possibility could be that the anion-sensitive

ATPase has different anion characteristics in both species. Since the anion-sensitive ATPase activity in the lizard is significantly stimulated by sulfate [12], this explanation is not valid in our case. It is more likely that the lizard gastric mucosa has a lower permeability for sulfate as compared with the amphibian gastric mucosa.

The lizard gastric secretion is also sodium-dependent. Complete replacement of serosal sodium by choline decreases the acid secretion rate by 60–82 %, the residual secretion being probably due to the relatively high sodium concentration remaining in the gastric mucosal cells. While Davenport [13] describes the acid secretion in the frog as sodium-independent, Sachs et al. [14] report that the acid secretion rate of the frog is significantly inhibited when the serosal sodium concentration is reduced to less than 1 % of the normal level. An increased acid secretion is observed, when the serosal side is bathed with a Ringer solution containing a high potassium concentration (80 mM). Under this condition the transmucosal potential is drastically lowered, very probably due to a depolarization of the serosal potential step [8]. This facilitates the chloride entry into the acid secreting cells. In view of the described chloride dependency of the secretion process the enhanced secretion rate can be understood. But why does this process return to the level existing at normal $K^+$ concentration, when ouabain ($1.5 \cdot 10^{-5}$–$10^{-4}$ M) is added rather than decreased to nearly zero, as expected at these ouabain concentrations? Upon addition of ouabain the cation-pump is inhibited, which normally results in a decrease of the intracellular potassium concentration. In this case, however, at high outside potassium concentration this redistribution process will be hindered, thus leaving the acid secretion process unaffected. In *Rana pipiens* [15], high extracellular potassium concentrations also abolish the inhibitory effect of ouabain on acid secretion. On the other hand the presence of sodium and chloride on the mucosal side is not required for normal acid secretion. As far as sodium is concerned this is also demonstrated by the amiloride experiment.

Amiloride, which inhibits passive sodium movement through the cell membrane [16–18], lowers the electric parameters dose-dependently and reversibly, when applied at the mucosal side of the resting mucosa, but not when applied at the serosal side. This suggests that a passive sodium influx on the mucosal side is essential for maintenance of these parameters. This finding agrees with our earlier conclusion that the mucosal potential step is mainly a sodium diffusion potential. The absence of an amiloride effect in the secreting mucosa may be due to the fact that the inhibitor is kept away from its receptor site by the volume flow during secretion.

The inhibitory effect of thiocyanate on acid secretion, but not on the electric parameters, is compatible with a role of the anion-dependent ATPase in the process of gastric secretion. The percentage inhibition of acid secretion (36 % for 6 mM, 53 % for 12 mM NaSCN) is nearly equal to that of the enzyme of the lizard gastric mucosa (33 % for 6 mM, 45 % for 12 mM NaSCN, with chloride as the only other anion; see Fig. 3 in ref. 12). It should, however, be pointed out that Davenport [19] has reported inhibition of carbonic anhydrase in human and dog gastric mucosa by thiocyanate (50 % inhibition by 0.6 mM thiocyanate).

The involvement of carbonic anhydrase in the process of acid secretion is indicated by the experiments with acetazolamide, a known inhibitor of carbonic anhydrase. Acetazolamide, added to the serosal side of the secreting mucosa, rapidly and partially inhibits the electric parameters and the acid secretion. These effects are

only slightly reversible, in contrast to Hogben's observation for acid secretion in the bull frog [20]. Inhibition is even stronger in the absence of bicarbonate in the medium, which may be due to the partial dependence of the acid secretion in the lizard on the presence of external bicarbonate (in the absence of bicarbonate 81 % of that in its presence). The effects of both inhibitors differ qualitatively as well as quantitatively, which strongly suggests that indeed two different enzyme systems are involved.

Although the changes in acid secretion rate roughly follow those in transmucosal potential and short circuit current, there are a number of noticeable differences. At $10^{-4}$ M serosal ouabain, $t_{\frac{1}{2}}$ for the latter is 10 min, and for the acid secretion 34 min. At $3 \cdot 10^{-7}$ M serosal ouabain, $t_{\frac{1}{2}}$ for the latter is 3 h, and for the acid secretion 1.4 h. While the electric parameters in these cases go virtually to zero, nearly a quarter of the acid secretion is retained. Addition of $5 \cdot 10^{-4}$ M ouabain on the mucosal side gives a time lag of 0.8 h for the electric parameters, but of 3.3 h for the acid secretion. Another difference is that amiloride affects the transmucosal potential, but not the acid secretion. Furthermore, thiocyanate inhibits acid secretion but has no influence on the electric parameters. Also the effects of ion substitution on these two parameters are clearly different. No immediate explanation can be given for these differences. However, they indicate once again that the acid secretion is not a simple function of the transmucosal potential.

Summarizing, it may be concluded that the transmucosal potential is composed of a mucosal sodium diffusion potential in series with a combined chloride and potassium diffusion potential on the serosal side. There is no indication for a significant involvement of an electrogenic sodium pump. The more sensitive and more rapid inhibition of transmucosal potential and acid secretion upon application of ouabain on the serosal side suggests that this membrane has the major $(Na^+, K^+)$-ATPase activity of the two sides of the mucosa. The effects of ouabain on potential and acid secretion appear to be indirect and due to the abolition of the cellular cation gradients. There does not seem to be a simple causal relationship between acid secretion and potential. The effects of acetazolamide and thiocyanate are compatible with a role of carbonic anhydrase and anion-dependent ATPase in the gastric secretion process.

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