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Acid Secretion and Intracellular pH in Isolated Oxyntic Cells

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Summary. This study demonstrates that isolated oxyntic cells are capable of secreting hydrochloric acid. Transitory peaks of medium acidification were observed when isolated oxyntic cells were stimulated with different secretagogues. The duration of these peaks was of about 3 min and had a magnitude of about 0.5 μEquiv/hr mg dry weight. These peaks were abolished by selective inhibitors of acid secretion and/or secretagogue action. Cell pH, as measured by the $DMO^{14}C$ technique increases 0.13 pH units upon stimulation with db-cAMP. This change was abolished by pretreatment with SCN^-. From acidification and cell pH experiments an increase of 5–10 mM in base concentration inside the cell upon stimulation is calculated. These results are interpreted on the basis of the existence of a neutral anion exchange mechanism at the serosal side that serves to control the cell acid-base balance.

Oxyntic or acid secreting cells have been isolated in more or less pure preparations from the stomachs of both mammalians and amphibians.

However, there are no reports on the properties of these cells as related to acid secretion, the assessment of ion transport being rather difficult. Where the secreted species is a protein, measurement of the biological activity is no more difficult than in the intact organ. Thus, secretion of enzymes and hormones has been demonstrated in a variety of isolated cell systems including exocrine pancreas, isolated pancreas islets, thyroid, adrenals and many others. Sometimes the process involves the transport of a molecule that can be radioactively labeled or a nonmetabolizable analogue that can be otherwise determined; at other times, the biological response may not involve the transport of a given species, but a change in a metabolic process that can be easily quantified.

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None of these is the case for the secretion of H^+ by the isolated oxyntic cells, where it is theoretically impossible to demonstrate such a secretion. It has been established that in the steady state the pumping of H^+ into the gastric lumen is accompanied by an stoichiometric release of base into the blood or serosal side [3]. Furthermore, Rehm and colaborators [15, 17] have shown that the release of alkalai into the serosal side takes place by means of an electrically neutral exchange, probably of HCO_3^- for Cl^-. Consequently, in the cell preparation there would be an immediate neutralization of the H^+ in the suspension medium.

Since H^+ pumping is an active process, and alkaline release through the exchange mechanism is supposed to be passive, accumulation of base inside the cell at the onset of secretion may be expected. This may then set up an electrochemical gradient that would drive the exchange mechanism. The initial accumulation of base (OH^- or HCO_3^-) would tend to shift the pH of the cytoplasmic compartment of the cell to the basic side. The magnitude of this shift will depend on the acid output, the volume and buffering capacity of the cytoplasmic compartment and the maximum rate of turnover of the exchange diffusion carrier. The change in intracellular pH may be large enough to be measured by conventional methods such as the analysis of the distribution of weak acids. On the other hand, for the same reasons expressed above, it may be possible to measure a change in outside pH at the onset of secretion, provided the concentration of buffer in the suspension medium were kept sufficiently low as to allow a quantification of the acid output.

This study concerns the measurements of changes in extracellular and intracellular pH in response to stimulation in oxyntic cells isolated from bullfrog gastric mucosa.

Materials and Methods

Solutions

Buffer solutions (TES-nutrient) used in this study contained in mM: 70 NaCl, 4.0 KCl, 0.8 $MgCl_2$, 1.8 $CaCl_2$, 1.0 Na_2HPO_4, 40.0 TES (N-tris-(hydroxymetthyl) methyl-2-aminoethane sulfonic acid, $pK_a = 7.5$ at 25 °C), 11 glucose. The pH was adjusted at 7.2. TES-HCO_3^- nutrient was prepared by replacing 18 mM TES (from a total of 40.0 mM TES) by 18 mM $NaHCO_3^-$. All other salts remained as in TES-nutrient. TES-HCO_3^- nutrient was always equilibrated with air +5% CO_2.

Low-TES-nutrient contained 105 mM NaCl, 5 mM TES and no phosphate or glucose. All other components remained as in TES-nutrient.

Enzyme solution was prepared in TES-nutrient by adding 5% fetal calf serum (Gibco) and 0.175% pronase (Merck, 70,000 PKU/g).

All solutions contained 1% bovine serum albumin (BSA, FV, Miles Labs.), 100 μ/ml penicillin, 100 μg/ml streptomycin and 0.25 mg/ml amphotericin B. Enzyme solution contained antibiotics but not BSA.

Cell Isolation

Oxyntic cells were isolated from the stomachs of *Rana catesbeiana* by the methods previously described [11]. Stomachs removed from pithed frogs were stripped from the muscular coat and the remaining mucosal layer was mounted as a diaphragm on lucite tubes. The surface epithelium and mucous neck cells were first removed by exposing the luminal side to a hypertonic solution (0.5 M NaCl) according to the methods described by Forte *et al.* [5]. Then the luminal side was exposed to enzyme solution at 32 °C for 90 min in a shaking waterbath. At this point shaking of the assembly in a vortex mixer brings about a massive detachment of cells from the mucosa into the solution bathing the luminal side. This suspension of cells was centrifuged at $60 \times g$ for 5 min in a refrigerated centrifuge at 0 °C. The cell pellets were washed twice with nutrient solution containing 10% fetal calf serum. The pellet was then resuspended in 5 ml nutrient + 1% BSA and layered on two tubes containing 5 ml nutrient + 20% FCS + 2% BSA and centrifuged for 3 min at $50 \times g$ in a swinging bucket rotor. The final pellets were pooled and resuspended in nutrient + 1% BSA and kept at 0–2 °C until use.

Oxyntic cells were identified by succinic dehydrogenase staining by a modification of the method of Nachlas *et al.* [12]. Viability was determined by the trypan blue exclusion test. The final preparation contained about 80% oxyntic cells ($81.3 \pm 4.5\%$; mean $\pm$ SEM; $n = 14$), with a viability always larger than 90%.

Acidification

Isolated oxyntic cells were suspended in low-TES-nutrient containing antibiotics and 1% BSA at an approximate concentration of 3 mg dry wt/ml. Four ml of the cell suspension were incubated in glass chambers with magnetic stirring, thermostatically maintained at 25 °C. The pH of the suspension was maintained constant by means of pH-Stat system (Radiometer). End-point of titration was pH 7.25–7.45. Acid output by the isolated cells was titrated with 7.7. mM NaOH.

After an incubation period of one hour, addition of secretagogues or inhibitors were made directly to the chamber. Substances were added as a $100 \times$ concentrated solution. Dibutyryl-cAMP (db-cAMP) was also added in the powder form. Usually there was a small deviation of the end point pH upon addition of secretagogues or inhibitors. The suspension was then rapidly titrated back to the end point or the end point reset to the new pH. Then the pH-Stat procedure was continued. Lactic acid was measured in deproteinized samples of both cell suspension and supernatant, by a modification of the method of Loomis [9]. Most of the lactate produced eluted into the suspension solution [4].

Intracellular pH

Preparation of samples. In order to measure intracellular pH, a modification of the method of Poole *et al.* [13] was used. This technique is based upon the equilibration of the weak acid 5,5-dimethyl-2,4-oxazolidinedione-^{14}C (DMO-^{14}C) across the membrane

of the cell. If one assumes that only the undissociated form is permeable, then the intracellular pH can be calculated by measuring extracellular pH, DMO-^{14}C in the cell pellet and the supernatant, and the cellular volume [22].

In the method described by Poole *et al.* [13] two parallel pellets are analyzed; one for DMO-^{14}C and one for inulin-^{14}C, each having a different extraction procedure.

In preliminary experiments it was found that this extraction procedure in two different pellets introduced an appreciable source of error, up to 0.1 pH units, that limited the sensitivity of the method. Thus, the procedure was modified so as to allow for the simultaneous determination of DMO-^{14}C and Inulin-^{14}C in a single pellet.

Isolated oxyntic cells were suspended in TES-HCO_3^- nutrient +1% albumin set approximately at a concentration of 2 mg dry wt/ml. DMO-^{14}C and Inulin-^{14}C were added to the cell suspension contained in a Falcon culture flask at final concentrations of 0.005 μCi and 0.1 μCi/ml of cell suspension, respectively.

The suspension was incubated in a shaking bath for 5 min and then transferred to glass chambers with magnetic stirring (Yellow Spring Instruments) at 25 °C; air +5% CO_2 was blown over the surface.

In experiments where the effect of stimulation on intracellular pH was studied, 7 ml of cell suspension was contained in each of two chambers. After an equilibration period of an hour, the pH of the cell suspensions was measured with a glass-calomel combination electrode and a pH-meter equipped with an expanded scale (Radiometer, PHM27). Then a 2-ml sample was placed in preweighted glass tubes; this was termed 0-min sample. Immediately after, 5 mM dibutyril-cAMP (db-cAMP) was added to one of the chambers. Samples were taken from control and experimental chambers at 20 and 60 min. Extracellular pH was measured prior to withdrawal of the samples.

Determination of DMO-^{14}C and Inulin-^{14}C. In order to determine the quantities of DMO-^{14}C and Inulin-^{14}C, each 2-ml sample was processed in the following way. Immediately after taking the sample, the tubes were spun at $3000 \times g$ for one min at 0 °C in a Sorvall centrifuge (RC-2B) equipped with an angle rotor. The supernatant was removed, the inside walls of the tube were wiped dry, and the tube with the pellet weighed. A 0.1-ml sample of the supernatant was then transferred to a glass test tube. The tubes containing the cell pellet and the supernatant sample were then dried in an oven at 95 °C for 24 hr. The dried cell pellet was then weighed in order to determine the total water content of the cell sample. To both supernatant and cell sample, 1 ml of 0.1 N HCl was added and they were incubated for 36 hr at room temperature. After this period, the samples were spun at $7700 \times g \times 3$ min in order to remove the digested tissue. A 0.1 ml sample was taken from both cell supernatant samples and was added to a scintillation vial containing 10 ml of a toluene-base scintillation fluid. In this sample total radioactivity contributed both by Inulin-^{14}C and DMO-^{14}C was measured. However, the counts due to Inulin were 20 times those contributed by DMO-^{14}C. In order to determine only DMO-^{14}C, a procedure similar to that of Poole *et al.* was used, except that two extractions were performed.

Five ml of 50% ethyl acetate-50% toluene v/v were added to the 0.9 ml. The tubes were shaken for 30 sec in a vortex mixer and then centrifuged at $3000 \times g \times 3$ min. Of the upper layer (ethyl acetate-toluene), four ml was transferred to a counting vial containing 10 ml of a toluene-base scintillation counting fluid. Another four ml of the ethyl acetate-toluene mixture was then added, and shaking and centrifugation were repeated. Four ml from the upper layer was transferred to a counting vial as before. These samples were termed D_1 and D_2 and total DMO counts were calculated from D_1 and D_2 after correction for background.

The extraction of DMO by this method is essentially complete. Because inulin is

not extracted into the organic phase, the inulin counts can be calculated by subtracting the DMO counts from the total counts.

The equation described by Wadell and Butler [19] as modified by Poole *et al.* [13] was used to calculate intracellular pH. Direct counts are used in this equation.

From the measurements of extracellular space in the cell pellet, the amount of cell water and wet/dry wt ratios could be derived. Wet/dry ratio in the isolated cell pellets (corrected for extracellular space) had an average of 3 in more than 100 samples analyzed. This value will be used for the calculation of intracellular buffers.

Results

Acidification

Results of single representative experiments in this series are presented. The effects of different secretagogues upon the rate of medium acidification in the isolated cell suspension is shown in Fig. 1. There was a basal rate of acidification prior to stimulation that was always about 0.1 μEquiv H^+/hr mg dry wt. About half of this rate could be accounted for by lactic acid production (in three experiments lactic acid production was 0.048 μmole/hr and total H^+ output in basal conditions

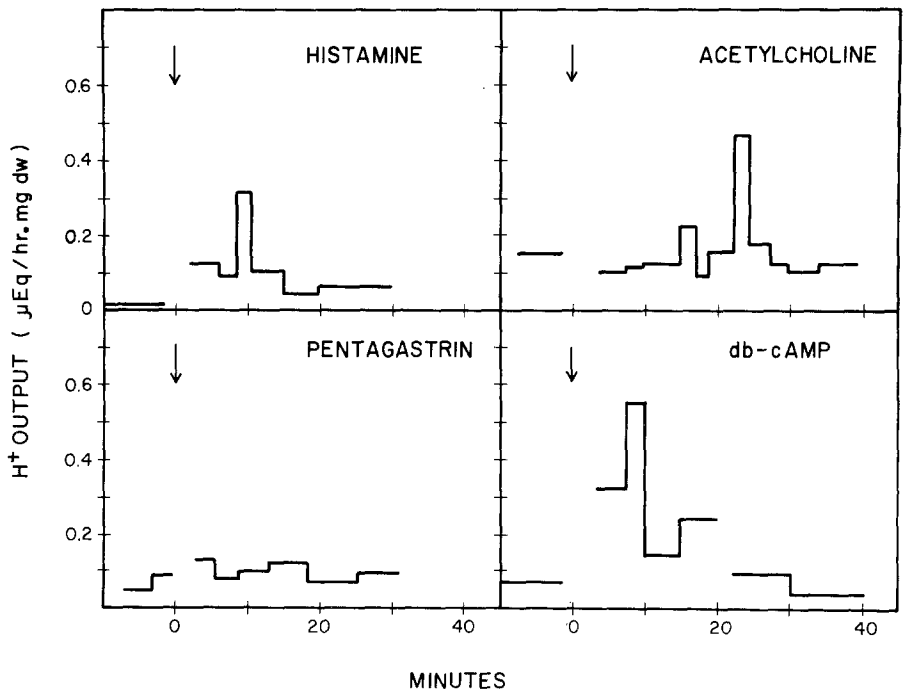

Fig. 1. Effect of secretagogues on H^+ output by isolated oxyntic cells. Acidification measured by continuous pH-stat. Histamine (10^{-4} M), acetylcholine (10^{-3} M), pentagastrin (10^{-6} M) and db-cAMP were added after basal H^+ output was measured

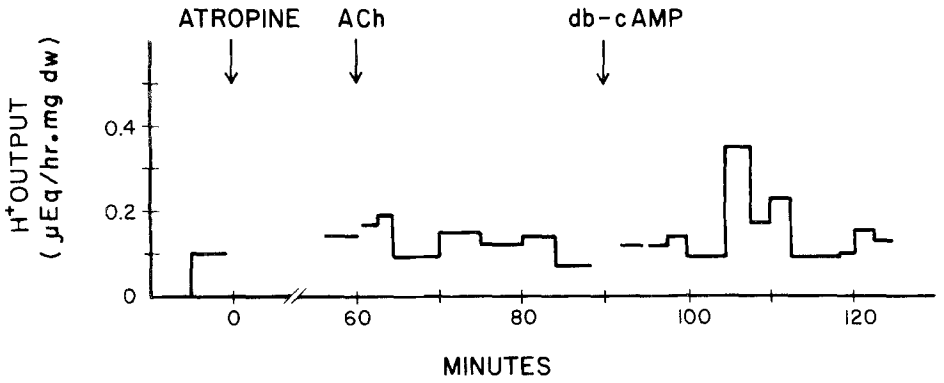

Fig. 2. Effect of atropine on the acidification response of isolated oxyntic cells to acetylcholine (ACh) and db-cAMP. Atropine (10^{-6} M) was present for 60 min before ACh (10^{-3} M) was added. Db-cAMP (5×10^{-3} M) added 30 min after ACh. Pretreatment with atropine blocks the response to ACh, but not to db-cAMP

was 0.087 μmole/hr mg dry wt). Histamine (10^{-4} M) acetylcholine (10^{-3} M) and db-cAMP (5×10^{-3} M) produced transitory peaks of acidification. The magnitude of these peaks was of 0.3 to 0.5 μEquiv H^+/hr mg dry wt and had a duration of 2 to 4 min, sometimes longer. They were observed 10 to 20 min after stimulation with the different secretagogues. Such transient peaks were never observed upon treatment of the cell suspension with pentagastrin (10^{-6} M). This is consistent with the hypothesis that this compound acts by liberating histamine from stores outside the oxyntic cell [7, 14].

To discard the possibility of these peaks being an experimental artifact, the effects of specific inhibitors were examined. Thus, atropine was used as an inhibitor of action of acetylcholine [20], burimamide as an H_2 receptor blocker [1, 19] and thiocyanate as an inhibitor at a more distal point.

The effects of atropine on the response to acetylcholine and db-cAMP are shown in Fig. 2. Pretreatment with atropine (10^{-6} M) for 1 hr completely blocks the transient peak in acidification produced by stimulation with acetylcholine. However, in these conditions db-cAMP was able to induce a response, indicating that atropine has no action on the acid production mechanism itself but blocks the stimulation by acetylcholine. On the other hand, this result indicates that db-cAMP acts at a more distal point in the chain of events that lead to the production of acid.

Similar effects were obtained when burimamide was used to block

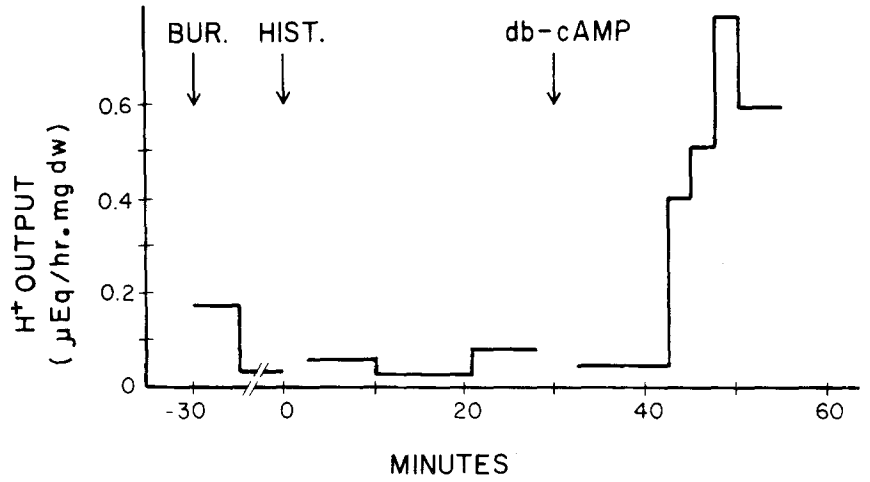

Fig. 3. Effect of burimamide (*BUR.*, 10^{-3} M) on the acidification response of isolated oxyntic cells to histamine (*HIST*) and db-cAMP. Burimamide was present for 30 min before histamine was added. Db-cAMP (5×10^{-3} M) added 30 min after histamine. Pretreatment with burimamide inhibits the response to histamine but not to db-cAMP

the action of histamine. Burimamide is a competitive inhibitor of histamine H_2-receptors [1, 19]. Fig. 3 demonstrates the effect of 30-min pretreatment with burimamide on the response to histamine on acidification. Nevertheless, db-cAMP produced a transitory peak as shown before in control experiments.

Inhibition of the transporting mechanism itself should block the actions of all secretagogues including db-cAMP. Thiocyanate has been considered to be a specific inhibitor of the H^+ pump in the stomach. This assumption is based on the observation that it inhibits acid secretion without affecting Cl^- transport. However, the mechanism of action is not known. Treatment with thiocyanate brings about a complete abolition of the response to all secretagogues. Fig. 4 shows the effect of pretreatment with SCN^- (20 mM) on the response to db-cAMP. This compound is able to induce a response in the untreated condition; however, pre-treatment with SCN^- for 30 min inhibits the transient peak of acidification obtained in the control. In the same manner, no response was obtained with either histamine or acetylcholine when cells were pretreated with thiocyanate.

In another series of experiments TES was omitted in the solutions and the pH maintained constant at pH 7.2 with the pH-stat. In these experiments, the acidification effect was markedly reduced and in many

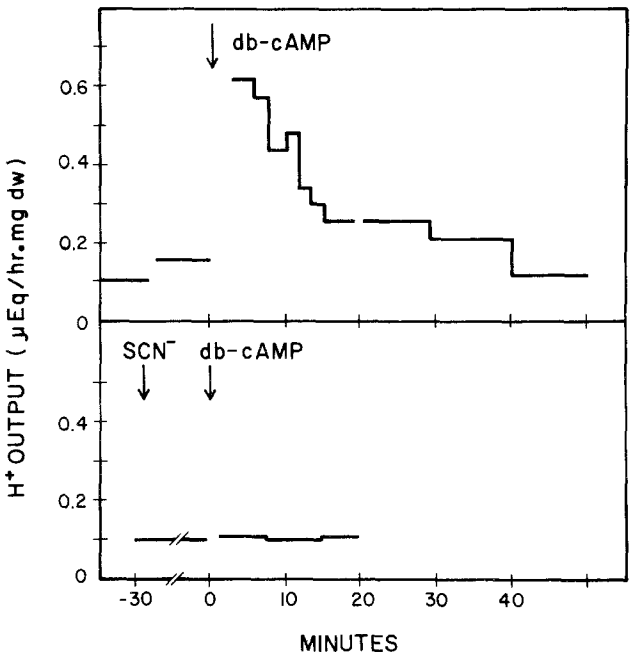

Fig. 4. Effect of SCN^- (10 mM) on the acidification response of isolated oxyntic cells to db-cAMP (5×10^{-3} M). Top graph shows the response to db-cAMP in control conditions (*see also* Fig. 1). Bottom graph shows clearly the inhibition of the response to db-cAMP by SCN^-

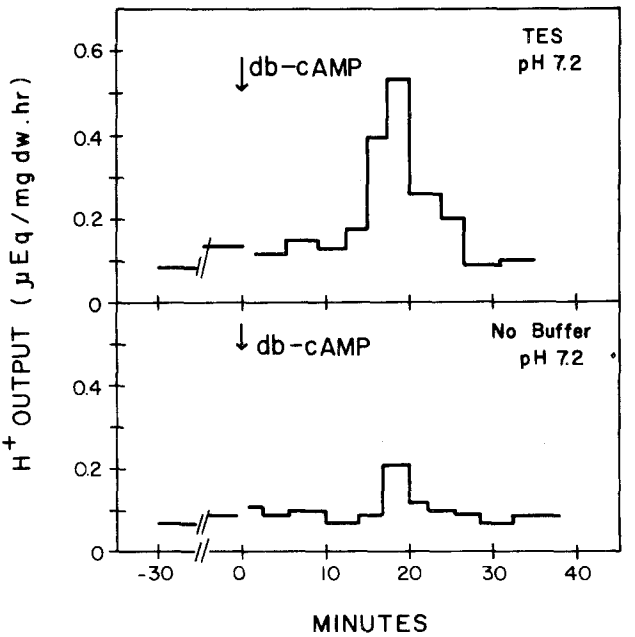

Fig. 5. Effect of withdrawal of buffer from suspension solution upon the acidification response of isolated oxyntic cells to db-cAMP. Cells were maintained at pH 7.2 with the pH stat method with and without 5 mM TES and the response compared in two aliquots

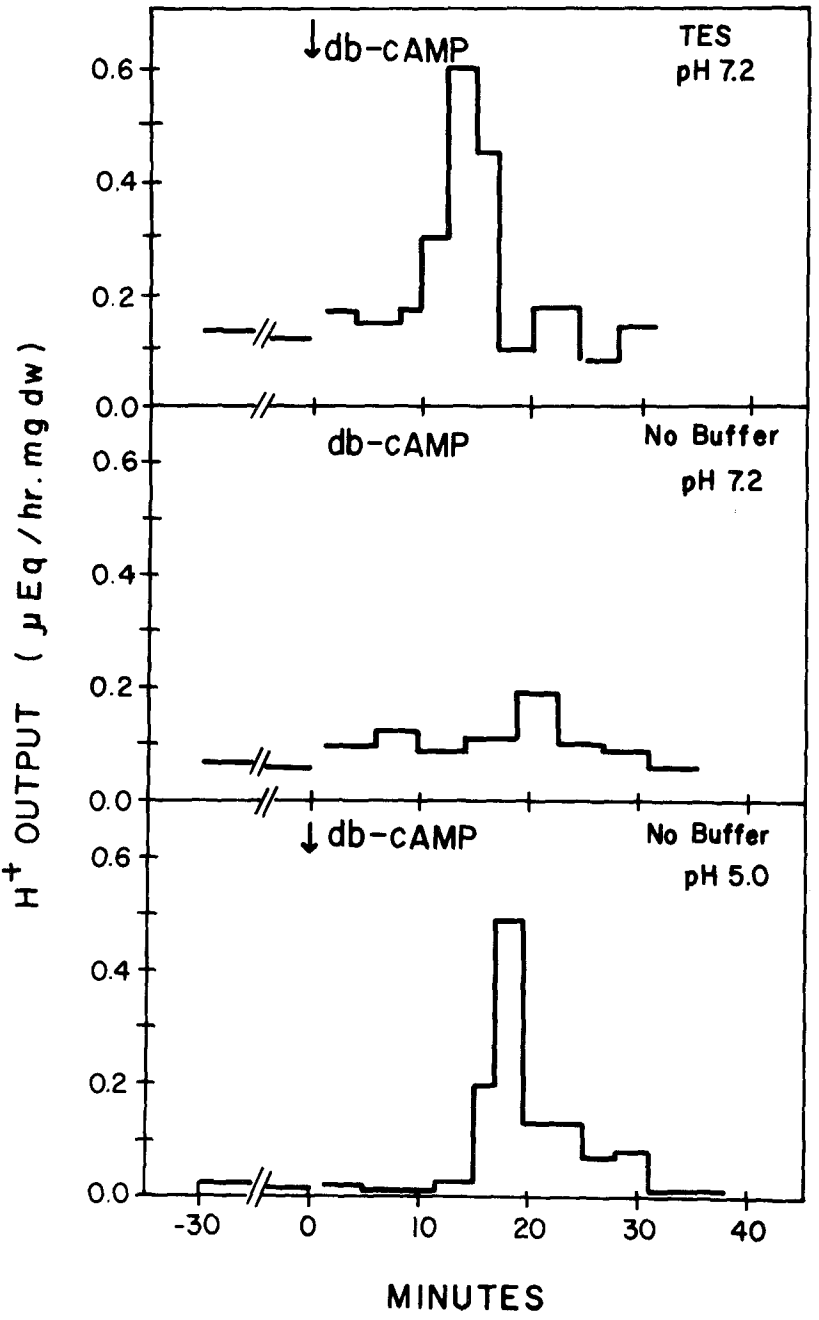

Fig. 6. Effect of different outside pH and buffer conditions upon acidification response of isolated oxyntic cells to db-cAMP. *See text* for details

cases disappeared. One of such experiments is shown in Fig. 5. In this figure, the response to db-cAMP is compared in cells with and without TES at pH 7.2. It can be observed that the acidification response is less than half in the cells without TES. Similar observations have been reported in the intact gastric mucosa by Sanders *et al.* [16]. These workers also reported that secretion could be reestablished if the pH of the nutrient solution was lowered. In the experiment in Fig. 6, the response to db-cAMP was compared in three different conditions: 5 mM TES at pH 7.2, no buffer at pH 7.2, and no buffer at pH 5.0. In the absence of buffer at pH 7.2 the response almost disappeared, as shown above in Fig. 5. However, an almost full response could be obtained in the no-buffer condition at pH 5.0. This effect at pH 5.0 was completely abolished by 20 mM SCN^- (not shown).

Intracellular pH

Since the method described originally by Poole *et al.* [13] prescribes the extraction of DMO into the organic phase (ethylacetate-toluene) from 5 M NaH_2PO_4, it was necessary to check how efficient the extraction of DMO from HCl is. Fig. 7 shows the relative extraction of a known amount of DMO into the organic phase as a function of the number of extractions and the nature of the inorganic lower phase. Part *A* of this figure shows that the extraction of DMO from 0.1 N HCl is not as efficient as from NaH_2PO_4. Thus, two extractions are performed to extract 98% of the radioactivity. Part *B* of the figure illustrates that the extraction from the digested cell pellet sample is less efficient than from the supernatant sample (87% *vs.* 92%). However, when two extractions are used there is no significant difference in the radioactivity extracted from both samples. Thus, the use of one sample for both inulin and DMO determinations presents the advantage of requiring half the amount of cell suspension and considering only one weight in the calculation, avoiding in this way one more potential source of error.

Extracellular pH was constant throughout the entire period of measurement of intracellular pH (60 min). In most of the experiments the value obtained for intracellular pH was somewhat higher than the measured extracellular pH. The mean value for intracellular pH in control conditions was 7.187, which is distinctly higher than the value of 7.110 ($p < 0.025$) recorded for extracellular pH.

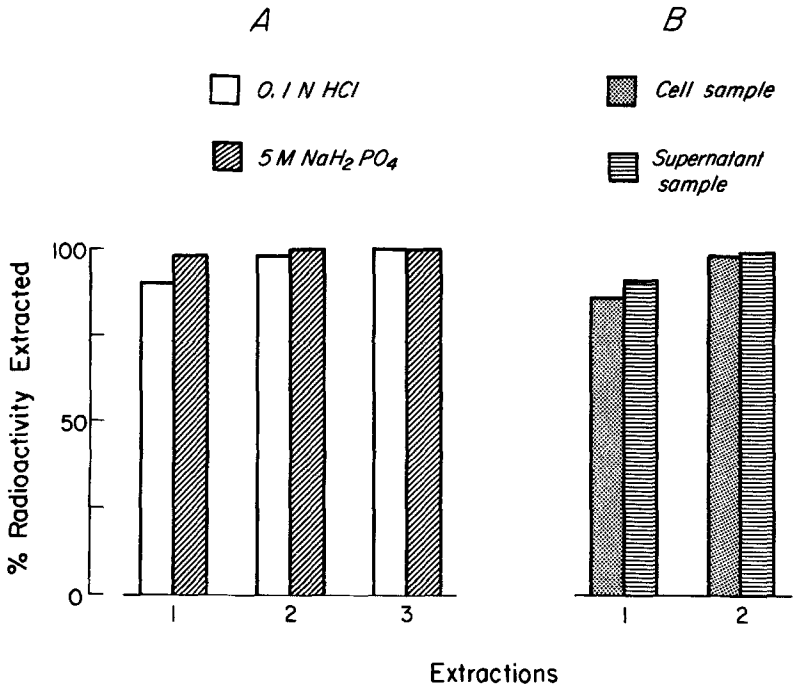

Fig. 7. Check of the efficiency of extraction of DMO-^{14}C into the organic phase. (*A*): Extraction from 0.1 M HCl as compared to 5 M $NaH_2PO_4^-$. (*B*): Extraction from cell pellet extract as compared to supernatant extract

Table 1. Effect of db-cAMP (5 mM) on intracellular pH (pH *i*) in isolated oxyntic cells

	pH*i*		*p*
	Control	db-cAMP	Control *vs.* stimulated
0′	7.183 ± 0.050	7.194 ± 0.047	(ns)
p(20′ − 0′)	(ns)	(< 0.02)	
20′	7.182 ± 0.051	7.317 ± 0.056	(< 0.005)
p(60′ − 0′)	(ns)	(< 0.01)	
60′	7.187 ± 0.055	7.311 ± 0.059	(< 0.05)

[a] $n = 5$. Intracellular pH measured at 0 min and 20 and 60 min after addition of db-cAMP. Cells pooled from 6 mucosae in each experiment. Values are means ± SE.

The effects of stimulation with db-cAMP on intracellular pH is shown in Table 1. Two aliquots of the same batch of cells were studied at the same time. In one chamber db-cAMP was added at 0 min (*cf.* Materials and Methods), keeping the untreated chamber as a control. Samples

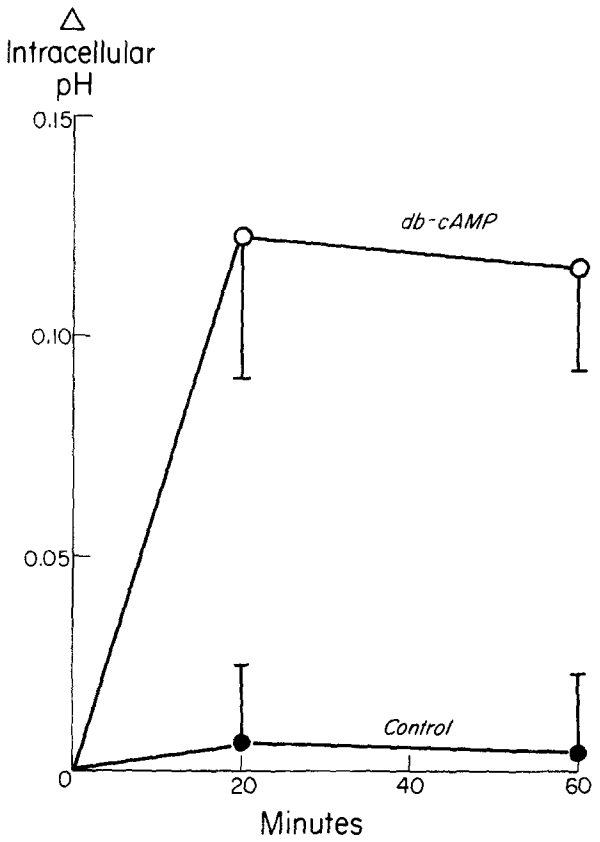

Fig. 8. Change in intracellular pH in isolated oxyntic cells as a function of time in control conditions (–●–) and stimulated with 5 mM db-cAMP (–○–). Bars represent the SE of the means; $n=5$

were taken at 0 min and 20 and 60 min after the addition of the secretagogue. As was expected, intracellular pH at 0 min was the same in both aliquots (control and stimulated). The values obtained for cell pH in the control chamber at 20 and 60 min do not differ from values at 0 min, which indicates the maintenance of a steady-state throughout the experimental period. Stimulation induced an increase in cell pH from 7.194 to 7.317 at 20 min. This change was maintained 60 min after stimulation with a cell pH of 7.311. These changes were statistically significant.

There was scatter in the absolute value of cell pH from experiment to experiment due to small variations in extracellular pH. Thus, changes in cell pH are plotted in Fig. 8. Addition of db-cAMP induces a change in cell pH of about 0.12 pH units. The magnitude of this change did

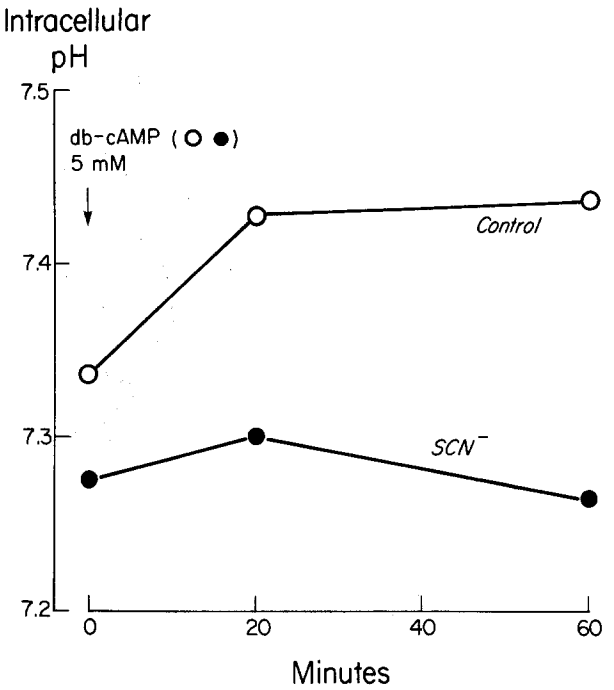

Fig. 9. Effect of SCN^- on the change in intracellular pH induced by db-cAMP in isolated oxyntic cells. One aliquot of cells was incubated for 60 min with 20 mM SCN^-. Intracellular pH measured at the end of this period (0 min) in control and SCN^- treated aliquots. db-cAMP was then added to both aliquots and intracellular pH measured 20 and 60 min after addition

not depend on the initial values of pH (7.032–7.302), at least in this small range. The error around the points in the controls is an indication of the sensitivity of the method, assuming a steady-state condition for the control aliquot.

It was of primary interest to know whether this change in cell pH was related to the stimulation of acid secretion by db-cAMP and not an effect of some other action of the compound. In an attempt to answer the preceding question the effects of pre-treatment with 20 mM thiocyanate on the cell pH change induced by db-cAMP were studied. These results are presented in Fig. 9. Db-cAMP induces an increase in intracellular pH in the untreated chamber, whereas this effect is abolished by pre-treatment with SCN^-. Although this result must be interpreted with caution, it should be pointed out that this anion inhibited acid secretion in the intact frog gastric mucosa and also abolished the transient acidification peak induced by stimulation with all secretagogues including db-cAMP, observed earlier in this study.

Discussion

The secretion of H^+ into the lumen of the stomach is an active process that requires the splitting of water into H^+ and OH^-. This fact implies that such secretion leaves the cell with an excess of OH^- that must be neutralized and/or released outside the cell. The most accepted view is that the OH^- and CO_2 form bicarbonate that leaves the cell at the serosal side in exchange for Cl^- [15, 17] in a carrier mediated mechanism similar to the one existing in the red blood cell. The Cl^- coming into the cell would accompany the H^+ pumped at the luminal side. The HCO_3^-/Cl^- exchange mechanism is supposed to be passive and the rate of exchange dependent on the electrochemical gradients of the species involved and the rate of turnover of the carrier. In the steady state the efflux of H^+ at the luminal face should be equal to the HCO_3^- efflux at the serosal face. In fact, it has been shown that the secretion of acid into the gastric lumen is accompanied by a stoichiometric release of an equal amount of base into the serosal side [3]. Thus, in the isolated cell suspension the H^+ would be immediately neutralized by the base release.

However, since H^+ secretion is an active process and the release of base depends on a chemical gradient, at the onset of secretion these two processes are not simultaneous, and a time lag ought to be observed between secretion and base release. Thus, in the case of isolated cells, a transitory modification of the suspension medium pH should follow stimulation of secretion; on the other hand, the initial accumulation of base would tend to shift the pH of the cytoplasmic compartment to the basic side.

Both of these phenomena have been demonstrated in the present report. Histamine, acetylcholine and db-cAMP induced transitory peaks of medium acidification in isolated cells. This is in agreement with the model discussed above. The H^+ output at the peak of acidification can be compared with the acid secretion in the intact mucosa by doing some calculations. The magnitude of this peak was of a maximum of about 0.5 μEquiv H^+/hr mg dry wt. The amount of cells contained in a chamber is about 12 mg dry weight which corresponds to 36 mg wet wt of isolated cells, being 28.8 mg oxyntic cells (80%). One cm^2 of intact mucosal preparation has a wet weight of about 80 mg and of these about 40% is submucosa and muscularis mucosa (32 mg). Of the 48 mgrs of mucosa properly 28% are oxyntic cells (or 13 mg). If we consider 5 μEquiv/cm^2hr to be the maximal rate of H^+ secretion

in *Rana catesbeiana,* there is more than twice of the weight of isolated cells needed to produce such a secretion (assuming maximal secretion) and the amount measured is within the expected range.

The duration of the observed peak of acidification represents the time lag in which base release at the serosal side of the cell becomes equal to H^+ output. It can also be considered as the time necessary to build up the electrochemical gradient that would drive the HCO_3^- or other base out of the cell at a rate equalling that of H^+ secretion. A preliminary calculation of the magnitude of this gradient can be made from the present data. The measured secretion of H^+ represents the acid secreted in excess of base release during the interval of time. On the other hand, it also represents the amount of base equivalents remaining inside the cell at the onset of secretion. If we take 5 μEquiv H^+/hr per chamber as the rate of acid production at the transient peak and 3 min as the mean duration of this peak, we can calculate that 0.25 μEquiv OH^- are left in excess inside the cell. Assuming that these OH^- are buffered by the CO_2/HCO_3^- system and dividing by the cell water (24 μl; wet/dry ratio is 3) we obtain that there is a 10 μEquiv/liter increase in $[HCO_3^-]$ at the peak of secretion, and this increment would be necessary to drive the exchange mechanism. However, this calculation assumes equal distribution of HCO_3^- in the cell water and the calculated number may vary according to the distribution of HCO_3^- across the membranes of the different organelles of the cell. Nevertheless, this number may be taken as the order of magnitude of the increase in anion concentration to drive the exchange mechanism.

On the other hand the situation in these experiments is a little more complicated since no added CO_2 was present in the gas phase. The amount of CO_2 present due to metabolism cannot be calculated. However, the rate of production of CO_2 can be estimated from oxygen consumption experiments (to be reported elsewhere). Basal QO_2 is 4.8 μl/mg dry wt hr and increases to 6.6 μl/mg dry wt hr under stimulation with db-cAMP. Assuming a respiratory quotient of 0.8, the CO_2 production would be of 0.17 and 0.24 μmole/mg dry wt hr. This amount of CO_2 is less than half of that needed to sustain a secretion of at least 0.5 μEquiv/mg dry wt hr, if one assumes that the whole amount of CO_2 produced is available for buffering. Taking in account only the increase in CO_2 production from the basal to the secreting state, this amount represents only about 12% of the CO_2 required.

Based on the work of Rehm and collaborators [16] a mechanism can be postulated to account for the needed CO_2. The presence of

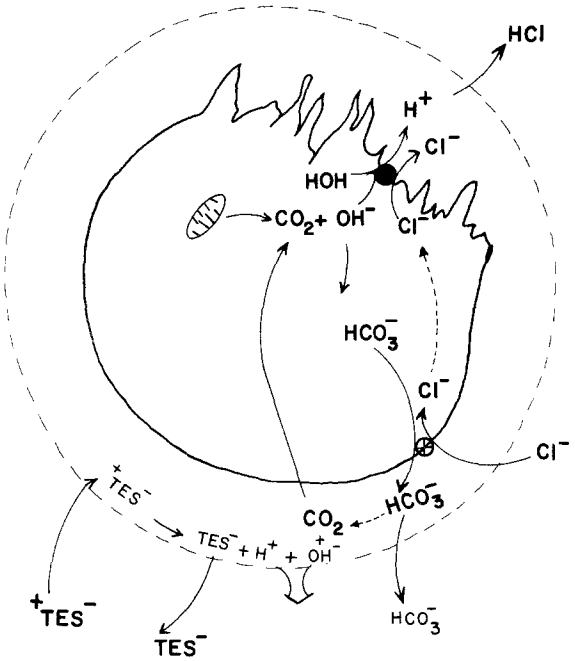

Fig. 10. Schematic representation of a hypothetical CO_2 recycling mechanism in the isolated oxyntic cell in the absence of buffer. Dotted line around the cell represents the unstirred layer-bulk phase boundary. *See text* for details

an outside buffer (TES) at a pH below its pK_a, would make CO_2 from the HCO_3^- that exits through the exchange mechanism. This CO_2 could then pass into the cell and recycle. Such a mechanism is pictured in Fig. 10. Such a scheme requires the presence of an unstirred layer between the cell and the bulk phase. With an outside buffer like TES present, the pH of the unstirred layer would be maintained close to that of the bulk phase. Part of the bicarbonate entering this compartment from the cell would be reconverted to CO_2 depending on the pH. This CO_2 could then pass into the cell and recycle, the cell thus acting as a sink for CO_2. If no buffer is available in the unstirred compartment, its pH would rise with secretion and as a consequence no CO_2 would be available for recycling. In this way the rate of acid production would be reduced according to the CO_2 production. On the other hand, the lack of CO_2 might bring a rise in cell pH. It is interesting to point out that acetozolamide, a carbonic anhydrase inhibitor, reduces acid secretion in the intact mucosa, and it has been proposed that it exerts its action by increasing intracellular pH [6]. Lowering the pH of the

bulk phase in the absence of buffer would also tend to lower the pH of the unstirred compartment, in this way allowing for a recycling of CO_2. These phenomena have been observed in the intact gastric mucosa [16] and also in the present study in isolated oxyntic cells, indicating that a recycling hypothesis is able to explain the observations.

The activity of H^+ in the cytoplasmic compartment depends on numerous variables, such as distribution of H^+ across the membranes of the cell and different organelles, and the buffering capacity of the cytoplasm. The value of 7.187 obtained for overall cell pH in controls in this study confirms earlier reports on isolated parietal cells from mammalian stomachs [2]. This value is high in comparison with the intracellular pH of skeletal muscle [18]. However, the relative alkalinity in the oxyntic cell may be related to its ability to secrete H^+. It is interesting that toad urinary bladder has a relatively high pH when measured by the same technique [8], and it is known that this epithelium secretes H^+ [10].

The increase in OH^- concentration inside the cell upon stimulation, predicts a shift in cell pH that will depend on a number of variables as mentioned before. It was calculated from the acidification experiments that the increase in OH^- concentration upon stimulation was of about 10 mM; this would increase the cell pH to about 9 if no buffering occurred. A change of 0.13 pH units was measured in other types of experiments (DMO-TES-HCO_3^-); if we assume that a similar change took place in these experiments, it can be concluded that most of the remaining OH^- was efficiently buffered.

Although H^+ output was not measured in these experiments, the change in pH, small as it may be, is an indication that isolated cells secrete acid in response to stimulation. Furthermore, the finding that inhibition with SCN^- abolishes the change in intracellular pH induced by stimulation indicates that proton translocation across the cell membrane is necessary for pH to change.

It is impossible to calculate the output of H^+ from the shift in intracellular pH. However, from the values of extra and intracellular pH and the known buffers, a calculation of the change in intracellular buffers can be made using the Henderson-Hasselbach equation, and assuming that the unionized form of the weak acid is in equilibrium across the membrane. These calculations are presented in Table 2. A change in total HCO_3^- concentration, of about 5 mM can be estimated from pH values in the experiments where cell pH was measured. This calculation assumes an intracellular PCO_2 in equilibrium with that of the bulk

Table 2. Calculation of the changes in intracellular buffers upon stimulation in isolated oxyntic cells (rows 1 and 2) and intact mucosa (row 3)[a]

	Medium		Cell				
	pH	Buffer (in mM)	pH		Buffer[c] (in mM)		Δ Buffer (ΔOH)
			Before[g]	After[h]	Before[g]	After[h]	
TES[d]	7.2	5[b]					10
TES-HCO_3^-[e]	7.110	20[b]–11.1[c]	7.187	7.317	13.3	17.9	4.6
HCO_3^-[f]	6.9	18[c]	7.47	7.47	25.5	47.42	21.9

[a] *See text* for details.
[b] [TES].
[c] [HCO_3^-].
[d] From acidification experiments.
[e] From cell pH experiments.
[f] Data from Villegas *et al.*
[g] Before stimulation.
[h] After stimulation.

phase. However, since the cell is an active source of CO_2, the cell PCO_2 might be expected to be higher than medium PCO_2 and the estimated bicarbonate would come closer to the value of 10 mM calculated from acidification experiments. Thus, in experiments where TES was used and increase of about 5–10 mM in [HCO_3^-] is estimated. This increase may be inferred to be the necessary one to drive the exchange mechanism.

Table 2 also presents some calculations based on data reported by Villegas *et al.* [21]. These workers found a larger shift in cell pH upon stimulation in intact mucosa. From these data it can be calculated that the increase in HCO_3^- concentration is of about 22 mM. In the intact mucosa, the size of the unstirred layer is much larger than in the isolated cells. Accumulation of HCO_3^- in this compartment may explain the larger gradient between cell and bulk phase estimated from their experiments.

The explanation given by Villegas *et al.* [21] for the change in cell pH in response to stimulation can be challenged on the basis of the experiments in isolated cells. They propose that the apparent change in cell pH is due to an exteriorization of the vesicotubules that are filled with a fluid of low pH. Although such phenomenon may occur, this implies that the change in pH is not real, but an artifact caused by the reduction in relative cell volume. If the change in cell volume occurs as a consequence of stimulation, it is not large enough to measure. Wet/dry ratios appeared to be the same in resting and stimulated cells

within experimental error. On the other hand, an increase in DMO counts in the cell pellet accompanies stimulation. If the explanation advanced by Villegas is correct, a change in wet/dry ratios with no change in total DMO counts in the cell pellet pould be predicted. Furthermore, the continuous active extrusion of H^+ ultimately requires the splitting of water into H^+ and OH^-, the latter remaining inside the cell until neutralized by CO_2 and exchanged for Cl^- as HCO_3^-.

In summary, based on medium acidification and cell pH experiments, we may conclude that isolated oxyntic cells can secrete acid. There exists a delay in base extrusion at the serosal side of the cell, which tends to raise the cell pH. It is possible that the change in cell pH may serve as a feedback regulator of the rate of acid secretion.

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