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REGIONAL GASTRIC MUCOSAL BLOOD FLOW IN MAN

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled REGIONAL GASTRIC MUCOSAL BLOOD FLOW IN MAN. Submitted by GARY MAYNARD SOENEN in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE IN EXPERIMENTAL SURGERY.

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ABSTRACT

Gastric mucosal blood flow was studied in six normal subjects and twelve patients using the new techniques of laser-doppler flowmetry and hydrogen gas clearance. Laser-doppler flowmetry was easily adapted to measurement of gastric mucosal blood flow whereas hydrogen gas clearance was not. No successful readings using hydrogen gas clearance were obtained due to drifting baselines and unidentified electrical interference. Laser-doppler readings in eighteen subjects demonstrated a regional variation in gastric mucosal blood flow. Fundal flow was highest and anterior antral flow the lowest ($p<0.00001$). Flow in the fundus, angularis and anterior antrum were statistically different from each other and every other area. This data strongly suggests the existence of a differential mucosal blood flow in the stomach.

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LIST OF ABBREVIATIONS

GMBF = gastric mucosal blood flow

LDF = laser-doppler flowmetry

HGC = hydrogen gas clearance

AA = anterior antrum

PA = posterior antrum

PP = prepylorus

A = angularis

F = fundus

MGC = mid-greater curve

AB = anterior body

PB = posterior body

SEM = standard error of the mean

CHAPTER ONE

INTRODUCTION

The role of gastric mucosal blood flow in normal and abnormal physiology of the stomach has long interested clinical investigators. That acid secretion, digestion and absorption are in part controlled by blood flow is known, but the specific relationships therein are poorly defined. Total blood flow to the mucosa as measured, for example, by aminopyrine clearance, increases when acid secretion is stimulated. As acid secretion falls, so does total blood flow. And yet, what does this data really mean? The stomach is not a simple organ. It has at least two areas that differ histologically, functionally and metabolically. The parietal cell bearing area is presumably more metabolically active. Does blood flow increase uniformly throughout these two regions, or can the stomach be divided into regions of different function and rates of perfusion each with their own controlling factors? It has also been speculated that mucosal blood flow subserves defense functions against injury, specifically acid-peptic attack. As far back as 1853, Virchow postulated that gastric ulcers might be secondary to alterations in the microvasculature, and a considerable body of evidence has arisen to support this basic premise. Can the focal occurrence of gastric ulcers along the lesser curve be explained by regional variation in gastric mucosal blood flow? Most evidence addressing this question has been indirect, however, as direct measurement of mucosal blood flow has been limited by a lack of appropriate methodology. The degree to which the pathogenesis of ulcers depends on changes in the microvasculature is therefore not known. Hence, many questions remain about the role of gastric mucosal blood flow under both normal and abnormal physiologic conditions.

Investigation of human gastric mucosal blood flow has been hampered by a lack of a reliable technique. Jacobsen (1981) has defined criteria for an ideal method of gastric mucosal blood flow measurement. These include the following:

1) Safety
2) Noninvasive
3) Capacity for continuous monitoring
4) Accurate
5) Reproducible
6) Inexpensive
7) Sound theoretical basis
8) Sensitive to rapid changes
9) Quantitative
10) Does not alter blood flow

Historically, available methods have not been able to fulfill all of these criterion. While several laboratory techniques have been very reliable in animals, they have not been suitable for application to humans because they involve either surgical trauma or the use of harmful substances. The accepted gold standard for blood flow measurement in the laboratory is the radioactive microsphere technique. As this involves the use of significant amounts of radiation as well as tissue sampling, it is not applicable to humans. Recently, the development of several new techniques has renewed interest in gastric mucosal blood flow measurement in man. These techniques are reflectance spectrophotometry, hydrogen gas clearance and laser-doppler flowmetry. All of these are noninvasive and applicable for repeated or continuous flow measurements through flexible endoscopes. Reflectance spectrophotometry gives a static picture of mucosal hemoglobin concentration and requires expensive equipment. Hydrogen gas clearance and laser-doppler flowmetry appear most promising as they have both been validated against numerous other standard techniques in the laboratory including radioactive microsphere methods. Both have been applied successfully to a variety of tissues for blood flow measurements including skin, testes, kidney, brain, bone and colon. More pertinent to the topic at hand, both hydrogen gas clearance and laser-doppler flowmetry have been successfully used for gastric mucosal blood flow measurement in the laboratory and in a few clinical studies. The purpose of this thesis then is to apply the relatively new technologies of hydrogen gas clearance and laser-doppler flowmetry to the measurement of human gastric mucosal blood flow. The aims of this study are as follows:

1) To apply laser-doppler flowmetry and hydrogen gas clearance to the measurement of gastric mucosal blood flow, and to determine their limitations and applications for human use.
2) To define intra-organ and inter-subject variations in gastric mucosal blood flow.
3) To establish if the laser-doppler flowmetry signal can be calibrated to yield absolute flow units (mL/100 g/min) using hydrogen gas clearance.

Before proceeding further, a review of the literature on the methodology of gastric mucosal blood flow is in order.

CHAPTER TWO

TECHNIQUES USED FOR GASTRIC MUCOSAL BLOOD FLOW MEASUREMENT IN MAN: A REVIEW OF THE LITERATURE

The criteria for an ideal method of gastric mucosal blood flow measurement has been defined by Jacobsen (1981) (5) and have already been described. None of the techniques to be reviewed, including laser-doppler flowmetry and hydrogen gas clearance, fulfill all of his criteria. A perfect method does not exist at present. The purpose of this section, therefore, is to define the limitations and inadequacies of each system as well as their applicability to the clinical setting. It will become apparent why laser-doppler flowmetry and hydrogen gas clearance were chosen as the techniques to be used in this study on gastric mucosal blood flow.

TECHNIQUES MEASURING TOTAL FLOW

1. Thermal Clearance

The first serious attempt at measuring gastric mucosal blood flow in man was performed in 1942 by Richards et al using a heat clearance technique (11). The equipment consisted of a duodenal tube running through the center of a balloon. At the balloon's greatest diameter were placed six thermocouples, while at the center, a heating coil and reference thermocouples were wrapped around the duodenal tube. The balloon was then swallowed and inflated. As the coil was heated to 10°C above body temperature, a uniform warming took place. The temperature gradient between the reference and peripheral thermocouples was recorded, resulting in a continuous record of temperature changes corresponding to blood flow. Although this technique held promise, it was not pursued further until 1968 when Bell and Shelley applied it to dogs (12). Focal gastric mucosal blood flow measurements were made using thermistors on suction cups. This was never applied to man.

Criticisms of this technique are many. Difficulty in controlling the probe position has been a significant problem. The units of flow obtained are arbitrary and recordings are disturbed by peristalsis. This method has never been validated against other techniques. To date, no further attempts have been made to improve methodology or overcome the aforementioned problems. Hence the technique has become an interesting sideline in the history of gastric mucosal blood flow.

2. C^{14} Aminopyrine Clearance

In 1966, Jacobsen et al determined canine gastric mucosal blood flow using an aminopyrine clearance technique (13). Briefly, this method is based upon the permeability of lipid membranes (cells) to the undissociated form of a weak base such as aminopyrine. In the plasma, aminopyrine is undissociated because of the weak alkalinity (pH 7.4). Aminopyrine readily diffuses across the gastric mucosa into the gastric lumen where the acidity causes it to ionize. Once ionized, aminopyrine cannot back diffuse across the gastric mucosa and is essentially trapped in the lumen. Experimental evidence has been obtained that demonstrates that aminopyrine is completely cleared from the blood on a single passage through the mucosa. Hence direct sampling of gastric contents and analysis for aminopyrine can be used to give an estimate of gastric mucosal blood flow.

Unfortunately, the large volume of aminopyrine needed in man to accurately measure clearance is toxic to bone marrow and hence cannot be used clinically. This difficulty has been overcome by Jacobsen and Tague by using minute amounts of aminopyrine labelled with C^{14}. Good correlation between aminopyrine clearance and C^{14} aminopyrine clearance has been demonstrated in dogs. Guth et al (1978) used the technique in ten human subjects, five of whom had duodenal ulcers (14). They were not able to demonstrate any difference in blood flow in response to pentagastrin between the ulcer group and normals, despite the fact that maximal acid output in the ulcer group was significantly increased. Guth concluded that C^{14} aminopyrine clearance reflected blood flow rather than gastric secretion.

Since this method depends upon an acidic environment in the gastric lumen to trap the C^{14} aminopyrine molecule, it is not applicable to all ranges of gastric physiology or pathology. Non-secretory states or achlorhydria for example, would prevent C^{14} luminal trapping and invalidate blood flow measurements. Other process that damage gastric mucosa may also invalidate the technique by allowing back diffusion to take place. In addition, C^{14} aminopyrine clearance reflects total gastric mucosal blood flow and cannot distinguish focal or even regional changes in flow. This would limit the technique to specific indications where measurement of total flow would be of value. For these reasons, it is unlikely that C^{14} aminopyrine clearance will ever gain wide clinical utility.

3. Neutral Red Clearance

Because of the inherent danger from radiation associated with the use of C^{14} aminopyrine and other radiolabelled indicators, a search was made for a safe, nontoxic substance with similar clearance properties. Neutral red was found to be both nontoxic and applicable to the clearance method (16).

The technique is similar to that of C^{14} aminopyrine. The patient is injected with an intravenous bolus of neutral red followed by a constant infusion. Samples of blood and gastric juice are taken at regular intervals and assayed for neutral red. A calculation of total gastric mucosal blood flow can then be made from this data.

Although initial studies in man were encouraging, neutral red was later discovered to have metabolites in blood which were poorly cleared through gastric mucosa thus invalidating the technique (15). A modification of the extraction process whereby none of the metabolites were extracted was subsequently developed by Knight et al (16-18). Using the modified technique, Knight was able to demonstrate similar clearance values for neutral red as Guth et al did for C^{14} aminopyrine in man. Since then, clinical studies with neutral red have yielded interesting results in normal subjects, ulcer patients and ulcer patients who have undergone highly selective vagotomy. Unfortunately, at no time has an attempt been made to validate the technique against another method for human measurement.

The advantages of this method are that it is nontoxic and inexpensive. Its limitations are similar to those of C^{14} aminopyrine - only total flow is measured, rapid flow changes cannot be detected, and damage to gastric mucosa may allow back diffusion. Because of these problems, it is unlikely that the neutral red technique will ever find wide usage.

4. ^{85}Kr Elimination

This method was originally developed in 1962 for the estimation of cerebral blood flow (19). Since then, it has been used along with ^{133}Xe to measure blood flow in the human small and large intestines. In the past decade, ^{85}Kr has been applied to human gastric mucosal blood flow measurement (22).

The use of radioactive inert gases for blood flow measurement is based on the Fick principle and was developed by Kety et al (20,21). After injection of the isotope intra-arterially and uptake by the target tissue has occurred, the rate of radioactive decay is recorded. Assuming that the disappearance of ^{85}Kr from the tissue is blood flow limited, the rate of decay will allow a calculation of flow. For a single compartment vascular bed, a monoexponential decay curve will result. For a multiple compartment vascular bed such as the stomach, a multiexponential decay curve will result necessitating a separation of the curves to derive mucosal flow. Such a process is known as curve stripping analysis and is complex and involved.

The single human study employing this method involved laparotomy and direct invasion of the gastric vasculature (22). While the results obtained were commendable, such an invasive procedure can hardly be expected to gain wide clinical use. The technique was not, unfortunately, compared with any other means of measuring gastric mucosal blood flow. A distinct advantage that emerged was the ability to calculate flow to the different layers of the stomach (muscularis, submucosa, mucosa). Other significant limitations are inability to measure focal or regional flow and measurements that are neither instantaneous nor continuous.

5. $^{99}Tc^{m}$ Clearance

Technitium is a well known substance used routinely in nuclear medicine departments for organ scanning. It has been proven to concentrate in and be cleared by the stomach. $^{99}Tc^{m}$ clearance in the dog was found in 1967 to correlate significantly with gastric blood flow measured by electromagnetic flowometer (24). Since then, only a few human studies have been performed (23). Like other studies on gastric mucosal blood flow in the human, comparison with another method was never made and instead attempts were made to apply comparative animal data.

While $^{99}Tc^{m}$ has the advantage of being relatively nontoxic compared to other radiolabelled indicators, problems have arisen with technical considerations. Back diffusion of $^{99}Tc^{m}$ into the mucosa may occur after having been cleared. Because this back diffusion is difficult to quantitate or control for, accurate measurements cannot be expected. Despite modifications of this technique developed in animals, no more human studies have been forthcoming.

TECHNIQUES MEASURING FOCAL FLOW

1. Iodoantipyrine Clearance

This technique is based upon the relation between blood flow and the uptake by a tissue of an indicator in the blood. The mathematical theory behind this method was developed by Kety between 1945-1951 (20,21). If the tissue uptake of this indicator is limited only by blood flow and not some other rate limiting process, then a calculation of flow can be made by assaying the tissue for the indicator content.

For the gastrointestinal tract, radiolabelled 4-iodoantipyrine is such an indicator (25). The substance is injected centrally and then biopsies of the target tissues are taken and a calculation made. Animal studies have demonstrated excellent correlation between this technique and that of microspheres (26). Using endoscopy and biopsy forceps, the technique has been applied to humans (27).

Advantages of this method include simplicity, speed of execution and ability to measure focal flow. Limitations are more numerous. A radioactive substance is used albeit in minute amounts. Tissue biopsy is necessary which would limit the number of measurements possible as well as the feasibility of repeated measurements. Moreover, the technique does not yield an instantaneous or continuous measurement of gastric mucosal blood flow. There is also evidence that iodoantipyrine is cleared into the gastric lumen in the presence of luminal contents. Because the disadvantages with iodoantipyrine clearance are not easily overcome, it is unlikely that it will be used much in the future.

2. Reflectance Spectrophotometry

This technique was originally applied to the measurement of oxidative processes in living tissues by Sato et al. It was later developed into a tool for measuring focal gastric mucosal blood volume in the human (50,51). Using a fiberoptic bundle passed through an endoscope, spectra are recorded of light reflected from the mucosa and stored in a computer. Since the absorptive spectrum of hemoglobin is a known constant, the quantity of hemoglobin in the tissue may be calculated by quantitating the amount of light that is absorbed.

To date, reflectance spectrophotometry has been used in clinical studies on thermal and head injured patients, gastric ulcer patients and on misoprostal treated normal subjects (50-52). The data is too extensive to review here, but are, for the most part, consistent with experimental data from animals, using other techniques.

There are several advantages to this technique. Collection of a single spectrum takes only 80 milliseconds, and thus provides an instantaneous blood volume value. Safety is not a problem and multiple sites may be recorded many times.

The major drawback to the system lies in the fact that it gives a static measurement of both hemoglobin concentration and oxygen saturation, but does not measure flow per se. Some situations may exist in which the values obtained reflect stasis rather than flow (eg. portal hypertension). In addition, the technique has been validated only against hydrogen gas clearance in a single comparative study (54). Perhaps as more comparative studies with it are done, its validity will be confirmed. Another limitation is the significant expense of the system, and to date, only its developers and one other group have studied its clinical utility.

3. Hydrogen Gas Clearance

The hydrogen gas clearance technique is based upon the washout of hydrogen gas from tissue saturated with hydrogen gas by the gastric mucosal blood flow. The measuring circuit consists of a reference silver/silver chloride skin electrode connected to a low resistance amplifier recorder and a platinum electrode placed in contact with the gastric mucosa. The subject breaths hydrogen gas until saturation is achieved at which point the hydrogen is shut off. The current generated by oxidation of molecular hydrogen to hydrogen ions at the platinum electrode is measured according to the Nernst equation:

$$E = \frac{0.59}{2} \log \frac{(A_{H+})^2}{A_{H2}}$$

where A_{H+} is the hydrogen ion activity in mol/L and A_{H2} is the partial pressure of hydrogen in atmospheres. Current generated is proportional to the amount of hydrogen present in the tissue. Aukland et al have demonstrated a linear relationship between the hydrogen concentration in tissue and the current generated (28). During the course of saturation and desaturation of the tissue with hydrogen gas, current is continuously recorded and plotted on a graph, producing a curve. The desaturation portion of the curve may then be used to calculate blood flow in cubic centimeters per minute per 100 g of tissue using the formula $F=0.693/_{T1/2}x100$. The assumptions behind these derivations have been detailed elsewhere (20,21.28).

Up to 1982, investigators had attempted gastric mucosal blood flow measurements with this technique using an electrode that had to be inserted into the gastric wall (30,31). This often resulted in measurement of submucosal blood flow rather than mucosal, or else measurement in multiple layers resulting in multiexponential curves. In 1982, Murakami et al developed a platinum contact which overcame these problems (29). Since then, the technique has been used endoscopically in dogs and humans (29,34). In numerous animal studies, hydrogen gas clearance has been validated against venous outflow, microsphere methods and aminopyrine clearance techniques with excellent correlations (29,32,33,35). In humans, a high degree or reproducibility between consecutive measurements has been demonstrated (29).

In the original technique developed by Murakami, 100% hydrogen gas was used for inhalation. Since then, the technique has been successfully adapted to the use of 3% gas, thus obviating the danger from flammability, explosion and subject hypoxia (33).

The major limitations to clinical use are few but real. A minimum period of 15 minutes is required to make a single measurement due to the time required for saturation and desaturation with 3% gas. Only a small number of measurements would therefore be possible on a patient undergoing endoscopy. Probe-to-mucosa contact has also proven to be a problem for some investigators attempting endoscopic measurements in canines. The long washout time and frequent gastric peristalsis have made constant contact a challenge. Recently, use of a suction cup apparatus has been tried with apparent success (55). In addition, because it takes a long time for a single measurement the blood flow values are neither instantaneous nor continuous and rapid changes in blood flow would be obscured.

4. <u>Laser-Doppler Flowmetry</u>

Laser-doppler flowmetry was first used by Riva et al (1972) for blood flow measurements in retinal vessels (36). It was found to be harmless, noninvasive and accurate. Its use was quickly applied to cutaneous blood flow measurements (37,44), and has been used to predict the outcome of free flap skin grafts in human subjects (41). Only within the last 5 years has laser-doppler flowmetry been applied to the study of gastric mucosal blood flow (38).

The [illegible] upon which laser-doppler flowmetry is built is derived from a phen[illegible]t described by astronomers. Through the study of light emitte[illegible]rs, the concept of the "Red Shift" was developed. Simply, this refer[illegible] to the fact that light back-scattered from a moving object will have its wavelength lengthened or shortened depending on whether the object is moving away from or towards the observer, respectively. The change in wavelength results in a different frequency of light. This phenomenon is known as the doppler shift. By illuminating tissue with a single frequency laser light and collecting the frequencies of back-scattered light, the doppler shift of moving red blood cells can be measured. The magnitude of the signal obtained is the product of the mean red blood cell velocity and the red blood cell concentration. An estimate of gastric mucosal blood flow can be made from this in arbitrary units (volts). The continuous signal obtained is linearly related to the flux of red blood cells (#RBC's x velocity) (41,53).

Laser-doppler flowmetry has been validated against other techniques in gastrointestinal blood flow in the human (49). In measurement of colonic blood flow at operation, laser-doppler flowmetry correlated well with total venous outflow measurements up to 45 mL/min/100 g after which it underestimated blood flow (49). Assessment of small bowel blood flow at operation has also revealed a linear relationship between laser-doppler flowmetry and total venous outflow (49). Validation of laser-doppler flowmetry in human gastric mucosal blood flow measurement, however, has been hampered by the absence of a reliable gold standard. Studies in animal gastric mucosal blood flow have demonstrated excellent correlation between laser-doppler and other methods such as electromagnetic flowometer, total venous outflow, ^{85}Kr elimination, hydrogen gas clearance and microspheres (42,43,45,46,48,49). Granger and Kvietys have summarized these results in a recent editorial (9).

Use of laser-doppler flowmetry at endoscopy and surgery have yielded interesting data as well as defined some of the limitations of the technique (38-40,56,59). Ahn et al demonstrated on forty-two patients at operation, . high correlation of laser-doppler flowmetry signal between the serosa and mucosa suggesting that laser-doppler measurements reflect blood flow to the entire gastric wall thickness. On ninety-eight patients undergoing laser-doppler flowmetry via endoscopy, differential blood flow was identified with the antrum having the lowest flow and the fundus having the highest. During the course of the study, the laser signals were stable, being disrupted only by the occasional peristalsis (49). Kvernebo et al studied thirty-four healthy subjects endoscopically to assess the effect of certain variables on the laser-doppler flowmetry signal (38). Such variables as reproducibility in time, spatial variation, the influence of peristalsis and the angle of the laser-doppler probe to the mucosa were made. On the basis of this study, specific technical recommendations can be made for the conduct of future clinical studies. Laser-doppler has also been applied to the study of gastric mucosal blood flow following highly selective vagotomy (49), as well as in patients with portal hypertension (58). In ten patients undergoing highly selective vagotomy, a reduction in laser-doppler signal (gastric mucosal blood flow) amounting to 57% was demonstrated in the dissected area along the lesser curve. By 3 to 4 weeks, blood flow had returned to normal. In patients with portal hypertension, compared to normal subjects, gastric mucosal blood flow was elevated (58).

While the laser-doppler in both animals and human studies has been found to be a safe, continuous, instantaneous and quick method for gastric mucosal blood flow measurement, problems do exist. It has not been possible to convert the arbitrary units (volts) to absolute flow values (mL/100 g tissue/minute) and in order to do this, direct comparison with other accurate techniques would be necessary. An absolute standard comparable to microspheres or total venous outflow in animal studies is lacking for human gastric mucosal blood flow measurement. Calibration in animals with subsequent application to human studies, although widely performed, is not an adequate means of comparison. Another limitation of laser-doppler flowmetry in the clinical setting has been the difficulty in maintaining probe-mucosa contact (49). Peristalsis frequently disrupts the probe's position. There has also been controversy about the spatial resolution of the probe. One study suggested that only the superficial mucosal layer to a depth of 1 millimeter was measured (45), while other reports have disputed this (43,47), citing evidence for transmural penetration. Only through further study and critical investigation will these and other problems be addressed.

CONCLUSION

As can be seen in the preceding review, relatively few attempts have been made to measure gastric mucosal blood flow in man. Without exception, no study has been performed in which a reference method was used for comparison. This is due in part to the lack of a suitable absolute standard in human gastric mucosal blood flow measurement and in part to an overzealous faith in the results of comparative animal studies. Conclusions arrived at by the different studies are, not surprisingly, often at odds.

The ongoing controversies surrounding ulcerogenesis and other processes where an ischemic etiology is suspected, demand a definitive technique that truly reflects gastric mucosal blood flow in all ranges of physiological parameters and pathologic states (5). Several of the aforementioned techniques hold particular promise, these being reflectance spectrophotometry, laser-doppler flowmetry and hydrogen gas clearance. All offer safety and the ability for focal flow measurements. Reflectance spectrophotometry, unfortunately, is almost prohibitively expensive and measures blood volume rather than flow. The relationship between gastric mucosal blood flow and gastric mucosal blood volume has not as yet been adequately defined.

Laser-doppler flowmetry and hydrogen gas clearance are techniques that have been successfully but individually applied to humans. Of any of the aforementioned techniques, both of these fulfill more of the criteria for an ideal blood flow measurement method as defined by Jacobsen than any of the others (5). Animal studies on both have demonstrated good correlation with more proven means of measuring gastric mucosal blood flow (29,32,33,35,42,43,45,46,48,49). A human study comparing the two would hopefully validate the two techniques against each other, but such has not as yet been performed. Of the two techniques, laser-doppler fulfills the most criteria, its most serious drawback being that it gives flow values in arbitrary units. By comparing hydrogen gas clearance with laser-doppler flowmetry, it may be possible to calibrate laser-doppler in absolute units. Gana et al (1986) has made such a comparison in the dog (42). A highly significant correlation between laser-doppler flowmetry and hydrogen gas clearance was found within each experiment. A conversion factor for converting laser-doppler flowmetry into absolute flow units using hydrogen gas clearance was not found from experiment to experiment however. Other limitations of laser-doppler flowmetry such as spatial resolution and probe-mucosa contact appear to be less critical at present but will still need to be overcome.

CHAPTER THREE

METHODS AND MATERIALS

STUDY GROUPS

Two groups of subjects were studied: 1) healthy controls (six in number); 2) patients undergoing gastroscopy in the course of routine investigation for upper gastrointestinal complaints (twelve in number).

Standard consent forms were used. Patients and controls were paid an agreed sum of money for participation in the study.

Healthy controls were recruited by advertisements in the University of Alberta newspaper and by posters displayed at the Charles Camsell hospital. Patients were recruited from cooperating endoscopists.

ENDOSCOPIC PROCEDURE

Routine endoscopic procedure was followed. Each subject was placed in the left lateral position, the throat sprayed with topical anesthetic and 10 mg of Valium and 40 mg of Buscopan were administered by intravenous injection. An Olympus GIF-IOJ flexible endoscope was inserted into the stomach and a routine examination carried out. Following this, measurements of gastric mucosal blood flow were carried out using laser-doppler flowmetry followed by hydrogen gas clearance. In the first nine patients, both laser-doppler flowmetry and hydrogen gas clearance were performed. In the final nine, only laser doppler flowmetry was done.

LASER-DOPPLER FLOWMETRY

A fiberoptic laser doppler flow-probe was attached to a Periflux-3 laser doppler flowmeter (Perimed KB, Sweden). Calibration of the flowmeter was carried out as described in Appendix 1. The probe was then passed through the biopsy channel of the gastroscope and the tip was placed gently in contact with the target areas of gastric mucosa. The probe was held in contact with each area for at least 1 minute. Excess pressure on the mucosa was prevented by ensuring that the red laser light was visible in the tissue immediately surrounding the probe[1]. Eight target areas were measured as follows:

1) Fundus
2) Body of stomach - anterior wall
3) Body of stomach - posterior wall
4) Lesser curvature at angularis
5) Greater curve opposite the angularis (med-greater curve)
6) Antrum - anterior wall
7) Antrum - posterior wall
8) Pre-pylorus

Additional measurements were taken on gastric ulcers when they were encountered, as follows:

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[1]Kvernebo et al has studied various technical factors that influence the laser doppler signal. Pressure does not influence the signal greatly unless it is so great that the laser light cannot be seen diffusing into surrounding tissues (38).

1) Ulcer center

2) Ulcer margin

3) 1 cm away from the ulcer margin in healthy appearing mucosa

The output signal from the flowmeter was fed into a Gould recorder. The average value of a stable 1 minute reading was calculated using computer digitalization and expressed in volts.

HYDROGEN GAS CLEARANCE

Prior to clinical use, each endoscopic platinum electrode was tested in the laboratory using 100% and 3% hydrogen gas in a chambered canine segment model as described by Gana et al. All electrodes were sterilized in Hibitane prior to clinical use. A silver/silver skin electrode (3M,MN) was placed on the subjects' anterior chest wall and served as a reference electrode. After finishing laser-doppler flowmetry measurements, the platinum contact electrode (Unique Medical Company, Japan) was passed through the biopsy channel of the gastroscope and placed in contact with the target area of gastric mucosa. Both the reference and platinum electrode were connected to a Gould recorder (2400S). The recorder was then calibrated as described in Appendix 3 to an electrode current of 0. The subject was then made to breath 3% hydrogen gas by face mask until a 3 or 4 cm deflection was achieved on the recorder. At this point the gas was shut off and washout allowed to occur. A computer program was then applied to the resulting curve for calculation of blood flow if the curve fulfilled all of the following criteria:

1) A stable baseline was present prior to administering hydrogen

2) A reasonable logarithmic curve was obtained

3) The recording returned to baseline after a reasonable time (fifteen minutes)

Three target areas were measured corresponding to the same areas measured by laser-doppler flowmetry:

1) Fundus
2) Lesser curve of angularis
3) Pre-pylorus

STATISTICAL ANALYSIS OF DATA

Average values ±SEM were calculated for each target area in both the healthy control and patient groups. Statistical differences between target areas were assessed using a one-way analysis of variance (ANOVA) and students t-test.

CHAPTER FOUR

RESULTS

HYDROGEN GAS CLEARANCE

1. Laboratory Testing of Electrode Function

Each of the three contact platinum electrodes used in the clinical study were first tested in the laboratory in a chambered canine segment model. Logarithmic curves were easily obtained using both 100% and 3% hydrogen gas after gas application times of thirty seconds and 2 to 3 minutes respectively (see Figure 1). The average time from start until the baseline was reached again was 15 minutes per measurement. A much more sensitive scale was necessary with 3% hydrogen in order to obtain a sufficient degree of deflection on the recorder. This resulted in considerable baseline fluctuation ("noise"). Despite this, calculations of flow were still possible.

2. Electrode Function in Patients

Curves of sufficient quality for calculation of gastric mucosal blood flow were not obtained in any of the nine patients. Several problems prohibited acceptance of the curve for the following reasons:

1) After the electrode was placed in contact with the mucosa and zeroed on the recorder, considerable baseline drift occurred in almost every case (Figure 2-A). In most instances, the drift eventually stabilized, but unfortunately this usually took 15 to 20 minutes.

2) Although increased electrode current was usually seen after 2 to 3 minutes of H_2 gas breathing the increases were irregular and sluggish, unlike the clean and swift increases seen in the laboratory. Moreover, the current usually levelled off and failed to exhibit a logarithmic curve during the washout phase.

3) In several instances where relatively good curves were obtained, return to baseline was not achieved (see for example Figure 2-B).

4) Considerable electrical interference from an unknown source was encountered, causing sudden and marked alterations in the baseline or developing curve. Such interference often caused off scale recordings.

In a few instances, tantalizingly good curves were obtained but these were rare and impossible to reproduce by repeat applications of gas. Hence our confidence in them was not sufficient to permit acceptance.

<u>LASER-DOPPLER FLOWMETRY</u>

1. <u>Technical Observations</u>

A) Signal Stability: Stable signals were obtained with relative ease. Abrupt fluctuations in the signal were caused by movement of the probe tip from either slippage or peristalsis. It was often necessary to repeat measurement in order to obtain a stable, continuous signal of at least one minute duration. Because of this, the average time for measurement of all areas was 30 minutes.

B) Target Areas: Several technical points pertaining to individual target areas are worthy of mention as follows:

i) Angularis - Despite premedication with Buscopan, the angularis persisted in demonstrating a slow rolling peristalsis that made probe placement difficult. It was necessary to place the probe on the anterior edge of the angularis where peristalsis was not quite so likely to dislodge it.

ii) Fundus - The probe was placed in the fundus after a J or U maneuver and adequate distension. The effect of distension on the laser signal was assessed in several patients. Regardless of whether all air was withdrawn or maximal distension performed, the laser signal remained constant.

iii) Anterior and Posterior Antrum - Perpendicular probe placement was not always possible in these regions because of the limited space. The side of the endoscope was used to slightly invaginate the wall, creating a small fold for the probe to rest on.

C) Reproducibility: Time constraints did not allow for the performance of two complete sets of readings. However, one and sometimes two target areas were randomly chosen in a number of patients for repeat measurements before terminating the procedure. On average, 15 minutes elapsed between the first reading and the repeat. In all cases, the two readings were identical.

2. <u>Regional Gastric Mucosal Blood Flow</u>

A) Healthy Controls (n=6): Mean gastric mucosal blood flow values for each region is depicted graphically in Figure 3 and pictorially in Figure 4. The standard error of the mean is depicted as a bar on top of each column of the bar graph. Gastric mucosal blood flow varies from region to region with the fundus demonstrating the highest flow and the anterior antrum the least. Table 1 expresses statistical significance between different regions (ANOVA, $p<0.000001$). The fundus is statistically different from every other region ($p<0.05$ to $p<0.001$). Both the angularis and anterior antrum are statistically different from most other regions. The PA, PB, PP and AB are not significantly different from one another.

B) Patients (n=12): Table 2 lists the patients and their respective endoscopic diagnoses. Those patients with a diagnosis of "normal" were kept in the patient group because all originally presented for investigation of upper gastrointestinal complaints. Figures 5 and 6 depict mean gastric mucosal blood flow for each region and Table 3 expresses statistical significance between regions (ANOVA, $p<0.000001$). The highest flow is again in the fundus and the lowest in the anterior antrum. Both of these areas are statistically different from most other areas. The PA, AB, PB and PP are again not statistically different from each other.

C) Combined Data From Health Controls and Patients (n=18): Figure 7 and 8 depict mean gastric mucosal blood flow values for each region and Table 4 depicts statistical significance. The fundus, angularis and anterior antrum are statistically different from each other and virtually every other area. Again, the PA, AB, PB and PP are not significantly different from one another.

D) Gastric Ulcers (n=2): Measurements were possible in two gastric ulcers. Both ulcers were prepyloric in location and at least 1 cm in diameter. A central crater covered by a layer of fibrinous exudate was present in both cases. The edges of the two ulcers were heaped up and surrounded by moderate hyperemia suggesting that they were in the healing phase. Blood flow values are depicted in Figure 9. Statistical analysis was not possible because of the small numbers involved.

E) Effect of Age on Blood Flow: Correlation analysis of age versus each target area was performed for a total of eight correlations. Age was found not to correlate with flow in any of the target areas.

CHAPTER FIVE

DISCUSSION

HYDROGEN GAS CLEARANCE

Hydrogen gas clearance has been applied extensively to the study of gastric mucosal blood flow in the rat, cat and dog. Results have correlated extremely well with other methods of gastric mucosal blood flow measurement under basal conditions as well as after stimulation with histamine and pentagastrin, so there can be little doubt that hydrogen gas clearance really does measure gastric mucosal blood flow. The one study in the literature successfully applying the technique to measurement of human gastric mucosal blood flow via endoscopy was by Murakami et al (1982). Utilizing 100% hydrogen gas and a noninvasive contact platinum electrode on nine patients, Murakami found repeated measurements to be highly reproducible. Comparison with another technique for validation was not performed. Unfortunately, no other reports of the hydrogen gas clearance technique applied clinically have appeared in the literature.

Concerns over flammability and subject hypoxia led to the application of 3% hydrogen gas for generation of the hydrogen current (33). Ashley and Cheung (1984) demonstrated that the lower concentration still enabled accurate readings although the signals obtained had a great deal more "noise" and required a much longer saturation time. The 3% technique has been successfully applied endoscopically to gastric and colon mucosa in anesthetized dogs (55).

Several factors may explain our failure to apply the 3% HGC technique to man. Tissue saturation with 3% hydrogen obviously takes longer than when 100% hydrogen is used, or when a larger animal is the subject of study. The average dog of 25 kg in our laboratory takes 2 to 3 minutes to achieve a reasonable degree of saturation using a very sensitive scale. Most of our patients averaged 70 kg; a comparable saturation to that of a 25 kg dog might be expected to take considerably longer than 2 to 3 minutes. In anticipation of this, an extremely sensitive scale (50 mv) was used for recording the current. The random baseline drift and spontaneous abrupt deflections may have represented underlying electrical activity in the muscularis propria that would otherwise have gone undetected if a less sensitive scale had been used. A major difference between clinical and laboratory studies is that animals are routinely anesthetized with sodium pentobarbital for the duration of the blood flow measurements whereas humans are obviously not. Motion artifact and neural stimulation induced by the awake state are therefore abolished in the anesthetized animal and barbiturates may exert a primary stabilizing effect on the underlying electrical activity of the gastric wall. Further study is required to characterize the electrical signal we observed. Perhaps these may be filtered out if properly identified.

Logarithmic curves were obtained in a few patients suggesting that hydrogen gas clearance may yet be successfully applied to man. Modifications of the electrode tip may be necessary to eliminate problems with contact loss and motion artifact.

LASER-DOPPLER FLOWMETRY

Laser-doppler flowmetry was found in this study to be technically quite easy to apply to the measurement of human gastric mucosal blood flow via endoscopy. This has consistently been the report of other investigators in the field (Kvernebo, Ahn, etc.). Signals were both stable and reproducible. Problems with signal disruption due to slippage or peristalsis were largely unavoidable and could probably be minimized by alteration of the probe tip design to include a small anchor. Precise probe placement on the regions of interest requires a thorough knowledge of endoscopy as well as variations in gastric anatomy.

Our results correlate well with other studies employing laser-doppler flowmetry to measure human gastric mucosal blood flow. Ahn et al (1986) measured different gastric regions in twenty-six normal subjects using a PF-1 flowmeter. A regional variation was found with fundus recording the highest signal and antrum the lowest ($p<0.001$). No difference between lesser and greater curve was noted. Kvernebo et al (1986) also demonstrated a regional variation again with fundus having the highest value. A statistically significant difference between the lesser and greater curves was found with the lesser curve demonstrating the lower flow ($p<0.01$). This differs from our results in that we found lesser curve flow at its midpoint opposite the angularis ($p<0.01$). The results are likely not comparable however, since they measured three regions along the lesser curve and three along the greater curve whereas we only measured one on each. In addition, Allen et al (1988) measured nine sites in thirty-eight patients via gastroscopy and revealed a significantly higher flow in the proximal stomach compared to the distal stomach ($p<0.001$) (56).

Studies utilizing other techniques have also demonstrated a regional variation in the human gastric microvasculature. Using reflectance spectrophotometry on fifty-one normal subjects, Kamada et al (1982) demonstrated higher mucosal blood volume values in the corpus than in the antrum ($p<0.05$). The angularis, however, was not specifically compared with the mid-greater curve as in our study. Several anatomic studies of the human gastric mucosal microvasculature utilizing scanning electron microscopy have yielded interesting results. Raschke et al (1987) has discovered the presence of a double capillary layer unique to the corpus mucosa. The submucosal plexus was observed to give off short and long mucosal arteries which contributed to a basal and apical mucosal capillary plexus respectively. Piasecki et al (1980) demonstrated the presence of mucosal-end arteries unique to the angularis and lesser curve. These vessels originate outside the gastric wall and pierce the muscularis and submucosa to supply discrete areas of mucosa. There is no communication with the submucosal plexus. Piasecki speculates that focal mucosal ischemia can be induced in mucosa supplied by these arteries by strong contractions of the lesser curve musculature. Virtually all vascular cast corrosion studies in recent years have concluded that arteriovenous anastomoses do not exist in the gastric mucosa. In view of the recent evidence of intra-organ variation in microvascular architecture, the need to invoke the presence of anteriovenous malformations indeed seems to be diminished.

It seems possible now to begin to paint a picture of regional variations in human gastric mucosal blood flow based on mucosal perfusion studies such as ours, and the microvasculature studies already mentioned. Mucosal blood flow is highest in the fundus. Dense capillary systems exist in this area to ensure adequate blood flow for the intensely metabolically active parietal cell mass. Flow is least in the antrum, where parietal cells do not exist and metabolic activity is comparatively low. The status of lesser versus greater curve flow is not at present clear. Evidence suggests the angularis, in particular, to have a unique microvasculature, but direct measurements of flow have yielded conflicting or at best uncomparable results. In our study, the angularis had the second highest flow in the stomach which could reflect the presence of Piasecki's numerous end arteries in this region. It is also possible, however, that our use of Buscopan to reduce muscular activity may have released these arteries from a physiologic tonus created by the surrounding thick lesser curve musculature. In this case, our values for angularis flow would be artificially high.

Blood flow dynamics around acute gastric ulcers were determined in two patients in our study. The ulcer crater was characterized by very low flow while the ulcer margin by very high flow. Flow 1 centimeter away from the margin yielded an intermediate value. This is precisely what one would expect in a lesion with a central necrotic area surrounded by an inflammatory response. Kamada et al (1986) had applied reflectance spectophotometry to the measurement of blood volume dynamics in gastric ulcers. Of interest is his finding that an initial high value for the ulcer margin correlated very well with complete ulcer healing at 3 months. Those patients with low values were more likely to have non-healing of their ulcers. It may well be possible to predict using laser-doppler flowmetry or reflectance spectrophotometry which patients with gastric ulcers would need more aggressive treatment such as surgery. Further study of ulcers using laser-doppler flowmetry is certainly required.

CONCLUSIONS

1) Laser-doppler flowmetry displays significant potential for exploring the regional mucosal blood flow in the stomach. The hydrogen gas clearance technique proved to be difficult and unreliable. However, in view of the partial success of some of our hydrogen readings, the technique may yet be applicable.

2) The human gastric mucosa has a regional variation in blood flow. The fundus demonstrates the highest flows and the anterior antrum the least. Other regions are intermediate between these two extremes.

FIGURE 1. LABORATORY TEST OF ENDOSCOPIC ELECTRODE USING 100% AND 3% HYDROGEN GAS

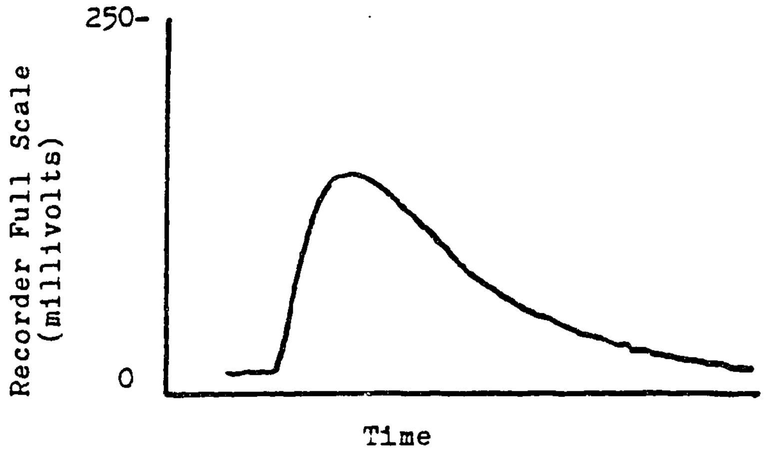

Figure 1-A. 100% hydrogen gas administered for 20 seconds. An exponential decay curve is obtained as the gas washes out.

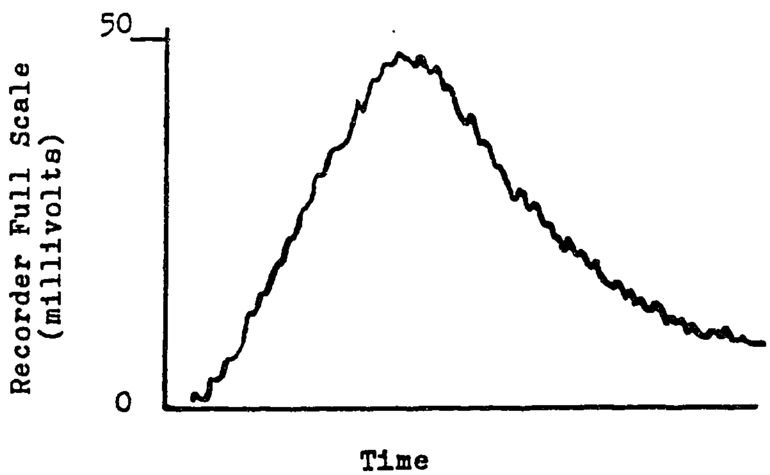

Figure 1-B. 3% hydrogen gas administered for 3 1/2 minutes. A good washout curve is obtained albeit with increased 'noise' due to the greater sensitivity required.

FIGURE 2. ELECTRODE FUNCTION IN PATIENTS

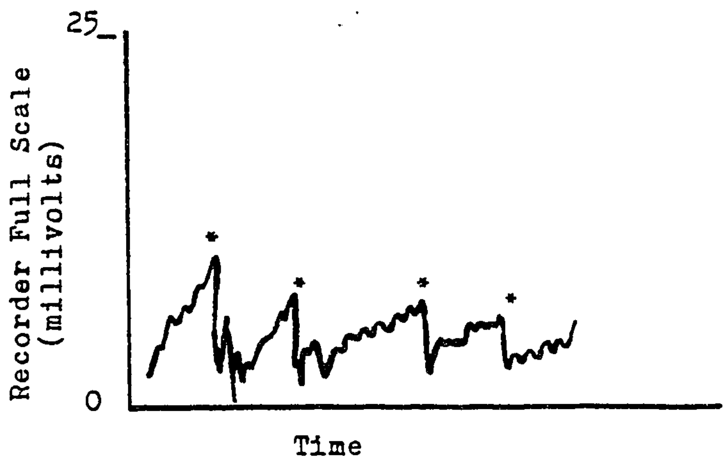

Figure 2-A. Upward baseline drift observed after electrode placement. After 15 minutes, the baseline has almost stabilized. * = zero adjustment.

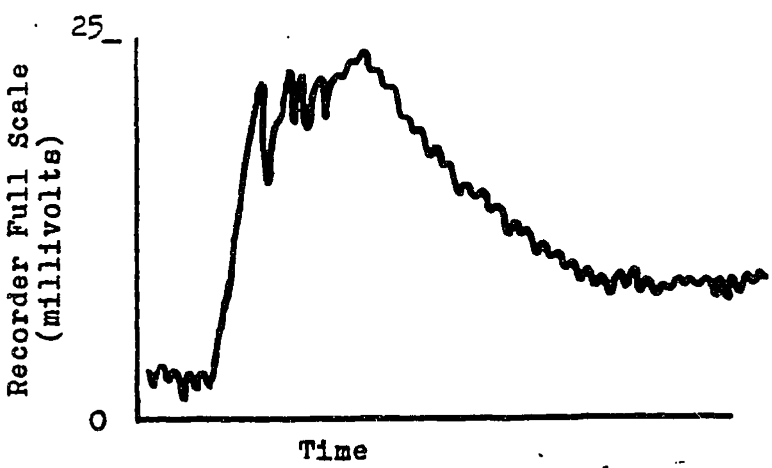

Figure 2-B. An exponential washout curve with failure of the curve to return to baseline. The first part of the curve has been altered by an unidentified electrical signal.

REGIONAL VARIATION IN GMBF IN HEALTHY CONTROLS (n=6)

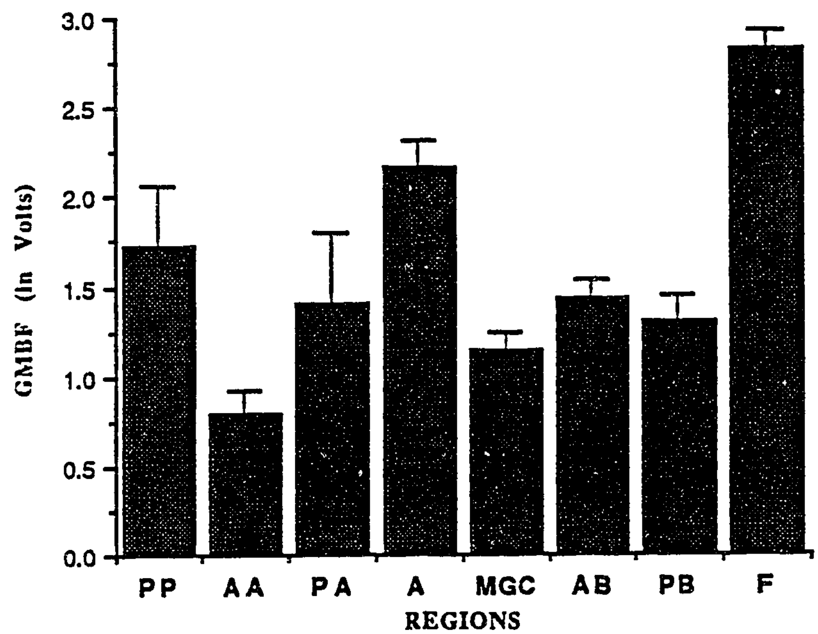

FIGURE 3: Gastric regions are arranged anatomically from caudad to cephalad. Statistically different areas are shown in Table 1. The fundic flow which exhibits the highest flow in the stomach, is statistically different from every other area.

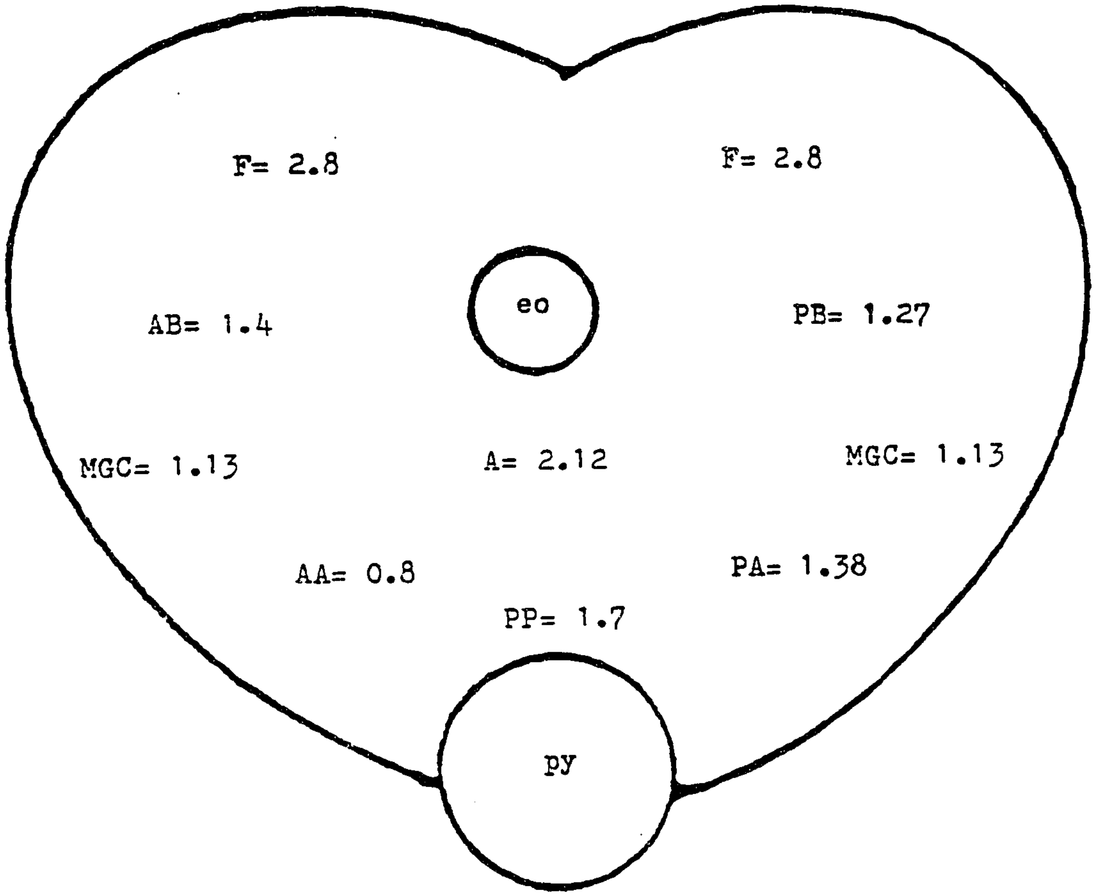

Figure 4 : Regional GMBF values in healthy controls. The stomach has been opened up along the greater curvature and laid open. The fundus exhibits a significantly higher flow than other areas of the stomach ($P < .01$). eo = esophageal opening, py = pylorus.

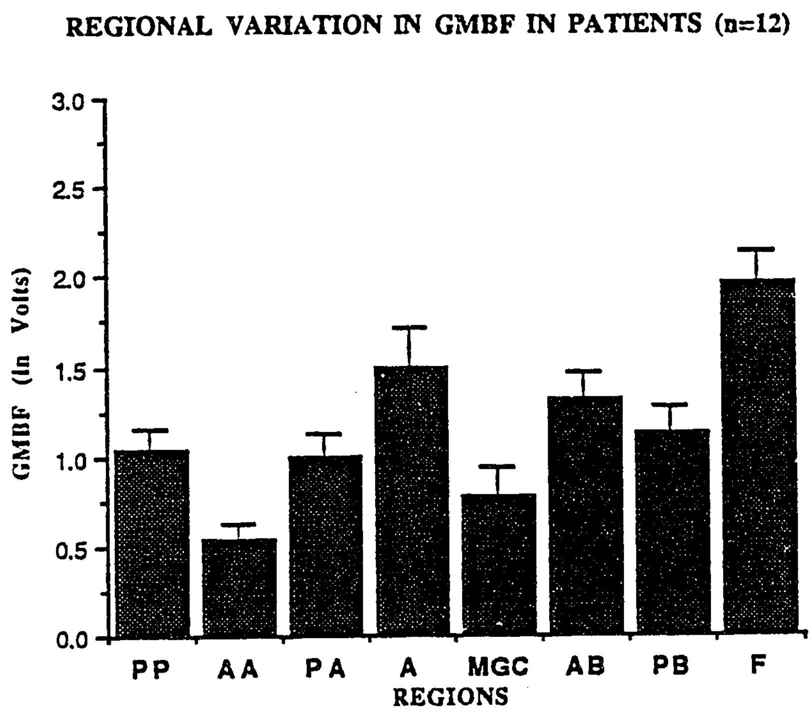

FIGURE 5: Gastric regions are arranged anatomically from caudad to cephalad. Statistically different regions are depicted in Table 2. Fundic flow is the highest and anterior antral flow the lowest. Both of these regions are statistically different from almost every other region.

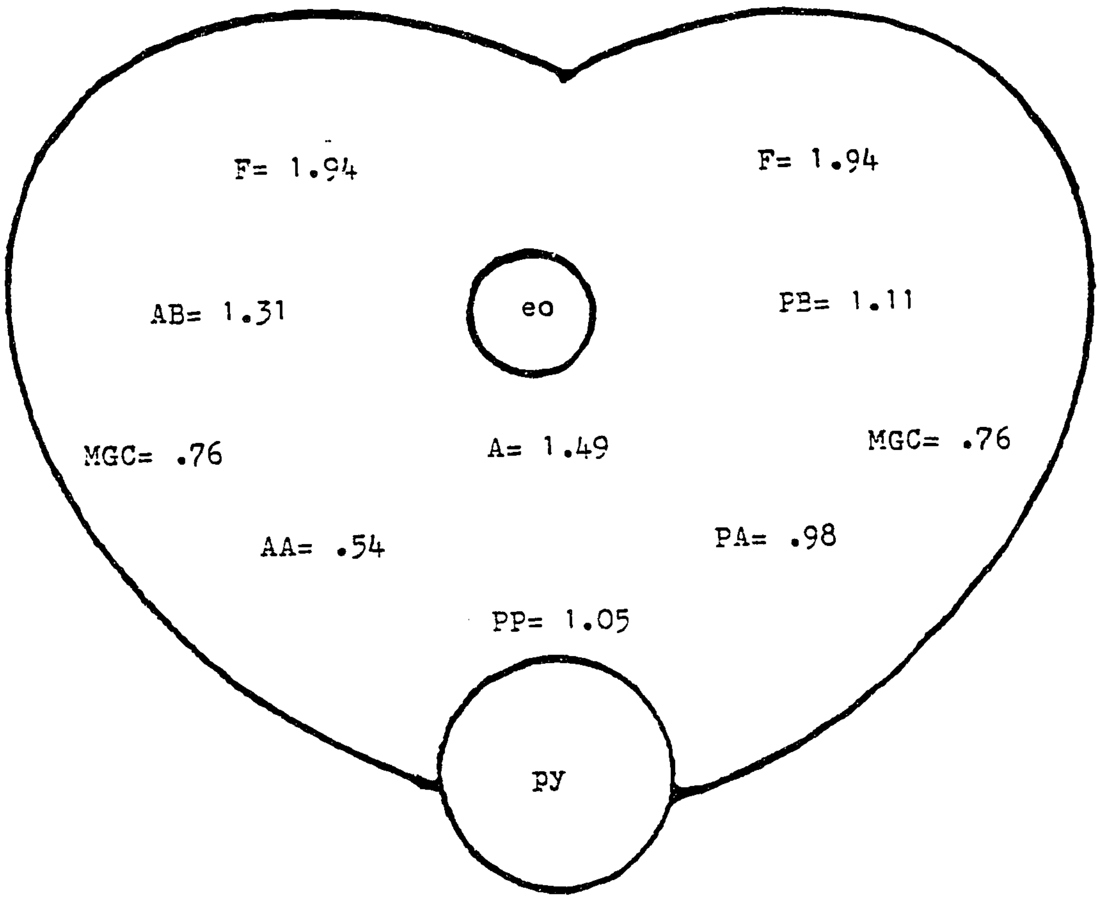

Figure 6 : Regional GMBF values in patients. The stomach has been opened up along the greater curvature and laid open. The fundus exhibits a significantly higher flow than other areas of the stomach ($P < .05$) except the angularis. eo = esophageal opening, py = pylorus.

REGIONAL VARIATION IN GMBF IN ALL SUBJECTS (n=18)

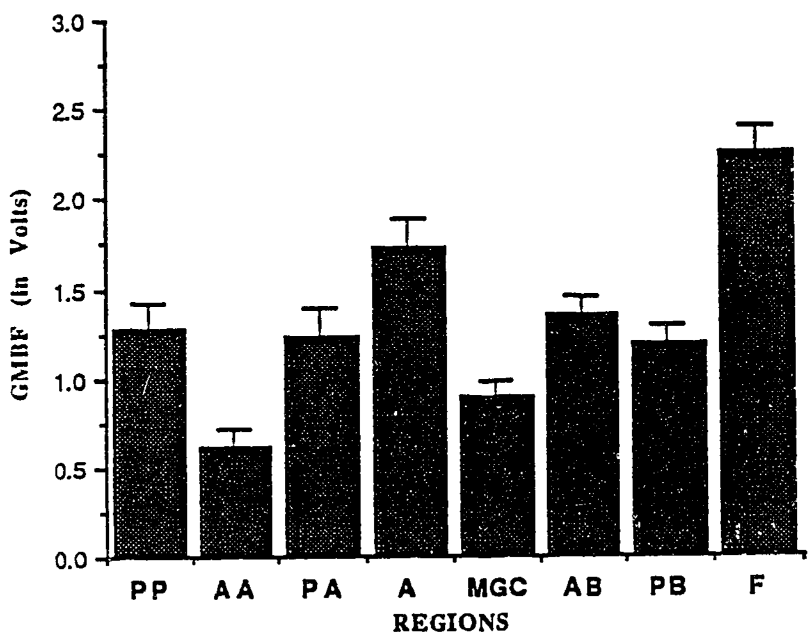

FIGURE 7: Gastric regions are arranged anatomically from caudad to cephalad. Statistically different regions are depicted in Table 4. The fundus, angularis and anterior antrum are statistically different from each other and virtually every other region.

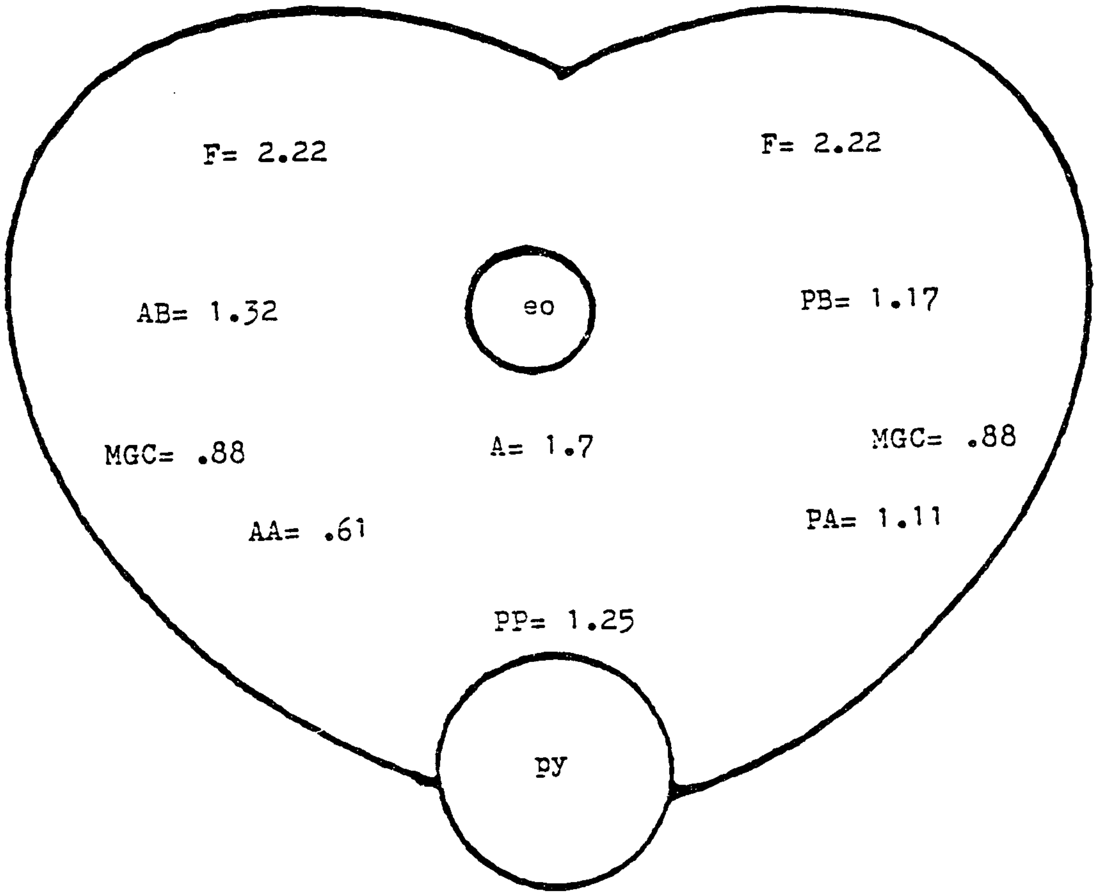

Figure 8 : Regional GMBF values in all subjects (n=18). The stomach has been opened up along the greater curvature and laid open. The fundus exhibits the highest flow while the antrum exhibits the least. eo = esophageal opening, py = pylorus.

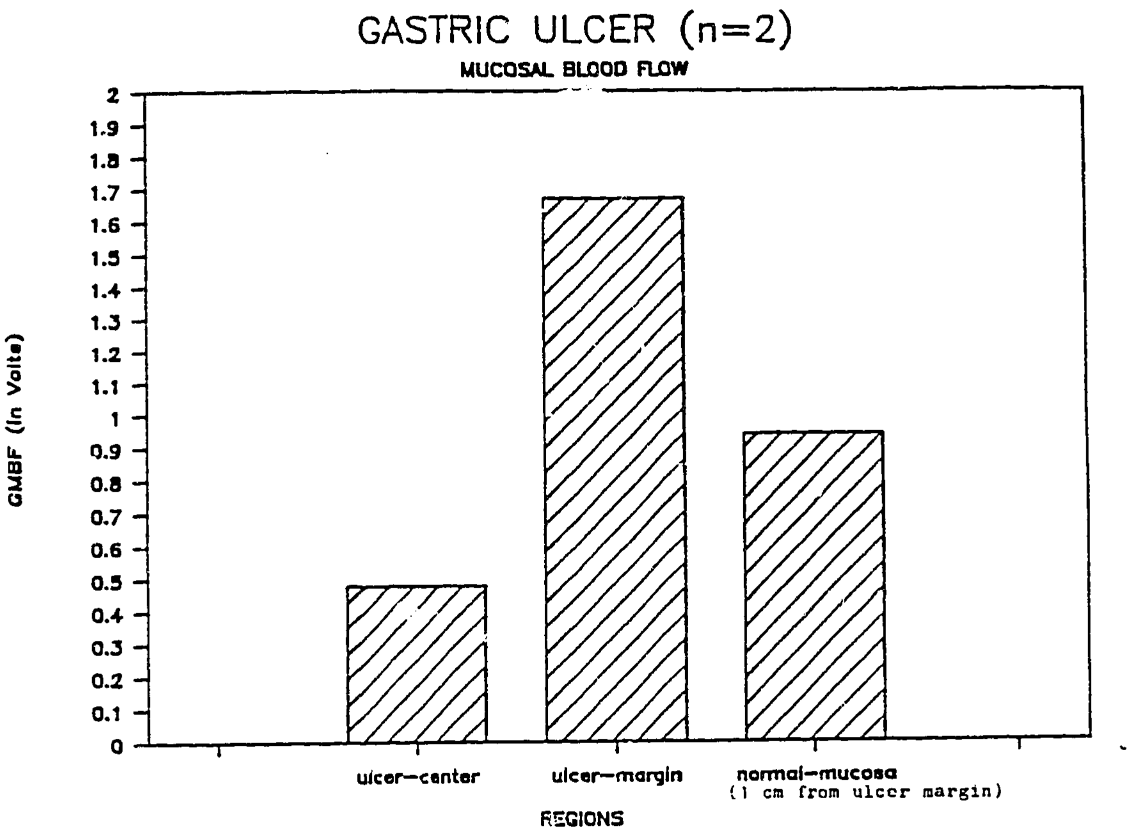

Figure 9. Gastric ulcer mucosal blood flow dynamics. The necrotic ulcer center exhibited a low flow value of .48V. At the heaped up ulcer margin, GMBF rose to 1.67V an increase of more than 300%. 1 cm away from the ulcer margin GMBF falls by almost half.

TABLE 1. Statistically different regions in healthy controls.

LEVEL OF SIGNIFICANCE	REGIONS	
$p<0.05$	AA vs PP	
	AA vs PB	
$p<0.01$	F vs PP	A vs AB
	F vs PA	A vs AB
	F vs A	
$p<0.001$	F vs AA	A vs MGC
	F vs AB	A vs AA
	F vs PB	
	F vs MGC	

TABLE 2. Endoscopic diagnoses in 12 patients.

PATIENT #	ENDOSCOPIC DIAGNOSIS
1	prepyloric gastric ulcer
2	gastric ulcer
3	normal
4	gastritis
5	normal
6	normal
7	normal
8	esophogeal varices
9	duodenal ulcer
10	gastritis
11	normal
12	duodenitis, esophagitis

TABLE 3. Statistically different regions in patients.

LEVEL OF SIGNIFICANCE	REGIONS
$p<0.05$	PA vs A
	F vs AB
	AA vs PA
$p<0.01$	A vs MGC
	AA vs A
	AA vs PP
	AA vs AB
	AA vs PB
	AB vs MGC
	F vs PB
$p<0.001$	F vs PP
	F vs AA
	F vs PA
	F vs MGC

TABLE 4. Statistically different regions in all subjects (n=18).

LEVEL OF SIGNIFICANCE	REGIONS
$p<0.05$	A vs PP
	A vs PA
	A vs F
	PP vs MGC
	AA vs MGC
$p<0.01$	PA vs AA
	PP vs AA
	A vs PB
	MGC vs AB
$p<0.001$	AA vs A
	AA vs AB
	AA vs PB
	F vs PP
	F vs AA
	F vs PA
	F vs AB
	F vs PB
	F vs MGC

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APPENDIX 1

LASER-DOPPLER FLOWMETRY

RECORDER SETTINGS

Calibrate the Gould recorder channel and adjust to the following preferred settings:

FULL SCALE: 10

CALIBRATE: 5-10 (depending on scale to be used on the recorder, eg. 5 for 5V full scale and 10 for 10V full scale)

CHART SPEED: 30 mm/min - Depress 50 plus -100 buttons.

NOTCH FILTER: Out

CUT OFF: Low - DC offset
High - 1K

OFFSET FINE/COARSE: Use to adjust the recorder pen to preferred baseline (zero) only after all the above settings have been made.

LASER-DOPPLER FLOWMETER SETTINGS

Hook up the flowmeter to the recorder. Ensure the flowmeter flashes LLL or zero. Use the following settings:

WIDE BAND: On (Depress switch at the back of the flowmeter. When on, red indicator light comes on)

ARTIFACT FILTER: On (switch at the back of the flowmeter)

TIME CONSTANT: L (switch at the back of the flowmeter)

APPENDIX 2

GOULD UNIVERSAL AMPLIFIER CALIBRATION FOR LASER-DOPPLER

1. Perform the following settings:
   FULL SCALE to OFF
   CALIBRATE to 10
   LOW CUTOFF to DC
   HI CUTOFF to 1K

2. With the recorder running, adjust pen zero to chart center.

3. mV X 100-mV-EXTmV to mV X 100.

4. Push (depress) zero button.

5. Full Scale Attenuator to 1.0.

6. Adjust mV balance to get the pen on center zero if not on it.

7. Release zero button.

8. Low Cutoff to DC Offset.

9. Use Offset (Coarse or Fine) to balance the transducer back to center zero.

10. Change Full Scale Attenuator, mV X 100-Mv-EXTmV, and Calibrate Settings to the desired Settings if necessary.

11. Use Coarse or Fine Offset to put pen on right edge of scale or desired baseline.

12. Connect LD Flowmeter or HGC input at the back of the amplifier and start recording. If it is desired to suppress the baseline signal (zero suppression) use only the Offset.

APPENDIX 3

HYDROGEN GAS CLEARANCE - RECORDER CALIBRATION

RECORDER SETTINGS

Calibrate the Gould recorder channel and adjust to the following preferred settings:

FULL SCALE: 0.5

CALIBRATE: 0.25/0.5/1 (depending on scale to be used on the recorder, eg. 0.25 for 12.5 mV full scale and 0.5 for 25 mV)

CHART SPEED: 6 mm/min (Depress 10 plus -100 buttons)

mV: mV X 100

NOTCH FILTER: Out

CUT OFF: Low - DC Offset
High - M

OFFSET FINE/COARSE: Use to adjust the recorder pen to preferred baseline (zero) only after all the above settings have been made.

2. Shave and abrade an area of skin on the patient's trunk. Apply some electrode gel and attach an Ag/AgCl skin electrode to this area of the skin.

3. Place the endoscopic Pt HGC electrode at a desired position on the gastric mucosa.

4. Hook up the electrodes (2 and 3) to the junction box:
   MGC - to RED terminal
   Ag/AgCl - to BLACK terminal

5. Hook up junction box to recorder.

6. Adjust the recorder pen using the OFFSET FINE/COARSE knob, back to baseline if necessary. Ensure patient has face mask in place and deliver 3% hydrogen gas and air. Obtain a H_2 saturation-desaturation curve.