

INTRACELLULAR pH IN GASTRIC AND RECTAL TISSUE POST CARDIAC ARREST

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1. INTRODUCTION

Hypoperfusion to the gut during cardiac arrest is an important clinical problem. The inability to control pH during metabolic stress, e.g. ischemia, leads to the disruption or halting of processes vital to balancing cellular metabolism. Alterations in cellular pH have been linked to changes in intramucosal permeability, which may result in the leakage of inflammatory mediators or bacteria, or both, into the systemic circulation and contribute to the development of organ failure, shock, or death.

A strong relationship exists between energy metabolism and cellular acid-base balance. Acidosis during ischemia is likely due to glycolytic accumulation of CO₂, lactic acid, and H⁺. Tissue PCO₂ is a recognized clinical marker of perfusion failure, resulting from a variety of conditions (hemorrhagic shock, sepsis, trauma) that arise when tissue O₂ requirements can no longer be met and anaerobic metabolism is initiated.^{1,2} The link between gastric mucosal hypercarbia and intracellular pH (pHi) has not been established. The relationship between gut tissue P_{CO2} and pHi is clinically important since cellular/tissue pHi monitoring technologies are not available at the bedside.

To our knowledge, gut pHi under hypoxic conditions has not previously been reported. This study was undertaken, then, to: 1) define baseline pHi of stomach and rectum, two clinically useful monitoring sites; 2) evaluate changes in pHi at these sites during ischemia; 3) identify differences in pHi in the gut layers; 4) compare pHi to mucosal P_{CO2}; and finally 5) estimate the buffering of pHi in the stomach and rectum and compare these values with arterial bicarbonate (HCO₃⁻).

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2. METHODS

2.1 Surgical Preparation

The surgical protocols for anesthesia, cannulation of vessels, and insertion of P_{CO_2} electrodes in rats has been previously described.³ While the abdominal cavity was open for instrumentation of the stomach, a small catheter (IP catheter) was inserted into the peritoneal cavity and the cavity was closed with sutures.

2.2 Experimental Protocol

Post-instrumentation, rats were injected via the IP catheter with 3 mL of 2% Neutral Red Dye (Sigma Chemical). The rat was repositioned several times during the first 5 minutes post-IP infusion to distribute dye to the gut tissue. All animals were stabilized for 30 minutes in order for mean arterial pressure to return to baseline. Arrest was induced by an intra-atrial injection of norcuron (.1-.2 mg/kg) followed by a potassium chloride bolus (0.5M/L; 0.12 mL/100 gm of body weight). Control animals remained under anesthesia while the stomach and rectum were harvested. Tissues were immersed in liquid nitrogen, and stored at -80°C . The rats were then euthanized by cutting the abdominal aorta. Organs from rats in the arrest group were similarly harvested and frozen.

2.3 pH_i Analysis

The pH_i is a measure of the acid-base status of cells within a specified area. The pH_i was determined by a reflectance histophotometric imaging technique using the absorption dye neutral red. Tissue was mounted on a cryotome chuck and maintained at -25°C . An unstained tissue blank was mounted next to each tissue block prior to photographing the sample monochromatically at 550 nm and 450 nm. The pH_i was calculated as: $\text{pHi} = -1.3(\text{OD } 550/\text{OD } 450) + 10.5$.⁴ Transmittance was determined by dividing pixel values from the 550 and 450 images by the 100% transmittance level obtained from the blank. Using Image Pro Plus (4.0 Media Cybernetics) images were processed by first converting them to floating point images, determining a ratio, and comparing values to a calibration curve. Grayscale tissue images were used to identify the area of interest (AOI) and these AOIs were identified within the pixel images. Mean AOI pH_i values were determined after eliminating values outside of the defined dye range, 6-8 units. Each tissue was sectioned 3-4 times and a separate determination was made of the pH_i for the whole tissue (all layers within the area) and for layers (mucosal, submucosal, muscularis mucosa).

2.4 Data Analysis

Statistical analysis was performed using JMP IN computer software (Version 4, SAS Institute). For each rat 3 to 4 slices of the same tissue were analyzed. The average pH_i (mean \pm SEM) for whole tissue and for layers, was determined for each slice by taking the average of pixel values within the AOI that fell within the pH range of 6-8 units. The AOI was drawn to include the largest possible area of each tissue sample. Because the same spot on each tissue was not sampled (e.g., the fundus of the stomach was not sampled consistently), tissue and group were treated as random effects in the model, thereby increasing the number of observations included in the analysis (whole tissue, $n=99$; layers, $n=283$). To evaluate changes in the pH_i by site (rectal vs. gastric) and group, ANOVA was used. To compare pH_i by layer over the experimental protocol a

nested ANOVA design was conducted (tissue type: rectal, gastric; by group: control, experimental; by site: mucosa, submucosa, muscularis mucosa). Pearson product moment correlation coefficients were calculated for pHi and P_{CO2} by site and group. Tissue buffer was calculated by modifying the Henderson Hasselbalch Equation whereby the pHi obtained in this study was substituted for pH and mucosal P_{CO2} was substituted for arterial P_{CO2} to produce a buffer value. Correlations were also used to determine the relationships between rectal, gastric, and arterial bicarbonate.

3. RESULTS

The anlaysis included data from 16 male Wistar rats (5 control; 11 15” Arrest). The mean arterial pressure (MAP) at baseline ranged from 85-119 torr. Baseline systemic variables for the control and 15” arrest group were similar and are summarized as group data in Table 1.

Table 1. Mean (±SD) of systemic variables at baseline (n = 16)

Variable	Mean	±	STD	Variable	Mean	±	STD
pH, units	7.40	±	0.06	K ⁺ , mmol/L	4.21	±	0.5
pCO ₂ , torr	37.21	±	7.2	Na ⁺ , mmol/L	137.42	±	1.2
pO ₂ , torr	141.38	±	29.8	Ca ⁺⁺ , mmol/L	4.68	±	0.2
HCO ₃ ⁻ , mmol/L	22.44	±	3.5	Cl ⁻ , mmol/L	102.33	±	2.4
O ₂ Sat, %	98.87	±	1.7	Lactate, mmol/L	2.33	±	1.1
tHb, g/dL	14.76	±	4.4	Glucose, mmol/L	9.97	±	2.3

P_{CO2}, partial pressure of carbon dioxide; P_{O2}, partial pressure of oxygen; HCO₃⁻ bicarbonate; O₂ Sat, O₂ saturation; tHb, total hemoglobin ; K⁺, potassium; Na⁺, sodium; Ca⁺⁺, corrected calcium; Cl⁻, chloride.

3.1 pHi in the Control and 15” Arrest Group for Whole Tissue

The pHi of the rectum and stomach under control and 15” arrest condition are shown for whole tissue in Figure 1. Stomach pHi was more alkaline than the rectum at baseline and acidified to a greater degree during 15” arrest. However, a similar change in pHi occurred between the control and 15” arrest group at the rectal and gastric site (NS, p = .10). The average control and 15” arrest pHi for rectal and gastric tissue were 7.27±.10 and 7.36±.08; and 7.38 ± .1 and 7.15 ± .08 units.

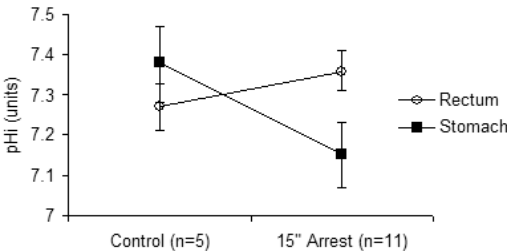


Figure 1. Rectal and stomach pHi at control and 15” arrest (n=16).

3.2 Relationship Between Layers of the Gut and Site

The mean rectal and gastric pHi of the mucosa, submucosa, and muscularis mucosa for the control and 15" arrest group are presented in Figure 2. Changes in pHi between the control and 15" arrest group were not the same in the stomach and the rectum ($p < .001$). Gastric layers were more alkalotic under control conditions with a rapid decline noted for the arrest group. Conversely, the rectal pHi changed only slightly and remained acidotic for both the control and arrest group. The change in pHi was similar across the layers (mucosa, submucosa, muscularis mucosa) in the control as well as the 15" arrest group ($p = .45$). Homogeneous changes were noted between the pHi of the layers in the stomach and rectum ($p = .69$).

3.3 Relationship of pHi to Tissue P_{CO_2}

Under control conditions, the rectal and gastric P_{CO_2} were highly correlated ($r = 0.95$; $n=5$), yet poorly correlated at 15" of arrest ($r = -0.39$; $n = 11$). Similarly, rectal and gastric pHi were moderately correlated for the control group but not for the arrest group ($r = .54$; $r = -0.05$). A comparison of rectal pHi to rectal P_{CO_2} and gastric pHi to gastric P_{CO_2} yielded poor negative correlations ($r=-.14$; $p=.60$; $r=-.33$; $p=.21$, respectively).

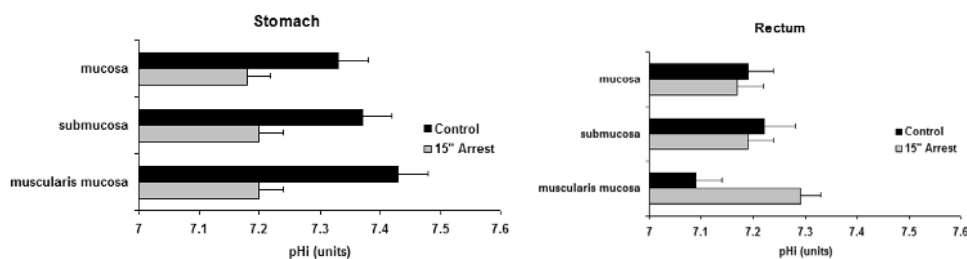


Figure 2. Rectal and stomach pHi (mean \pm SEM) by layer during 15" Arrest and control condition ($n=16$).

3.4 Calculating the Buffering Component

In the control group, the average calculated buffer value (mean \pm SEM) was similar at both sites (rectal 48.1 ± 15.4 ; gastric 48.9 ± 10.5) but more than double the value of arterial HCO_3^- (22.3 ± 1.1). While the amount of calculated buffer at the rectal site nearly doubled at 15" arrest, gastric buffer increased less dramatically (84.8 ± 20.3 vs. 57.14 ± 8.02) and no statistical difference from control was noted. Rectal buffer was moderately correlated with both gastric buffer ($r = .66$) and arterial HCO_3^- ($r = .52$) under control conditions, but an inverse correlation was found between the calculated buffer value at the two sites at 15" arrest ($r = -0.40$).

3.5 Dye Considerations

Tissue samples were pink in color on visual inspection. The percent of pixels within the physiologic range of 6-8 units was slightly greater in the stomach than the rectum (53.7% vs. 45.9%). Under arrest conditions the amount of in-range pixels was reduced in the rectum while increased in the stomach, both in the direction of a lower pHi. The percent of pixels in the range was similar for all layers ($p = .20$), with the greatest number of pixels in range within the mucosal lining for both sites.

4. DISCUSSION

The pHi for gastric and rectal tissue exceeded the values reported for muscle (7.00 ± 0.01)⁵ and brain (hippocampus, 7.01 ± 0.15 ; cerebral cortex, 7.04 ± 0.02).⁶ Changes in pHi in relation to acid secretion in the gastric mucosa have been reported in several studies.^{7,8} However, in these reports, using the dye bromocresol purple in which the pK was unreported, allowed for only a qualitative report of changes in pHi. We found no values for gastric or rectal pHi in the literature.

It may be that fifteen minutes of cardiac arrest was insufficient time to produce significant changes in pHi in gut tissue. Whether the pHi analysis was done using whole tissue or by layer, we found no difference in pHi by site (rectal, gastric) or condition (control, 15" arrest). However, the downward trend for pHi noted between all layers of the stomach and between the mucosal and submucosal layer of the rectum at 15" of ischemia suggests an extended period of time would have yielded significant reductions in pHi from baseline.

Heterogeneity of microcirculatory blood flow to different regions of the gut and the different layers has been proposed by other scientists based on varying oxygen consumption needs during hypoxic and ischemic challenges. Reductions in O₂ and flow may reduce pHi as well as produce unequal changes in pHi between the layers. During the hypodynamic phase of sepsis, Hildebrand's group reported reductions from baseline in microcirculatory blood flow to the muscularis of the stomach (55%) and colon (70%), yet the colon mucosa blood flow remained constant. In contrast, during the hyperdynamic phase of shock, microcirculatory flow exceeded baseline in both the gastric and colonic mucosa while flow to the muscularis mucosa returned to baseline in the stomach. In the colon however, there was reduced flow to the muscularis mucosa suggesting autoregulation favored the mucosa.⁹ If we link changes in pHi to blood flow, these findings are not wholly consistent with our results. The pHi was reduced in the muscularis in the stomach during arrest, yet increased in the rectum at 15" arrest.

The layer that exhibited the highest gastric pHi was the muscularis mucosa. Although rats were fasted 14-18 hours, stomachs contained food/stool/bedding. Because gastric acid was likely to be secreted in response to stomach contents, the findings may be explained by acid secretion, where HCO₃⁻ enters the diffusion barrier (from lamina propria, muscularis mucosa, and part of the submucosa) at a rate equal to the rate of hydrogen ion secretion through the mucosa.¹⁰ The raised pHi at baseline may be linked to alkalization within the muscularis mucosa in response to H⁺ secretion. Under anoxic conditions the reduction in pHi preceded the cessation of H⁺ secretion. We noted a greater reduction in pHi from baseline for the muscularis mucosa when compared to the gastric mucosal and submucosal pHi. For the rectum, the submucosal and mucosal layers had a greater pHi value for the control group. Unlike the stomach, the pHi of the muscularis mucosa in the rectum increased during arrest.

High levels of CO₂ were generated during flow stagnation induced by cardiac arrest. The high correlation in the control group between the two sites suggests comparable generation of CO₂ occurs. However, during arrest the site differences may not be attributable to differences in CO₂ generation alone. A difference in tissue buffering capacity or impaired removal of CO₂ cannot be ruled out.

In our study the gastric site had the greater reduction in pHi hence, less buffer during arrest. The buffer values reflect an estimate of intracellular buffering, extracellular buffering, or a combination thereof. Both intracellular (pHi) and extracellular

components (tissue PCO_2) were entered into the Henderson Hasselbalch equation to obtain a buffer value. Changes in PCO_2 and pHi may be buffered to a different degree depending on the tissue type.

5. CONCLUSION

We directly measured pHi using the pH sensitive dye, neutral red. We defined pHi for rectal and gastric tissue in whole tissue and by layer under control and arrest conditions. Fifteen minutes of arrest was not sufficient time to alter the pHi at the rectal or gastric site. On initial inspection, the stomach may be more sensitive to ischemic changes than the rectum. Understanding the mechanism by which PCO_2 generation is used to track clinical changes is vital to the early detection of tissue dysoxia in order to effectively treat and manage critically ill patients.

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7. REFERENCES

1. Gutierrez G, Brown SD. Gastrointestinal tonometry: a monitor of regional dysoxia. *New Horiz* 1996; 4:413-419.
2. Maynard N, Bihari D, Beale R, Smithies M, Baldock G, Mason R, McColl I. Assessment of splanchnic oxygenation by gastric tonometry in patients with acute circulatory failure. *JAMA* 1993; 270:1203-1210.
3. Fisher EM, LaManna JC. Gut dysoxia: Comparison of sites to detect regional gut dysoxia. *Advances in Experimental Medicine and Biology* In press.
4. Hoxworth JM, Xu K, Zhou Y, Lust WD, LaManna JC. Cerebral metabolic profile, selective neuron loss, and survival of acute and chronic hyperglycemic rats following cardiac arrest and resuscitation. *Brain Res* 1999; 821:467-479.
5. Zhang RG, Kelsen SG, LaManna JC. Measurement of intracellular pH in hamster diaphragm by absorption spectrophotometry. *J Appl Physiol* 1990; 68:1101-1106.
6. Crumrine RC, LaManna JC, Lust WD. Regional changes in intracellular pH determined by neutral red histophotometry and high energy metabolites during cardiac arrest and following resuscitation in the rat. *Metab Brain Dis* 1991; 6:145-155.
7. Hogben CA. Gastric secretion of hydrochloric acid. Introduction: the natural history of the isolated bullfrog gastric mucosa. *Fed Proc* 1965; 24:1353-1359.
8. Hersey SJ, High WL. Effect of unstirred layers on oxygenation of frog gastric mucosa. *Am J Physiol* 1972; 223:903-909.
9. Hildebrand LB, Krejci V, tenHoevel ME, Banic A, Sigurdsson GH. Redistribution of microcirculatory blood flow within the intestinal wall during sepsis and general anesthesia. *Anesthesiology* 2003; 98:658-669.
10. Sanders SS, Hayne VB, Jr., Rehm WS. Normal H^+ rates in frog stomach in absence of exogenous CO_2 and a note on pH stat method. *Am J Physiol* 1973; 225:1311-1321.

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