THE POTASSIUM ACTIVITY OF THE FROG GASTRIC PIT

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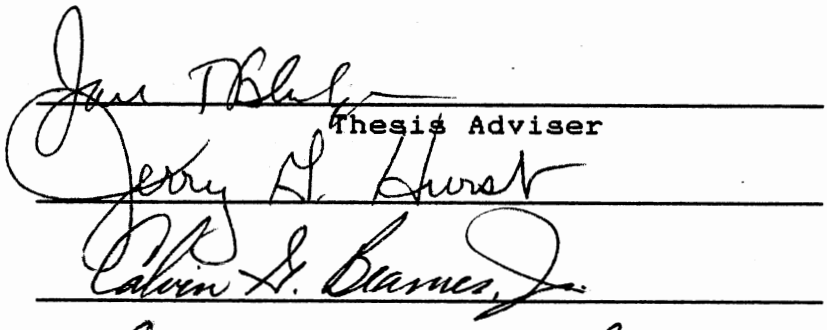

Thesis Adviser

Dean of the Graduate College

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And finally I would like to extend my appreciation to the people who made it all possible, my family. Mom, Dad, and Dana, it was because you first believed in me that I am able to believe in myself. Thank you for all the encouragement.

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CHAPTER I

INTRODUCTION

ULCER TREATMENT

Most attempts to heal gastric ulcers have revolved around controlling acid secretion. In order for ulcers to heal they must be spared from long term exposure to low pH's. Antacids are effective in lowering the gastric pH but their affect is short lived. Muscarinic antagonist, like atropine, can inhibit as much as 50% of the meal-stimulated acid production, but they have side effects affecting cholinergic receptors throughout the body. The histamine H_2 receptor antagonists, cimetidine, potently inhibit acid production and are selective for the stomach. It may be difficult to completely inhibit acid secretion because there exists a multiplicity of known receptors on the parietal cell and there is a variety of second messenger systems, which involve cAMP in the H_2 receptor pathway and intracellular Ca^{++} in the acetylcholine and gastrin pathways (22). There exist a need for more effective means to control gastric acid secretion.

A NEW CLASS OF DRUGS

Drugs of the benzimidazole class may be the answer.

These compounds are potent and very specific for inhibition of acid secretion. Under normal physiological pH's these compounds are inactive but in extremely acidic regions the active compound is formed. The active molecule is believed to bind directly to the enzyme hypothesized to be actively involved in acid secretion, the H^+/K^+-ATPase. The covalent intermediate is incapable acid secretion. The high specificity of this class of compounds can be explained by the fact that only in the stomach can the activating pH be reached.

THE FUTURE

The development of these compounds was made possible by a furthered understanding of the acid secretory mechanism. Even though a great deal is known about this mechanism, questions still remain. One of the questions results from two conflicting bodies of electrophysiological data. Experiments performed on the microsomal preparation suggest that acid production by the H^+/K^+-ATPase is electroneutral (23). On the other hand, data gathered on intact stomachs point toward an electrogenic mechanism (18). There exists a need for new methods of investigation. New research avenues may provide data required for further elucidation of the acid production mechanism. A better understanding of this mechanism will hopefully lead to more effective means of treating ulcers and other disorders whose treatment requires acid production to be tightly controlled. It is the hope of

all those involved in this research project that information from these experiments will be helpful in this endeavor.

CHAPTER II

LITERATURE REVIEW

It has long been accepted that the gastric mucosa is responsible for the acidification of the stomach lumen. The burden of this task falls upon the parietal (or oxyntic) cell of the gastric gland. One of the questions that remains to be settled is whether the gastric pump is electrogenic or electroneutral. Two models for gastric acid secretion have emerged: (a) the K diffusion model which supports the electroneutral hypothesis and (b) the seperate site theory which supports the electrogenic hypothesis.

In 1948 Conway and Brady (5) modified an existing model for acid production that contained organic acids as storage forms for hydrogen ions. The problem with the existing model was that the theoretical pH produced by this system could not reach the pH found physiologically. Since they knew yeast could acidify regions by exchanging hydrogen ions for inorganic cations, they proposed a similar mechanism for the gastric mucosa; whereby, physiological pH's could be attained; thus, the Conway-Brady theory was born.

The K^+ diffusion model for acid secretion is a modification of the Conway-Brady theory. In this model KCl diffuses into the lumen down its concentration gradient.

The H^+-K^+ ATPase enzyme then exchanges K^+ions for H^+ ions with a 1:1 stoichiometry. No net charge is transfered and the pump is said to be electroneutral.

In the seperate site theory H^+ and Cl^- are pumped into the lumen by seperate but parallel mechanisms. Each pump generates a potential; thus, this model is electrogenic. A large amount of data has been amassed supporting both sides. In this brief review I will attempt to present the most relevant data and arguments for both theories.

Rehm (18) provides evidence that the gastric pump is elctrogenic. His experiments are performed on frog mucosa whole mounts on an Ussing chamber. Under normal conditions the transmucosal PD of the nutrient side is positive with respect to the secretory side. When sulfate replaces chloride as the primary anion in the solutions the sign of the PD becomes inverted. (i.e. In Cl^- media the nutrient side is positive and in sulfate media it is negative.) The reason for this is that the potential for Cl^- masks the potential for H^+ under normal conditions. Since the two potentials are of opposite sign, when the Cl^- potential is removed, by removing Cl^- from the bathing solutions, the transmucosal PD undergoes an inversion of sign. Cl^--free media makes the dissection of the transmucosal PD into its two components possible. In the Cl^--free media the transmucosal PD is a reflection of the potential generated by H^+ secretion. Using inhibitors of acid secretion Rehm was able to establish a linear relationship between the PD

and the secretory rate. All of this data points to an electrogenic acid pump.

Most of the data gathered in support of the electroneutral model rallies around the discovery of a K^+-stimulated, H^+-translocating ATPase. Forte et al. (7) found K^+ dependent ATPase activity in microsomal membranes derived from fundic mucosa. This observation was extended to several different mammalian species by Ganser and Forte (9). It was later discovered that these vesicles could accumulate protons (16). Sachs et al. (23) found that vesicles enriched 35-fold in K^+-stimulated ATPase activity could acidify the vesicle interior without the production of a potential. If the pump is electrogenic then lipid-permeable ions and ionophores will increase ATPase activity by short-circuiting the system. An increase in activity with a K^+ ionophore , but not with lipid-permeable ions, suggests a requirement for intravesicular K^+ and not an increase in ATPase activity due to an increase in membrane conductance; valinomycin (.01 mM) stimulated ATPase activity; protonophores and lipid permeable ions had no effect on the ATPase activity. Intravesicular K^+ is needed for acidification (21). Lee and Forte (15) showed that gastric microsomes were relatively impermeable to K^+; therefore, vesicles had to be incubated in a high K^+ concentration solution or valinomycin, the K^+ ionophore, had to be added to the solution.

The H^+/K^+-ATPase was localized by cyto- and

immunochemical methods to the apical area of the oxyntic cell in the fundic epithelium (20). Berglindh et al. (1) provided direct evidence that the site of acid secretion is the canalicular and apical surfaces of the oxyntic cell.

During stimulation of the oxyntic cell morphological transformations take place within the cell (8, 11). There is a rapid reduction of the tubulovesicular membrane area, the total reduction can be as great as 90% (24). At the same time the tubulovesicular membrane area is decreasing, the microvilli increase in size and number. Measurements have shown increases of four-fold and greater in the microvillar surface area of the parietal cell in mice during stimulation. Conversely, in resting, or non-secreting cells, the tubulovesicular system becomes extensive while the microvilli decrease in number and size (12, 24, 28, 32). This suggests that some sort of membrane transposition occurs when going from the resting state to the secreting state.

Wolosin and Forte (30) discovered that stimulation prior to slaughter resulted in a redistribution of the K^+ ATPase activity that was reduced to less than half in the microsomal pellet and concomitantly increased in the membrane fractions normally associated with nuclei and mitochondria. Density gradient fractionation of the mitochondrial pellet yielded a preparation rich in H^+/K^+-ATPase. Stimulation associated (s.a.) vesicles did not need to be pre-incubated in KCl solution or treated with

valinomycin to take up protons (29). The ionophore-independent acidification appears to be explained by the presence of a KCl diffusion pathway in the s.a. vesicles. These observations are consistent with the membrane transposition theory.

Hersey et al. (14) found that inhibition by omeprazole (a highly specific inhibitor of the H^+/K^+- ATPase) did not significantly change the conductance of the stomach. They concluded that the gastric pump is electroneutral. Rehm et al. (19), attacking the electroneutral model, concluded that their experiments with high K^+ solutions on the secretory side supported the electrogenic model for acid secretion.

At the present time, the results of experiments performed on whole stomach mounts can be explained by either of the two models. Although there is plenty of evidence gathered to support the electroneutral model in the form of vesicle experiments it is very difficult to make the correlation to the electrophysiological data of the whole stomach experiments. Those that support the electrogenic model face the same type of problem. The electrophysiological data correlates well with their hypothesis but how do they explain the vesicle experiments which provide strong evidence in favor of the other model? I hope that the experiments that I plan to run will provide a new avenue for acquiring data in support of either side.

CHAPTER III

THE POTASSIUM ACTIVITY OF THE FROG GASTRIC PIT

The gastric mucosa has long been believed to be responsible for acidification of the stomach lumen. Microsomes isolated from the fundic mucosa are capable of H^+ uptake in the presence of K^+, Mg^{++}, and ATP and are rich in K^+-Mg^{++}-dependent ATPase (9). It has been shown that H^+ accumulation by the gastric microsomes is the result of a K^+-H^+ exchange pump requiring intravesicular K^+ (23). Due to the microsomes' impermeability to K^+, the microsomal vesicles had to be incubated in a high K^+ solution or valinomycin added to the solution (15). The internal face of the vesicle corresponds to the luminal side of the secretory membrane. It has been hypothesized that a KCL diffusion pathway exists in the secretory membrane (23) and shown experimentally (29).

The gastric pit is continous with the gastric gland where acid production by the parietal cells occur. Acid produced down in these glands must pass through and out the gastric pit to reach the stomach lumen. The flow out of the gastric pit will carry any ions that are free in solution down in the gastric glands to the opening of the gastric pit

where the ions' concentration (or more accurately activity) can be measured. By measuring the change in K^+ activity just inside and at the opening of the pits in response to various treatments we hope to gain a better understanding of the processes involved in acid production by the gastric mucosa.

METHODS

The stomachs were removed from anesthetized (10% urethane) Rana pipiens. The external muscle layer was removed by blunt dissection and the mucosa was placed on the chamber previously described (3). Microelectrodes were pulled from standard capillary glass. The tips were broken off to the appropriate diameter (approximately 25 microns) and silanized with Sigmacote. The tips were then filled with K^+ sensitive liquid ion exchanger. The microelectrodes were backfilled with 1 mM KCl solution. A micro-manipulator was used to guide the microelectrode into the gastric pit where K^+ activity measurements were made under various acid secretory conditions.

Unless otherwise specified, the mucosal solution contained (in mM): 4 KCl and 156 NaCl. The serosal solution contained (in mM): 25 NaHCO3, 4 KCl, 1 Na2HPO4, 1 CaCl2, 75 NaCl, 0.8 MgSO4, 0.5 adenosine, 10 glucose, 0.1 histamine. The serosal solution was bubbled with 95% O_2-5% CO_2. Thiocyanate and cimetidine were used to inhibit gastric acid secretion. Microelectrode glass was purchased from World

Precision Instruments. The liquid ion exchanger came from Corning. All other chemicals came from Sigma Chemical Company.

RESULTS

Table I list the K^+ concentrations of gastric pits from six different frogs. As can be seen there is some variability between frogs as well as between gastric pits from the same frog. However, the pit K^+ concentrations for a given frog were fairly close to each other. Since the mucosal solution used in this experiment contained no K^+, whatever K^+ found had to be coming from the pits.

TABLE I

POTASSIUM ACTIVITY OF
FROG GASTRIC PITS

Frog	1	2	3	4	5	6
Pit K^+ Activities	6.5 6.5	2.5 3.2 2.5	1.6 1.7 2.5	6.8	5.0 5.0	12.9

The results in Figure 1 (see Appendix) were obtained with the microelectrode just above the opening to the gastric pit. When the mucosal flow was shut off (labeled "A" in the figure) the K^+ activity in the region near the microelectrode tip increased. When the flow was started again (labeled "B") the K^+ activity returned to the same value as before the solution flow was cut off.

Figure 2 shows another tracing of the K^+ activity of the gastric pits but this time the microelectrode was placed just inside the opening of the pit (A). However before the microelectrode was positioned the mucosal solution had to be drained off to allow a good view of the pits. The period between "A" and "B" contains very little solution on the mucosal side. The readings obtained during this period are attributed to the increase in resistance caused by low solution volume thereby greatly decreasing the conductance. However, at the point labeled "B" the mucosal solution flow was reestablished and readings between "B" and "C" are valid. Prior to point "A" a baseline for 4 mM K^+ was established by placing the microelectrode in the edge of the flowing mucosal solution. As can be seen, the pit K^+ activity for both pits was greater than the 4 mM baseline. The activity continued to increase between points "B" and "C" until the microelectrode was backed away from the pit opening and out into the flowing mucosal solution where the tracing slowly returned to the 4 mM baseline.

Figure 3 contains three tracings of an experiment conducted exactly like the experiment represented by figure 2. But there is a difference in the two. In figure 3 the serosal side of the skin had been exposed to serosal solution containing 10^{-4} M cimetidine for approximately 25 minutes. When the mucosal flow was reestablished the K^+ activity immediately dropped to the 4 mM baseline and remained there; unlike the tracing in figure 2 where the K^+

activity rose between periods "B" and "C." Upon removing the microelectrode from its position near the pit opening, the tracing became very noisy. However, the pit activity fluctuated around the 4mM baseline.

Figure 4 shows yet another experiment conducted in the same manner as that of figures 2 and 3. But this time the serosal solution did not contain any histamine. The top tracing corresponds to the gastric pit activity after the stomach had been on the chamber for approximately 10 minutes. The period between "B" and "C" shows the K^+ activity recorded by the microelectrode just above the pit opening was greater than the 4 mM baseline. This suggest that there is K^+ near the pit opening. The bottom tracing was recorded 35 minutes after the top tracing. The period between "B" and "C" shows a pit K^+ activity of 4mM (the same as the baseline value of the mucosal solution) and does not change when the microelectrode is withdrawn at point "C." Thus it seems that any K^+ that was initially present near the pit opening is now gone.

Thiocyanate, a compound used to inhibit acid production by the gastric mucosa, did not have any effect on the pit K^+ activity. See table II on the next page for a summary of the K^+ activity measurements of the gastric pits under the experimental conditions presented.

TABLE II

SUMMARY OF THE GASTRIC PIT POTASSIUM ACTIVITIES UNDER DIFFERING EXPERIMENTAL CONDITIONS

Treatment	Pit K^+ Activity[a] (mean +/- S.E.)	n
Stimulated Stomach and 0 mM K^+ Mucosal Solution	4.7 +/- 3.1	12
Stimulated Stomach and 4 mM K^+ Mucosal Solution	10.7 +/- 2.9	4
Treated with 0.1 mM cimetidine and 4 mM K^+ Mucosal Solution	4.0 +/- 0	3
Treated with 10 mM SCN^- and 4 mM K^+ Mucosal Solution	10.2 +/- 1.0	6[b]
Unstimulated Stomach and 4 mM K^+ Mucosal Solution	4.2 +/- 0.2	5

a Measured in mM.
b These measurements were made on the same pit at five minute intervals.

DISCUSSION

The presence of K^+ in the gastric pit in excess of that found in the bathing solution is clearly demonstrated by the data of Table I. By not including K^+ in the mucosal bathing solution, any K^+ activity registered by the microelectrode will have to come from the stomach. Potassium secretion by the chambered gastric mucosa has been reported to accompany acid secretion (25). Their experimental design did not provide any evidence as to where the K^+ was coming from other than it usually followed acid secretion. Our

experiments show that it is highly probable that the K^+ is coming from the gastric pits.

With the microelectrode above the pit opening, an increase in K^+ activity was seen when the mucosal solution flow was clamped off (see figure 1). If the parietal cells do produce acid by the H^+/K^+-ATPase method, then tight coupling between KCl diffusion into the lumen and exchange back across the membrane for a H^+ would be expected. However, tight coupling does not seem to be the case as shown by the data. In order to detect K^+ coming out of the pits the solution flowing across the mucosal surface had to be clamped off. The reason being that as the solution carrying the K^+ out of the pits reached the surface it was diluted by the flowing mucosal solution and/or swept away. With the microelectrode tip closer to the pit opening a time dependent increase in K^+ activity was seen (see figure 2). The positive sloping of the line between points "B" and "C" indicate that the microelectrode is in contact with higher K^+ activities. Since the microelectrode was held stationary, the K^+ must be accumulating in this area. The location of the microelectrode suggests that the source for this K^+ is the gastric pit.

Cimetidine, an H_2-receptor antagonist, competitively inhibits acid secretion stimulated by histamine (2). After treatment with cimetidine, the there was no increase in K^+ activity at the pit opening. This can be interpreted as a decrease in K^+ in the gastric pit. During histamine-induced

acid secretion morphological changes have been shown to take place within the parietal cell (11) which involves movement of the H^+/K^+-ATPase to the apical membrane (27) and the appearance of a K^+ conductance pathway hypothesized to provide a pathway for the net movement of KCl to the luminal site of the exchange enzyme (10). Inhibition of acid secretion by cimetidine would prevent the formation of the K^+ conductance. Since cimetidine diminishes the K^+ flow out of the pit, it is believed that the source of the K^+ must be the K^+ conductance of the apical membrane that is initiated by stimulation. It seems that the K^+ flow coming out of the pits correlates well with the appearance of a KCl conductance in the apical membrane.

To test whether stimulation by histamine is responsible for the K^+ flow out of the gastric pits, histamine was left out of the serosal solution. The top tracing of figure 4 shows that there was some initial K^+ activity associated with the pit (the section between "B" and "C" remains above the 4 mM baseline). However, the tracing does not slope upward between these two points as does the corresponding sections of figure 2 where histamine was present in the serosal solution. The bottom tracing was taken 35 minutes after the top one and shows that the K^+ activity between "B" and "C" is now absent. The K^+ activity measured in the top tracing is probably the remnant of acid secretion that was stimulated when the stomach was removed and placed on the chamber. This type of spontaneous secretion has been

reported before(25).

Inhibition of acid secretion by thiocyanate had no effect on the K^+ activity measured at the pit opening. This can be explained given its two modes of action: thiocyanate can act as a protonophore and an ATP synthesis inhibitor. The determining factor seems to be the concentration. Thiocyanate at concentrations higher than 10 mM inhibits ATP synthesis but at concentrations less than 10 mM it acts as a protonophore (13, 17). If ATP synthesis is inhibited, energy consuming processes in the cell will have to slow down or stop. Two important enzymes, the Na^+/K^+-ATPase and the H^+/K^+-ATPase, consume a lot of ATP. If ATP stores are depleated the functioning of theses two enzymes will be affected. Protons and K^+ will not be exchanged at the apical membrane, nor will K^+ be replenished in the cell. If the intracellular concentration of K^+ falls below some critical level in a part of the parietal cell associated with the KCl conductance , then this conductance will shut down (25). This would show up as a decrease in the K^+ activity measured at the pit opening. Since SCN^- produced no effect on the K^+ activity it is believed that the 10 mM SCN^- used to inhibit acid secretion was acting as a protonophore and destroying any pH gradient at the apical membrane of the parietal cell.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The results of the experiments presented here seem to agree with the KCl diffusion model for acid secretion by the gastric mucosa. The presence of K^+ in the gastric pits and the changes associated with no histamine in the serosal solution, cimetidine, and the lack of effect of SCN^- all point toward KCl diffusion into the lumen of the gastric gland followed by exchange back across the apical membrane for a H^+ resulting in the net secretion of HCl.

However, the exact nature of the KCl conductance still remains elusive. It is highly likely that the KCl conductance is modulated by intracellular messenger systems. In this paper a new method for investigating the formation of acid by the gastric mucosa has been proposed. It is hoped that the experiments presented here, combined with other methods of investigation, may lead to further elucidation of the mystery of acid formation.

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APPENDIX

Figure 1. Increase in K^+ Activity of Mucosal Solution Caused by Stopping the Mucosal Solution Flow. At point "A" the mucosal solution flow was cut off and at point "B" it was started again. The mucosal solution contained 4 mM K^+.

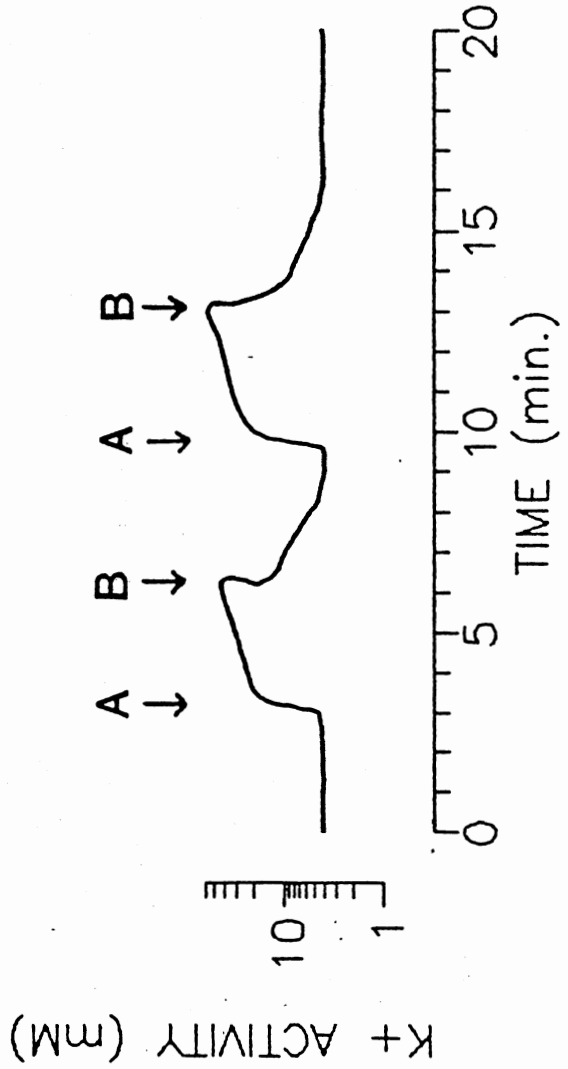
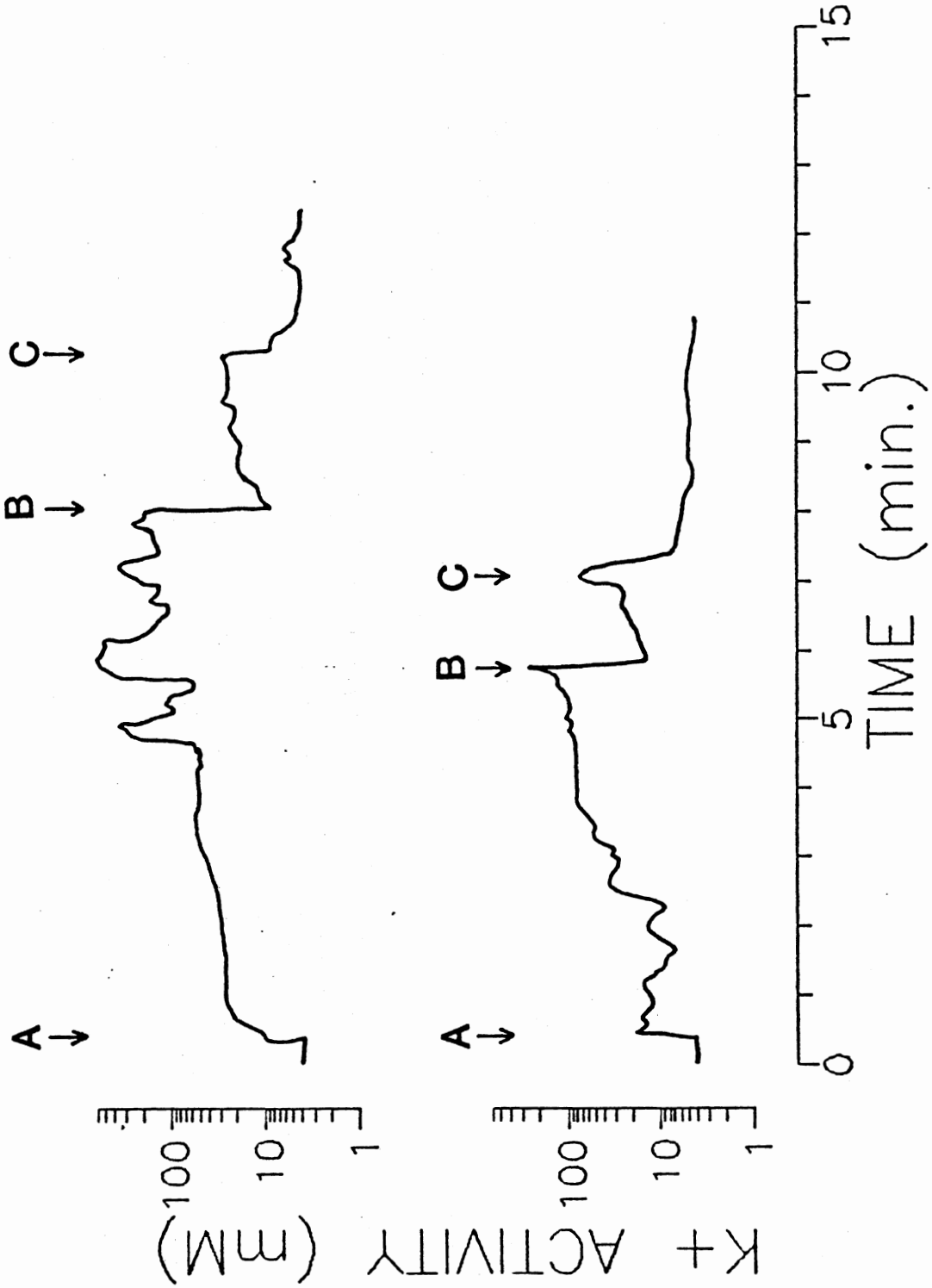

A
B
A
B
0
5
10
15
20
TIME (min.)
10
1
K+ ACTIVITY (mM)

Figure 2. Tracings of the Gastric Pit K^+ Activity. At point "A" the K^+ sensitive microelectrode was placed near a gastric pit opening. "B" designates the resumption of solution flow to the mucosal side. At point "C" the microelectrode was backed away from the pit opening and into the 4 mM K^+ mucosal solution.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B
C
A
B
C
100
10
1
100
10
1
K+ ACTIVITY (mM)
0
5
10
15
TIME (min.)

Figure 3. Tracings of the Gastric Pit K^+ Activity After Treatment with Cimetidine. The serosal side of the stomach was exposed to a 10^{-4} M cimetidine solution for 25 minutes prior to the tracings shown. "A" corresponds to the microelectrode being placed near the gastric pit opening. "B" denotes the time at which the mucosal solution flow was resumed. "C" is the point at which the microelectrode was backed away from the pit and into the 4 mM K^+ mucosal solution.

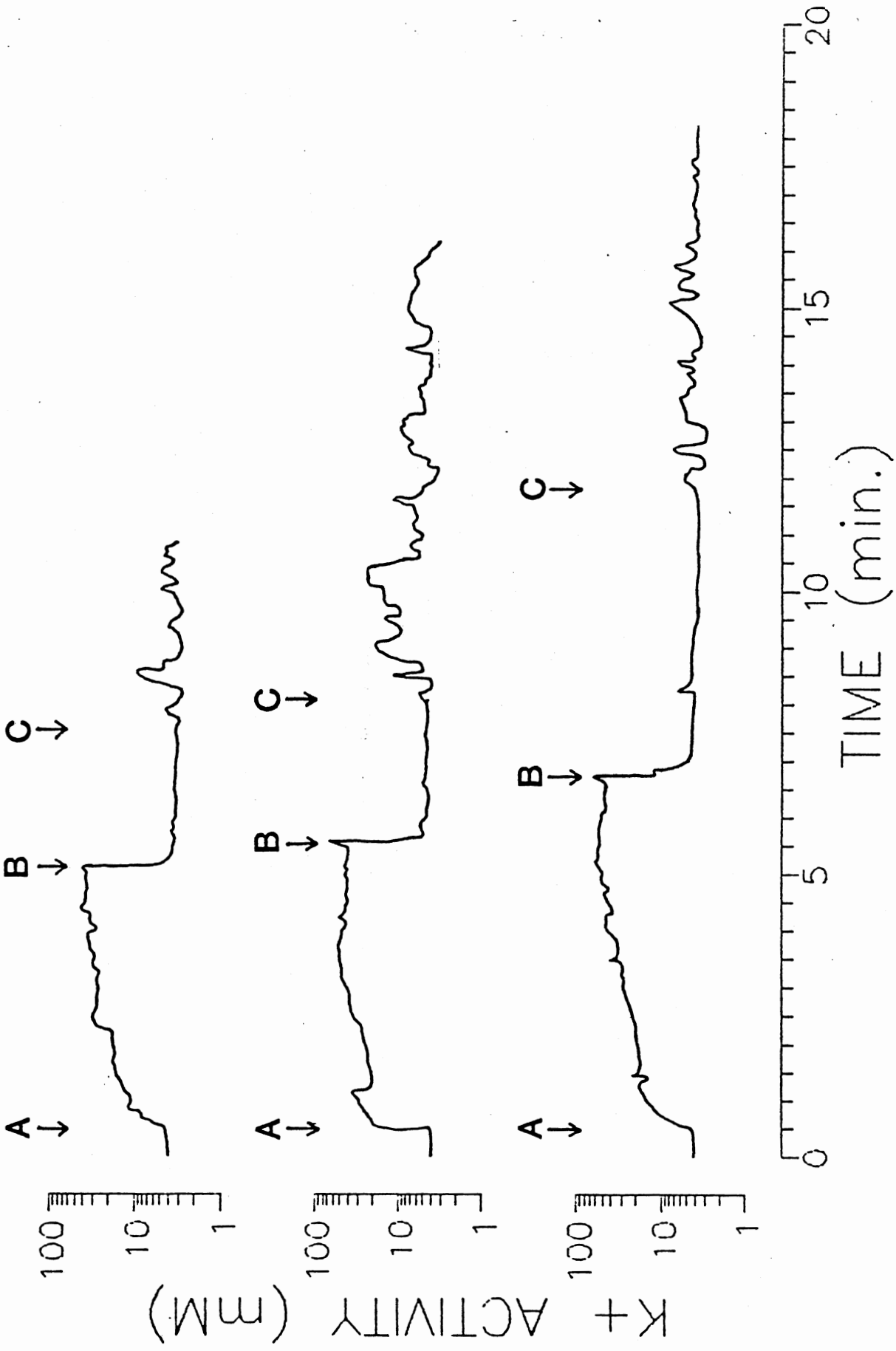
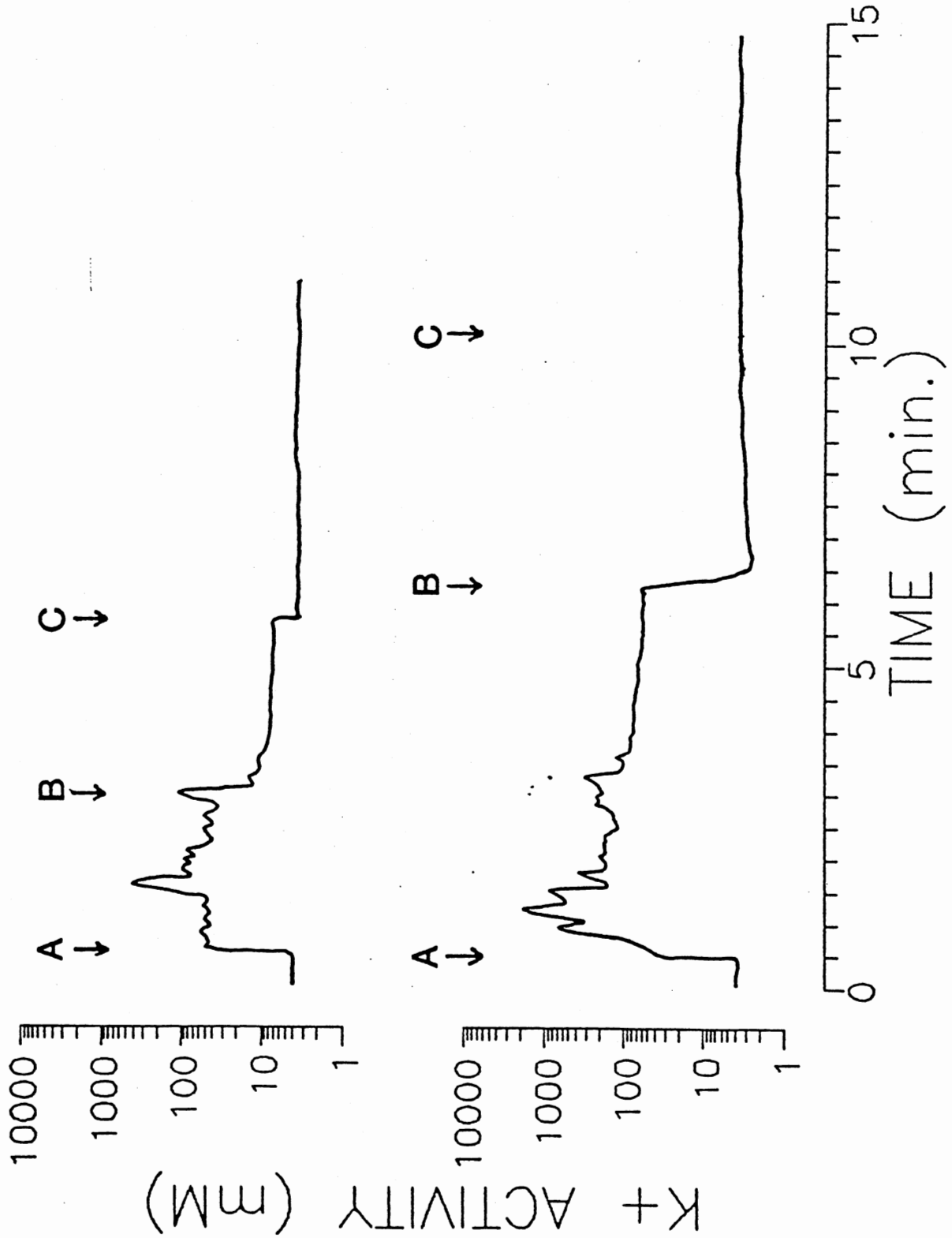

A
B
C
100
10
1
K+ ACTIVITY (mM)
0
5
10
15
20
TIME (min.)

Figure 4. Demonstration of the Spontaneous Secretion of the Gastric Mucosa. The top tracing corresponds to the gastric pit K^+ activity after 10 minutes in serosal solution without histamine. The bottom tracing is the K^+ activity 35 minutes after the top tracing. "A" corresponds to the microelectrode being placed at the opening to the gastric pit. "B" is the point where the flow to the mucosal side was resumed. "C" is the point at which the microelectrode was backed away from the gastric pit and into the 4 mM K^+ mucosal solution.


A
B
C
A
B
C
10000
1000
100
10
1
10000
1000
100
10
1
K+ ACTIVITY (mM)
0
5
10
15
TIME (min.)

VITA[2]

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Master of Science

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