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LASER-DOPPLER FLOWMETRY AND HYDROGEN GAS CLEARANCE: COMPARISON AND APPLICATIONS IN THE MEASUREMENT OF FOCAL GASTRIC MUCOSAL BLOOD FLOW

BY

THEOPHILUS JONES GANA

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY**

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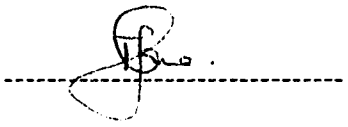

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THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED **LASER-DOPPLER FLOWMETRY AND HYDROGEN GAS CLEARANCE: COMPARISON AND APPLICATIONS IN THE MEASUREMENT OF FOCAL GASTRIC MUCOSAL BLOOD FLOW** SUBMITTED BY **THEOPHILUS JONES GANA** IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **DOCTOR OF PHILOSOPHY** IN EXPERIMENTAL SURGERY.

Dr. Jarley Koo (Supervisor)

Dr. R.L. Jones

Dr. W.W. Lautt (External Examiner)

Dr. J.C. Russell (Committee Chairman)

Dr. O.G. Thurston

Dr. G.L. Warnock

Date: April 20, 1992

DEDICATION

This work is dedicated to my parents, Joseph Umaru Gana, M.B.E., and Hannah Adisatu Gana, for giving us (his children) a good education and especially to my late father who through his exemplary life taught us that hard work is the only way to achieve success.

ABSTRACT

Laser-Doppler flowmetry (LDF) and hydrogen gas clearance (HGC) are two recent, validated and clinically most promising techniques capable of endoscopic measurement of focal gastrointestinal blood flow. However, LDF is not calibrated in absolute flow units and 3% HGC is associated with a small, unstable signal with a low signal-to-noise ratio. This study was aimed at comparing, refining, and evaluating LDF and HGC, and applying them to study the role of blood flow in aspirin-induced mucosal injury and in misoprostol (G.D. Searle) cytoprotection of such injury in canine chambered gastric segments. Simultaneous basal gastric mucosal blood flow (GMBF) measured by LDF and HGC at the same sites were significantly correlated in individual experiments but the regression lines obtained in the latter did not corroborate a common regression line hypothesis and the slopes were significantly different. An amplifier/filter was developed that significantly increased the magnitude and quality of the 3% HGC signal and reduces baseline drifts. Washin GMBF were not significantly different from the corresponding washout blood flow values, making it feasible to obtain 2 measurements from one HGC curve, thereby reducing measurement time. Misoprostol dose-dependently increases GMBF. In contrast to misoprostol, aspirin significantly decreased GMBF in all measured areas of the mucosa with significantly greater reduction (66% of basal) in the grossly ulcerated areas. Misoprostol pretreatment induced a transient 18% increase in GMBF but did not prevent the subsequent decrease in GMBF induced by aspirin. Misoprostol pretreatment was cytoprotective and also induced a sustained alkaline nonparietal secretion. In conclusion, LDF and HGC are comparable in the measurement of focal GMBF, however, a universal calibration factor (slope) for the LDF signal to absolute flow values is not feasible. An improved HGC technique is presented. The usefulness of LDF and HGC are demonstrated in the application studies of the role of focal blood flow in aspirin-induced gastric mucosal injury and prostaglandin cytoprotection. In those studies, misoprostol was vasodilatory, cytoprotective and induces a sustained alkaline nonparietal secretion. In contrast, aspirin induces focal ischemia of varying

degrees and areas with blood flow reduced below a "critical GMBF value" develop gross damage. Increase or maintenance of GMBF does not appear to be the direct mechanism of gastric cytoprotection by prostaglandins.

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LIST OF ABBREVIATIONS

ASA = Aspirin (acetylsalicylic acid)

GMBF = Gastric Mucosal Blood Flow

HGC = Hydrogen Gas Clearance

IAP = Iodoantipyrine Clearance

LDF = Laser-Doppler Flowmetry

MP = Misoprostol

PG = Prostaglandins

Chapter 1

Techniques Used for Gastric Mucosal Blood Flow Measurements: A Review of the Literature

INTRODUCTION

In the resting state, the gastrointestinal tract and portal system, excluding the liver, receives 21% of the normal cardiac output making it the single most important peripheral vascular bed in that state.[1] It seems logical, therefore, that there is sustained interest in the role of the circulation in gastrointestinal physiology and pathophysiology. This indicates the need not only to develop newer and better techniques for assessing blood flow in the gastrointestinal tract but to continuously seek ways to improve on existing techniques. Consequently, the last three decades has witnessed both a qualitative and quantitative improvement in the techniques used to measure gastrointestinal mucosal blood flow. Before Jacobson and his co-workers[2] developed the aminopyrine clearance technique for the measurement of gastric mucosal blood flow (GMBF), only the direct collection of timed venous effluent and the indicator-dilution technique[3,4] were used for organ blood flow measurements in the gastrointestinal tract.[5] These techniques require surgery and measure only total organ blood flow respectively.

At the onset of the surge in techniques, Jacobson proposed criteria that an ideal blood flow-measurement technique should meet.[6] These should include: quantification of the data in absolute circulatory units (ml/min/g), accuracy, reproducibility, capability of estimation of total and intra-organ distribution of blood flow, minimal disturbance of the physiology or psychology of the subject, sensitivity to rapid changes, capacity for continuous monitoring, a minimum of unproven assumptions, independence from other measurements, sound theoretical basis, low cost, and ease of performance. The purpose of this review is to examine the body of current knowledge of the principles, advantages, limitations and applications of the techniques

used for measuring gastric blood flow with particular interest in those capable of intramural or regional and focal blood flow assessments. Blood flow in the context used in this dissertation includes tissue blood flow, the total blood flow through vessels of all sizes in a given volume of tissue, and capillary or exchange vessel flow (in microvessels of 100 μm diameter or less).[7] Before proceeding to discuss the techniques of measuring gastric blood flow, it is worthwhile to briefly enumerate the importance of GMBF in gastric physiology and pathophysiology.

WHY MEASURE GASTRIC MUCOSAL BLOOD FLOW?

The gastric mucosa receives about 80% of the total resting gastric blood flow.[3,8] This rich vascular supply is necessary for the mucosa to achieve its physiological functions of producing secretions for the digestion of food and absorption of the digested foods.

Relationship Between Gastric Blood Flow and Acid Secretion. The relationship between gastric secretion and GMBF has been extensively studied.[9-13] Most of the information available about this relationship has been obtained using the aminopyrine clearance technique to measure GMBF in studies of gastric secretory function.[12] However, the aminopyrine clearance technique has been found to be inaccurate in the absence of active gastric acid secretion[14-18] and during gastric mucosal damage.[18-20] Consequently, the relationship between GMBF and gastric acid secretion established by investigations using this now obsolete technique[12,18] has to be re-examined in studies employing the newer, more accurate and valid techniques.

Gastric Mucosal Defense. Gastric mucosal blood flow plays an important role in protecting the gastric mucosa against injury.[13,21-24] Studies have shown that a decrease in mucosal blood flow predisposes the gastric mucosa to marked lesion formation while an increase protects it against injury.[13,22] The precise mechanism(s) of this protection is not known. However, it has

been hypothesized that an increase in GMBF will: (i) maintain adequate supply of oxygen to the tissues and prevent a deficit in energy metabolism[25]; (ii) buffer H^+ that back-diffuses into the lamina propria[21,23,26]; and (iii) dilute and remove both back-diffused H^+ and any damaging agent that may gain access into the lamina propria.[23] Availability of better techniques of assessing GMBF especially focally, will allow verification and further illumination of these hypotheses.

Ulcerogenesis. Although there is overwhelming evidence that focal mucosal ischemia is a basic pathogenic feature of all acute[24,27-29] and chronic[30-32] ulcer disease in the gastrointestinal tract, the precise sequence of events between the presence of the etiological factors and the appearance of the ulcers is still not known. Investigation of focal ischemic phenomena in the gastrointestinal tract has been hindered by lack of techniques capable of assessing mucosal blood flow in focal areas. Furthermore, these techniques if available, can help in predicting areas of acute gastric mucosal ulceration in predisposed subjects, further the understanding of the pathogenesis of the lesions, and facilitate the development of better preventive and treatment regimens for the management of these conditions.

Gastric Cytoprotection. The ability of prostaglandins to protect against gross and histological gastric mucosal injury by a mechanism other than inhibition or neutralization of gastric acid secretion has been defined as gastric cytoprotection.[33-35] The precise mechanism(s) of this gastric cytoprotection remains unknown. One hypothesis is that this prostaglandin effect may be mediated by an increase in GMBF.[36,37] However, there are conflicting reports from studies on both the effects of prostaglandins and mucosal damaging agents on GMBF[36,38], due in part to the techniques used for blood flow estimates in those studies. Mucosal lesions produced by mucosal damaging agents are focal in nature.[34,39,40] Techniques like the aminopyrine clearance and radioactive microspheres used in most previous studies lack the capacity to

assess focal blood flow and the former technique is inaccurate during gastric mucosal damage.[18-20]

Clinical. A large body of data has been obtained from animal studies on the role of mucosal blood flow in gastrointestinal physiology and pathophysiology. However, there is a dearth of information from comparable human studies. This is due to the lack of a gold standard, a technique which is safe, noninvasive and can be accurately applied in human subjects.[29] Clinically applicable techniques will enable us to substantiate or dismiss the bulk of data obtained from previous laboratory studies.[29]

Splanchnic Ischemia and Organ Failure. Recently there has been an increasing awareness of the important role of splanchnic ischemia in single and multiple organ failure.[41] The importance of early diagnosis and treatment as critical factors in the management of occlusive and nonocclusive intestinal ischemia if a fatal outcome is to be avoided has been stressed.[42] Techniques of measurement of gastrointestinal mucosal blood flow will aid in the early detection of these conditions, enable the institution of treatment early in the disease and avert an otherwise fatal outcome.

THE TECHNIQUES

Most of the techniques used for measurements of gastric blood flow, with the exception of the clearance techniques, have been developed for use as well either in the intestine or other organs of the body.

Clearance Techniques

The clearance techniques are based on the pH partition hypothesis described by Shore, Brodie and Hogben in 1957.[43] The principle involves the partitioning of weak organic bases between

two compartments, plasma and gastric juice, as a function of the pH gradient between them. Briefly, the unionized weak base freely diffuses across a lipid membrane at the high pH of blood (which they calculated to be 7.0), but at the markedly lower pH of gastric juice in the gastric lumen, it dissociates, becomes lipid insoluble and is trapped in the gastric juice. The validity of the technique is certain only when the luminal pH of the stomach is maintained below 2.5 and the dissociation constant, pKa, of the weak base is 5 or less. From the concentrations of the weak base on either side of the partition, its clearance, which is assumed to be an accurate estimate of GMBF is calculated. These clearance techniques can not be used in the intestine, since it does not have the acidic medium to trap the weak base molecule.

The use of the gastric clearance of aminopyrine as a measure of GMBF was first reported by Jacobson, Linford and Grossman in 1966,[2] making it the first noninvasive technique to estimate GMBF along with gastric secretion in anesthetized and nonanesthetized animals (dogs). Jacobson subsequently described assumptions which must be satisfied by a substance to be suitable to act as a clearance marker in the measurement of GMBF.[44] These include: (i) the substance must be completely cleared from the circulation being measured; (ii) the substance must not be synthesized, destroyed, or retransported by the organ after clearance has taken place; (iii) the substance must be physiologically inert; (iv) the substance must be capable of quantitative and accurate detection in appropriate biological fluids; (v) the clearance of the marker must be constant and independent of its plasma concentration over a wide range of plasma values; and (vi) the clearance of the marker must be identical with the clearance of other markers which differ widely from it in their physical and chemical properties.

The use of the aminopyrine clearance (APC) technique was limited to experimental animals initially and could not be extended to humans because of the danger of bone marrow toxicity associated with the large doses required to give spectrophotometrically measurable serum concentrations.[45] However, the subsequent labelling of the aminopyrine molecule with radioactive carbon (^{14}C) and validation in dogs[46] led to its application in humans in smaller

doses.[47] In addition to aminopyrine, the use of other clearance markers have been reported. These include ^{14}C-aminopyrine,[46] ^{14}C-aniline,[48] ^{99m}technetium,[49] and neutral red.[50] Aminopyrine clearance, the prototype of the clearance techniques, has been the most widely used and was previously used as a standard for the validation of other newly developed techniques.

Advantages of the clearance technique[51] are: it measures mucosal blood flow of the entire stomach and not the total flow to the whole stomach; it can measure mucosal blood flow continuously; it can be used in conscious subjects, and it can be used to obtain simultaneous gastric acid secretion data. The method has been extensively used to study the relationship between GMBF and gastric acid secretion.[9-13] The limitations include: over a decade after its development, it was discovered that the APC technique does not accurately reflect gastric mucosal perfusion in all situations in which the stomach is not actively secreting acid;[14-18] some of the assumptions for clearance markers are not directly proven; and day to day variations have been reported[51] in R-ratios (ratio of the concentrations of aminopyrine in gastric juice to plasma) within the same animal. Recent studies have shown that ^{14}C-aminopyrine clearance reflects both GMBF and parietal cell activity.[52-54] More recently, APC has been found to be inaccurate in the presence of gastric mucosal damage.[18-20] Because of these major limitations, the APC technique has become obsolete[18] in the measurement of GMBF in the last few years.

Limitations of Other Clearance Markers: Use of ^{14}C-aniline clearance to measure GMBF was first reported by Curwain and Holton.[48] Aniline may produce methemoglobinemia, so it has not been used in man. Also, due to its high tendency to bind to plasma proteins, it underestimates flow and is best used to reflect relative changes in blood flow.

A year after the introduction of the neutral red clearance technique, its developers reported that with its use in humans, a metabolite of it was formed which not only cleared poorly into gastric juice but interfered with the accuracy of the measurements.[55] The extraction

technique was later modified to extract only diffusible neutral red from the plasma making it possible to use the technique more accurately in dogs and humans.[56,57]

Technetium 99m (^{99m}Tc) clearance has been used to estimate GMBF in rat and man,[49,58] but the values obtained were significantly less than those obtained for aminopyrine clearance. This is thought to be due to gastric back-diffusion of technetium which can occur if allowed to accumulate in the stomach and the fact that it is also reabsorbed in the duodenum.[58] Recently, technetium-4-methyl-aminophenazone clearance has been reported to give similar results to aminopyrine clearance in anesthetized dogs.[59] It was also suggested that this substance would be more suitable and convenient for use in man than ^{14}C-aminopyrine because the subject would receive less radiation, the measurements would be easier to make, and will be less expensive.

Clearance of pH Trapped Compounds in the Intestine: This technique which was developed for use in the intestine, is based on a similar principle and methodology like the clearance techniques used in the stomach to measure mucosal blood flow. It was first described by Csaky and Varga[60] in 1975 and is based on the pH partition hypothesis.[43] A weak acid, barbital, diffuses across the lipid membrane in the undissociated form from the high pH of blood (7.4) to the higher pH of the intestinal lumen (pH 9.75) where it dissociates and is trapped. Assuming intestinal perfusion is the rate-limiting factor in the delivery of the weak acid to the intestinal mucosa, the clearance of the weak acid is a measure of mucosal blood flow.

Although, generally the technique is similar to the clearance techniques discussed above, so little has been done with the technique that its validity, advantages and disadvantages have not been established.[61] The technique has been used mainly in rats.[60,61]

Microsphere Technique

The use of the microsphere technique, a form of the indicator-dilution technique described by Delaney and Grim,[3,4] was first reported by Archibald et al.[8,16] Both radioactive and nonradioactive microspheres can be used but the former has been more widely used.[62] The principle of the technique assumes that after central arterial injection (i.e., into the left ventricle of the heart), microspheres are trapped in vessels in the microcirculation of an organ or part of an organ in direct proportion to the fraction of the cardiac output reaching it. At the end of the study the experimental animal is killed. By counting the number of spheres (for nonradioactive microspheres) or determining the radioactivity (for radioactive microspheres) in the organ or tissue of interest, knowing the cardiac output, and the total number of spheres or radioactivity respectively injected, a flow value can be calculated for the organ or tissue.

The minimal requirements that must be met for this technique to yield valid data have been enumerated.[63] These are: (i) microspheres must be uniformly distributed in blood and travel with blood so that sphere flow approximates blood flow; (ii) the law of conservation of mass must be observed, i.e., all spheres entering the circulation must be accounted for; (iii) the microspheres must be trapped within a tissue layer in vessels which function as parallel circuits among the individual layers; (iv) if any spheres reach venous blood, the fraction "shunting" through each layer must be known; (v) once trapped in a tissue layer, microspheres must not move to another layer nor be released into venous blood as a result of subsequent pertubations. Although the fulfillment of these minimum criteria by the microsphere technique has been questioned,[63] this method and the APC technique have been the most widely used methods in gastrointestinal mucosal blood flow measurements. The radioactive microsphere technique is also being used as a gold standard in animal models in the evaluation and validation of other techniques which include the aminopyrine clearance technique,[16] hydrogen gas clearance technique,[64] and laser-Doppler flowmetry.[65,66] This technique has been used more extensively to measure blood flow in the intestine[12] than in the stomach.

Compared to the APC technique, this method can measure intramural blood flow or flow to a small segment of tissue, the flow values obtained are independent of the gastric secretory state,[16,45,67] hence the technique can be applied in conditions of hypochlorhydria or during gastric mucosal injury when the clearance methods give unreliable results. It permits a number of accurate measurements without tissue manipulation. Although a lot of reports have appeared in the literature on its use in animals, the method has absolutely no clinical potential as the animal has to be killed at the end of the experiment and the tissue or organ excised for counting of spheres or radioactivity. Shunting of injected microspheres[12] into the venous blood, a problem that has been reported both in the intestine (about 20-30%) and to a much lesser extent in the stomach, and the movement of previously lodged microspheres[68] may lead to an underestimation of blood flow. Sphere size (>12 μm) may interfere with the distribution of spheres within the tissue being measured especially in series-coupled vascular beds;[69] also sphere sizes of <12 μm when injected may not be trapped in the capillaries but may travel through the organ being studied making it impossible to determine which part of the gastrointestinal wall is bypassed and hence the true intramural distribution of blood flow.[69b] Other limitations of the technique include: it cannot offer continuous measurements as it only permits a limited number of instantaneous measurements; it is expensive; and it requires the use of a significant amount of hazardous radioactivity. Furthermore, although the technique is used for regional blood flow measurements, it is inaccurate to measure mucosal blood flow at small foci because a single sample must contain a minimum of 400 spheres in order to avoid statistically significant inherent variability in the measurement.[70]

Heat Clearance Technique

The use of heat clearance from a heating coil placed on the gastric mucosa as a measure of blood flow was first reported by Richards et al, in 1942.[71] Subsequently, the technique was improved by Bell and Shelley[72] and others.[7] The principle[45,72] is based on the assumption that altered

thermal conductivity of a tissue (mucosa in this case) gives an estimate of its blood flow. The changes in the thermal conductivity of the tissue are picked up by a thermistor containing a heater coil which is fixed by suction to the mucosa.

Advantages of the method are it can measure focal GMBF, hence, it can be used to compare blood flow changes at specific points in the mucosa; it can measure blood flow instantaneously and continuously; and it can be applied in conscious animals and in human subjects. Major disadvantages of the technique are the inability to precisely control the position of the probe and the possible effect of the applied heat on blood flow.[7] Other disadvantages are that it offers only qualitative measurements of blood flow (the results are expressed in arbitrary units, millivolts), gastric contractions interfere with the recordings, and the suction required to attach the measuring probe to the mucosa may interfere with blood flow.[7,45] The method has not been widely used.

Washout of Radioactive Inert Gases

The use of inert gases to measure blood flow was first introduced by Kety and Schmidt,[73-75] and is based on the Fick principle. Thereafter, reports on the use of radioactive inert gases krypton (^{85}Kr)[76] and xenon (^{133}Xe)[77] in estimating blood flow appeared. The principle assumes that the transcapillary exchange after intra-arterial or direct injection of a radioactive inert gas into tissues is instantaneous and its removal from a site is proportional to and dependent on blood flow.[78,79] The theory of tracer (inert gas) washout further assumes that tracer elimination only occurs via the blood and that blood flow remains constant throughout the entire period of the tracer washout curve (i.e., entire measurement time). Due to their high lipid solubility, inert gases are highly diffusible into tissues and are removed almost completely after a single passage through the lungs.[13]

To perform blood flow measurements,[13] a bolus of the isotope is injected intra-arterially or placed in the gastric lumen, and its disappearance with time noted. The rate of radioactive decay versus time is plotted on semilogarithm paper. A monoexponential curve is

usually expected for a single compartment vascular bed but most organs are heterogeneous and have more than one flow rate to different compartments resulting in multiexponential curves. A complex curve peeling or curve stripping analysis of the multiexponential curve is required to separate the latter into its component monoexponentials. The rate of radioactive decay of each monoexponential curve gives an estimate of blood flow for the compartment it represents. The method is capable of estimating blood flow to a part or whole organ. Applications of the inert gas washout techniques in the gastrointestinal tract have been limited almost entirely to measurements of blood flow in the intestines.[78]

The advantages of the technique are: it does not involve sampling of tissue or blood;[79] it can be applied clinically; the inert gas can be instilled luminally into the small intestine and also intraperitoneally to evaluate ischemia in parts of the bowel.[69] Its limitations[69] include: the complex curve peeling analysis requires a lot of commitment; it is important that blood flow remains constant during the entire period of measurement; it offers discontinuous measurements; tissue injection may cause significant injury;[78] the countercurrent exchange mechanism may affect the results obtained in the small intestine;[80] and the equipment required is expensive.

Hydrogen Gas Clearance Technique

In 1964, Aukland, Gower and Beliner[81] applied Kety's theory[73-75] to local blood flow measurement with hydrogen gas. Mackie and Turner were however, the first to apply hydrogen gas clearance (HGC) in the gastrointestinal tract to measure gastric submucosal blood flow.[82,83] Semb later described a hydrogen washout technique based on the same principles, used it to measure GMBF in conscious animals and also pointed out the technical difficulties associated with the method.[84,85] It was not until 1982 that Murakami and co-workers[86] noted that in all previous studies, investigators inserted the hydrogen electrode into the stomach wall actually measuring submucosal blood flow,[82,83] or reported considerable difficulty in inserting it into the mucosal layer.[84] They therefore modified the inserted electrode used previously into a

contact electrode which yielded monoexponential washout curves and better values of GMBF.[86] That report also represented the first application of HGC to measure GMBF through the endoscope in human subjects. Hitherto, the method had been applied clinically only to measure cerebral blood flow.

The principle is based on the assumption that the rate of desaturation of hydrogen gas, a highly diffusible, biologically inert gas, from a homogenously perfused tissue is blood flow limited.[81,86] To perform hydrogen gas clearance, the experimental animal or subject is made to breathe in hydrogen gas. The current generated on the surface of a platinum electrode placed at a particular point in the tissue to be assessed, by the oxidation of molecular hydrogen to hydrogen ions and electrons, during saturation and desaturation of the tissue with hydrogen gas is measured. A curve of the electrode current versus time during saturation and desaturation is obtained. The desaturation part of the curve is plotted on semilogarithm paper to obtain a straight line. Assuming that: (a) for the highly diffusible, biologically inert gas (H_2), the tissue is in instantaneous diffusion equilibrium with the venous blood from that particular tissue throughout the whole saturation and desaturation period; (b) that the current generated on the surface of the platinum electrode is proportional to the concentration of hydrogen gas in the tissue; and (c) that arterial concentration of hydrogen gas at the beginning of desaturation is zero; by some complex mathematical derivations,[73-75,81] blood flow is calculated from the formula $F = 0.693/t_{1/2} \times (\lambda)$, where F is the flow in ml/min/gram of tissue, $t_{1/2}$ is the time taken, in minutes, for the decay current to be reduced to half its numerical value, and λ the tissue-blood partition coefficient for hydrogen gas which has been determined to be 1 ml/g.[81] Blood flow is usually expressed per 100 g of tissue for ease of comparison with values reported by other workers in the literature. The HGC technique has been used in conscious animals,[85] anesthetized dogs, rats and rabbits[64,86-90] and in humans[86,91] with good results. In the stomach, the technique has been validated against the venous outflow,[87,90] aminopyrine clearance[86,88,89] and radioactive microsphere[64] techniques with significant linear correlations.

The method is noninvasive, offers accurate measurements of blood flow to a focal area of the mucosa and can be applied through the endoscope in animals.[92,93] Although its application in humans has been limited,[86,91] it has the potential for safe, widespread clinical application through the endoscope. The technique is one of the cheapest currently available,[94] and is not affected by the gastric pH.[95] Initially, the potential hazards of flammability or explosion associated with the use of pure hydrogen gas posed a serious threat to its widespread clinical and laboratory use. In an attempt to circumvent the problem of flammability, the contact electrode method has been modified by Leung and co-workers[88] to permit the use of 3% hydrogen gas plus air as opposed to 100% hydrogen, since mixtures of 4% (or less) hydrogen gas with air are not flammable. Also, the platinum electrode has been modified, making it more sensitive to 3% hydrogen gas and allowing 20% oxygen to be added to the gas mixture thus eliminating the transient hypoxia noted otherwise.[64] These modifications were expected to usher in a new era of acceptability for a safer hydrogen gas clearance technique. However, 3% H_2-20% oxygen gas mixtures are associated with a much smaller, unstable, electrode current with a low signal-to-noise ratio. As a result, the only clinical study using HGC in the stomach reported to date is the initial validation study of the contact electrode method performed endoscopically.[86] Subsequent attempts by the same investigators[91] and others[96] to apply HGC in the measurement of focal GMBF through the endoscope in humans have been unsuccessful. Another major limitation of the technique is the inability to measure blood flow instantaneously and continuously, a period of 15-30 minutes is required to make one measurement, thus rapid changes in blood flow may be missed. The depth resolution of hydrogen gas clearance in the gastrointestinal tract is believed to be within the superficial layers of tissue[69,97] but this has not been verified experimentally.

Hydrogen Gas Clearance by Electrochemically Generated Hydrogen Gas: The use of locally generated as opposed to inhaled hydrogen gas to measure blood flow in the brain was first reported by Stosseck and Lubbers in 1970.[98] Hydrogen gas is generated by electrolysis on the

surface of a platinum electrode (200 μm diameter) placed at a point in the tissue of interest.[99] Another platinum electrode (the measuring electrode - 15 μm diameter) is placed 300 μm away to measure the H_2 produced by the generating electrode. A polarizing voltage of between +100 to +200 mV is applied to the measuring electrode. To obtain blood flow values or "microflow", current generated in the tissue of interest in the perfused and nonperfused states has to be obtained. The latter for the determination of hydrogen gas carried away from the measuring site by routes other than blood flow (nonconvectional losses). The principle assumes that all the generated hydrogen gas is taken away from that site by the blood flow.

Koshu et al,[100] have modified the equation used by Stosseck et al[99] to calculate cerebral blood flow, prolonged the generation time of H_2 and hence the amount of H_2 generated, and have obtained blood flow values that are correlated with those obtained by hydrogen inhalation. Recently, the technique has been applied in the gastrointestinal tract of dogs in a hybrid hydrogen gas clearance/laser-Doppler probe[101] to simultaneously measure focal mucosal blood flow by the two methods. The flow values obtained by the two methods were correlated. This technique has also been applied in the measurement of skin[102] and bone[103] blood flow with good results.

Advantages and limitations of the technique are the same as for the standard inhalation method except for the following points. This method obviates the need for the inhalation of H_2 which could be uncomfortable for a human subject, and the method is extremely fast, requiring less time (15 seconds) to perform one measurement. The limitations are: (i) to obtain flow measurements, nonconvectional losses of H_2 from the generation site have to be determined usually following cardiac arrest or killing of the animal, a requirement that leaves the method with a doubtful clinical potential; (ii) the presence or absence of local tissue damage by the generating electrode has not been firmly established; (iii) the theory of the technique is fraught with some questionable assumptions; and (iv) the flow values obtained differ markedly from those obtained by the standard inhalation HGC technique.[94]

Iodoantipyrine Clearance Technique

Described as a snapshot capillary clearance technique, iodoantipyrine (IAP) clearance is based on the principle that the tissue uptake of a highly diffusible indicator, 4-iodoantipyrine, following its injection into the central circulation is blood flow limited.[104,105] By measuring the concentration of the indicator in the tissue, an estimate of blood flow in absolute flow units is obtained.

To perform IAP clearance measurements of blood flow,[13,105] 4-iodoantipyrine is infused intravenously over a period of 1 min. During infusion, arterial blood samples are obtained every 3-5 seconds to monitor the arterial concentration of the indicator. Tissue samples are then quickly obtained and rapidly frozen. The concentration of the indicator in the blood is determined. To obtain tissue concentrations of the indicator, two methods have been used: (i) tissue sampling technique, in which the concentration of the indicator within the frozen tissue sample is determined, and (ii) autoradiography technique, in which frozen sections are cut and autoradiographs are prepared.[106,107] The latter is believed to offer a higher degree of spatial resolution. In human subjects, tissue has been obtained for determination of indicator concentrations by forceps mucosal biopsy through the endoscope.[108] This technique has been validated against the radioactive microsphere technique in measurements of total wall and mucosal blood flow obtained at different sites in the gastrointestinal tract with good correlations.[104] It has also been used in studying gastric mucosal blood flow in portal hypertensive rats.[109]

Advantages of the technique are: it is clinically applicable in humans through flexible endoscopy;[108] its spatial resolution is very precise, hence, it can assess blood flow in small volumes of tissue and intramurally. Blood flow can be measured without the use of anesthesia or surgery, and the method can be used to study and correlate microvascular anatomy with blood flow.[106] Limitations of the technique include: it does not offer continuous blood flow measurements; and presence of liquid lumenal contents in the gastrointestinal tract may lead to

underestimation of blood flow since the principle of the method is dependent on high diffusibility of an indicator in liquid media.[105] Furthermore, the use of radioactivity and the requirement for tissue biopsy specimens in humans constitute major disadvantages of the technique. Lastly, a theoretical disadvantage of the technique is that changes in capillary permeability and diffusion surface area may lead to changes in blood flow.[7]

Reflectance Spectrophotometry Method

This method involves the use of a computer-equipped reflectance spectrophotometer to measure an index of mucosal hemoglobin concentration which is related to blood volume.[110-112] Reflectance spectrophotometry involves the sequential collection of spectra from the mucosa at many points, its storage in a computer, the expression of the spectral intensity (absorbance and partly, scattering) as a function of the local hemoglobin concentration and hence, mucosal blood volume. The spectrophotometer is coupled to the mucosa by an optic guide which has two coaxial fiber optic bundles which carry monochromatic light (1.0 nm half-spectral bandwidth) to it and a central bundle which collects the reflected light. The computer is programmed to subtract the spectrum obtained from a reference standard or under standard conditions from each reflected spectrum.[112] The method also provides an index of oxygen saturation of hemoglobin from the spectral analyses of the reflectance spectra. Hence the method has been a valuable tool in assessing tissue hemoglobin oxygenation levels in studies of ischemic conditions in animals and humans.[111,113-116] Blood volume is expressed in arbitrary units.

Attempts to validate reflectance spectrophotometry in simultaneous measurements of gastroduodenal mucosal perfusion, during vagus nerve stimulation, with LDF and HGC revealed no changes in blood flow by the technique, i.e., both indices of hemoglobin concentration and hemoglobin oxygen saturation did not change; whereas laser-Doppler flowmetry and hydrogen gas clearance showed expected increases of comparable magnitude in GMBF.[116] The investigators also reported that during hemorrhagic shock, there was good

correlation between measurements of GMBF by HGC and the corresponding values obtained simultaneously by reflectance spectrophotometry. They attributed the lack of response of reflectance spectrophotometry to possible interference from laser light. The method has been used to measure mucosal hemodynamics mainly in the stomach and colon, and has been applied endoscopically in human subjects.[113,114,116-119]

Advantages are: it is relatively non-invasive; it offers focal mucosal measurements and can be applied clinically. The technique is fast, the whole procedure along with endoscopy is completed in about 20 minutes.[113,114] A major disadvantage with this method is that it has not been validated against most of the previously existing blood flow measurement techniques, like the radioactive microspheres, in the gastrointestinal tract. Another disadvantage is that it relates static tissue hemoglobin concentration to blood volume and does not actually measure blood flow. The optic guide can compress the tissue during measurement reducing the tissue hemoglobin concentration.

Laser-Doppler Flowmetry

Details of the history of this technique have been published.[120,121] Laser-Doppler flowmetry (LDF) or laser-Doppler velocimetry as it is also called, is a common technique for measuring the velocity of fluid flow in industrial research but its use for blood flow measurements was first presented by Riva et al.,[122] who used the technique to measure red blood cell velocity in single rabbit retinal vessels. Stern[123] was however, the first to demonstrate its potential for use clinically. He used the method to measure skin blood flow in human finger tips after inflating a blood pressure cuff around the upper arm and during the intake of alcohol. A fiber optic laser-Doppler blood flowmeter for clinical use based on the laser-Doppler principle was later presented by Watkins and Holloway.[124] The clinical use of the method had been limited either by the size of the necessary equipment[125] or because of mode competition and laser wide-band beam amplitude noise.[126] Recently, the development of a new instrument in which the

latter problems are suppressed[127,128] has provided the impetus for studies with this method.

Laser-Doppler flowmetry[120,127-131] is based on the principle that laser light illuminating a perfused tissue is scattered by both the static tissues and the moving red blood cells. However, only the laser light scattered by moving red blood cells undergo a shift in frequency according to the Doppler effect, which is proportional to the average velocity of the red blood cells. The back scattered light from the tissue is carried to a detector system where it is processed by electronic circuits (analog analyses) to give a stable, continuous signal linearly related to the tissue perfusion, defined as the average blood cell velocity multiplied by the blood cell concentration within the scattering volume of tissue. The laser light to and from the tissue is conducted via flexible fiber optic guides. Spacing between the transmitting and receiving fibers within the optic probe is in part responsible for determining the measurement volume of LDF.[131] Although still a subject of controversy, the measurement depth of LDF is believed to be a volume of tissue of approximately 1 mm diameter in most tissues.[120,127,128,130] Blood flow is expressed in arbitrary units, volts.

Laser-Doppler flowmetry was first applied in the gastrointestinal tract by Shepherd and Riedel[132] to measure intestinal blood flow in dogs. Subsequently, more reports have appeared in the literature on the use and validation of this technique in intestinal blood flow measurements in animals[66,132-135] and humans[136] with good results. Similarly, the technique has been used and validated in GMBF measurements in animals[65,134,137] and humans[91,138,139] with good results. Although endoscopic measurements of human GMBF by LDF[139,140] have been reported, consecutive endoscopic measurements of focal mucosal blood flow by LDF and HGC (for comparison) in human subjects were unsuccessful due to difficulties in maintaining stable contact between the HGC electrode and the mucosa.[91] In addition, LDF has been applied to microcirculatory blood flow measurements in other tissues and in different animals which include, human skin, testis of rat, skeletal muscle and bone in pigs, kidneys of rats and the brain of rhesus monkeys.[120] Use of LDF in cutaneous blood flow measurements has

been reviewed recently.[141] LDF is now being used in plastic and reconstructive surgery as a routine method for the postoperative surveillance of free flaps.[142,143]

The advantages of LDF are: it is noninvasive; it offers instantaneous and continuous measurements of focal blood flow. The flowmeter is easy to use and transport. The flow values obtained by the method are reproducible and the technique is applicable clinically through the endoscope. Furthermore, it is not affected by the countercurrent exchange mechanism in the small intestine. The main limitations of the technique include: (i) sensitivity to motion artifacts; merely placing the probe on the mucosal surface gives erratic readings with movement of the probe or contraction of the gastrointestinal wall.[144] Also due to the inherent motility of the gastrointestinal tract, optical coupling may be lost during measurements especially in laboratory experiments. Hence the recommendation that the optic probe be used in light contact with the tissue being assessed;[139] (ii) lack of calibration in absolute blood flow units; LDF does not give blood flow values in absolute flow units. Efforts to calibrate the LDF signal in absolute flow units have not met with any success;[97,129,144] (iii) spatial resolution of the technique; this issue has generated a lot of controversy in the recent past following a report in which reactive hyperemia in the intestinal mucosa was not simultaneously picked up by a serosa-muscularis LDF probe. It was concluded that LDF only measured blood flow in the superficial layer of the mucosa.[132,145] However, more recent reports have disputed this claim and suggested a contrary view, that LDF measures blood flow across the entire thickness of the gastrointestinal wall, corresponding to a depth of at least 6 mm.[66,134,136,144,146] The differences observed have been attributed in part to differences in the instrument types available commercially.[66,97] Tissue optical properties (both scattering and absorption) and the fiber separation within the optic probe theoretically affect the depth of tissue sampled by the laser beam of light.[131] More recently however, Johansson et al,[147] using isolated segments of feline small intestine, have shown that the measuring depth of LDF in the gastrointestinal tract is highly dependent on the fiber diameter and geometry of the measuring probe. They

suggested that taking these two factors into consideration, it may be possible to design probes that are suitable for superficial and intramural measurements. There is a need to further define and resolve the measurement depth of the different commercially available laser-Doppler flowmeters. Lastly, the laser-Doppler optic probe may compress the tissue and alter local blood flow.[69]

CONCLUSION - THE PROBLEM

The last decade has witnessed a significant increase in the number of techniques used for GMBF measurements. Most of these techniques have also been applied in the measurement of intestinal blood flow. Notably, there has been a progressive shift from invasive to noninvasive techniques. Methods capable of focal, instantaneous and continuous blood flow measurements, those with the capability or potential for clinical application and those with the additional advantage of endoscopic clinical application have all emerged. Most of the currently used techniques show varied experimental and clinical objectives, and limitations, so that virtually every method presented may be the method of choice under some given set of experimental or clinical conditions.[7] This leaves the investigator with an onerous task of selecting the most appropriate technique for his study. Although progress has been made, there are unresolved problems, theoretical considerations to be further illuminated, and an ideal blood flow-measurement method as proposed by Jacobson[6] has remained elusive. The method of the clearance markers first reported about three decades ago offers only total gastric mucosal blood flow values, does not accurately reflect mucosal perfusion in the absence of active gastric acid secretion and during mucosal damage. The radioactive microsphere technique, although it is more accurate than the clearance techniques and offers regional blood flow values, has absolutely no clinical potential. The heat clearance technique offers focal GMBF measurements but the flow values are qualitative; and gastric contractions, the suction required to stabilize the probe to the mucosa and the probe itself interfere with the recordings and blood flow. Other

investigators have reported their experience with the use of the washout of the radioactive inert gases, ^{85}Kr and ^{133}Xe, to measure gastrointestinal mucosal blood flow. However, the complex curve stripping procedure of the multiexponential curves, radioactivity, and the expense of the technique has not credited it with wide usage. The iodoantipyrine clearance technique, although has been used in humans and is capable of offering total organ, intramural and focal mucosal blood flow values, it cannot measure blood flow continuously, requires the administration of radioactive material and biopsy specimens in humans. Blood flow values obtained by the method are also inaccurate in the presence of luminal contents in the gastrointestinal tract. The reflectance spectrophotometry method developed for the measurements of human gastric mucosal blood volume through the endoscope, only gives qualitative values of the static hemoglobin concentration and requires the use of expensive equipment. Hydrogen gas clearance on the other hand is cheap, offers focal mucosal blood flow values and is potentially clinically applicable through the endoscope; however, it cannot give instantaneous and continuous blood flow values, requires that the blood flow remains constant throughout the entire measurement period, requires up to 30 minutes to make one measurement and has a small unstable signal when performed with 3% H_2. Laser-Doppler flowmetry, although measures focal GMBF instantaneously and continuously, has problems of spatial resolution, sensitivity to movement artifacts and lack of calibration in absolute flow units. Despite their limitations, however, HGC and LDF hold great promise because they satisfy more of the criteria for an ideal blood flow measurement method proposed by Jacobson. Also, they are noninvasive and safe for clinical application through the endoscope. These techniques may eventually approach the ideal, but no matter how much promise they wield, their success and future will depend on the ultimate resolution of their present limitations. This can be achieved only through further evaluation and refinement of the methods.

OBJECTIVES

This study was therefore designed to address some of the limitations of HGC and LDF, and apply the techniques to study the role of focal mucosal blood flow in gastric pathophysiology. The primary objectives were:

1) **Techniques** - Compare, refine and further evaluate HGC and LDF:
   a) Calibrate the LDF signal to absolute flow units by simultaneously measuring GMBF by LDF and HGC at the same focal mucosal site.
   b) Improve the magnitude and quality of the HGC signal in the measurement of focal GMBF with 3% hydrogen gas.
   c) Decrease the time required to obtain HGC measurements.

2) **Applications** - Apply HGC and LDF to study:
   a) The response of focal GMBF to prostaglandins.
   b) The role of focal GMBF in drug-induced acute erosive gastritis by aspirin.
   c) The role of focal GMBF in prostaglandin cytoprotection against aspirin-induced mucosal injury.

To achieve these objectives, the experiments reported in the following chapters were performed. The experiments were not necessarily performed in the sequence reported. Experiments that address the objectives related to "techniques" are reported in the early chapters (Chapters 3 & 4) followed by those adressing the "applications" (Chapters 5, 6 & 7) of the techniques.

EXPERIMENTAL MODELS

In selecting experimental models for achieving the objectives of this study, prime consideration was given to the fact that the ultimate goals are the extrapolation of the results to the human

situation and the safe application of LDF and HGC in humans. The experiments in this study were performed using two experimental models:

1) **Canine Chambered Gastric Segment Model.** Availability, relative ease of handling, and size which permits studies similar to those performed in man, are the main reasons for selecting the dog for splanchnic blood flow work.[148] Hence, the canine chambered segment model of the gastric corpus with an isolated vascular pedicle preparation of Moody and Durbin [23,149] was used for this study.

Surgical preparation: Some aspects of the surgical preparation of this model described below have been modified for convenience. Briefly, under pentobarbital anesthesia, a midline laparotomy is performed. The spleen is mobilized and a total splenectomy is performed using the classical technique for dogs described by Crane.[150] This involves the ligation and division of each of the short perpendicular branches of the splenic artery that enter the body of the spleen at the hilus (Figure 1: A & B). One of the advantages of using this technique is that it preserves the short gastric arteries and veins which will ultimately provide the vascular pedicle for the wedge of the gastric corpus that will be isolated for the chambered gastric segment.

Next, the stomach is mobilized by: a) dividing the omentum; b) ligating and dividing the right gastric and right gastroepiploic vessels; and c) the left gastric vessels medial to the upper end of the lesser curvature. Demarcate a wedge of the gastric corpus, along the greater curvature, supplied by the short gastric branches of the splenic artery (Figure 1B). In demarcating the wedge, a suitable vascular pedicle, i.e., an artery and vein supplying and draining it from the splenic artery and to the splenic vein respectively, should be identified. Two de Petz clamps are placed across the stomach at the distal margin of the demarcated wedge of the corpus. The stomach is divided between the clamps with a scalpel. The gastric antrum is usually removed to reduce possible hormonal effects on secretion from the gastric segment.[149] The crushed duodenal stump is transected with a scalpel just distal to the clamp

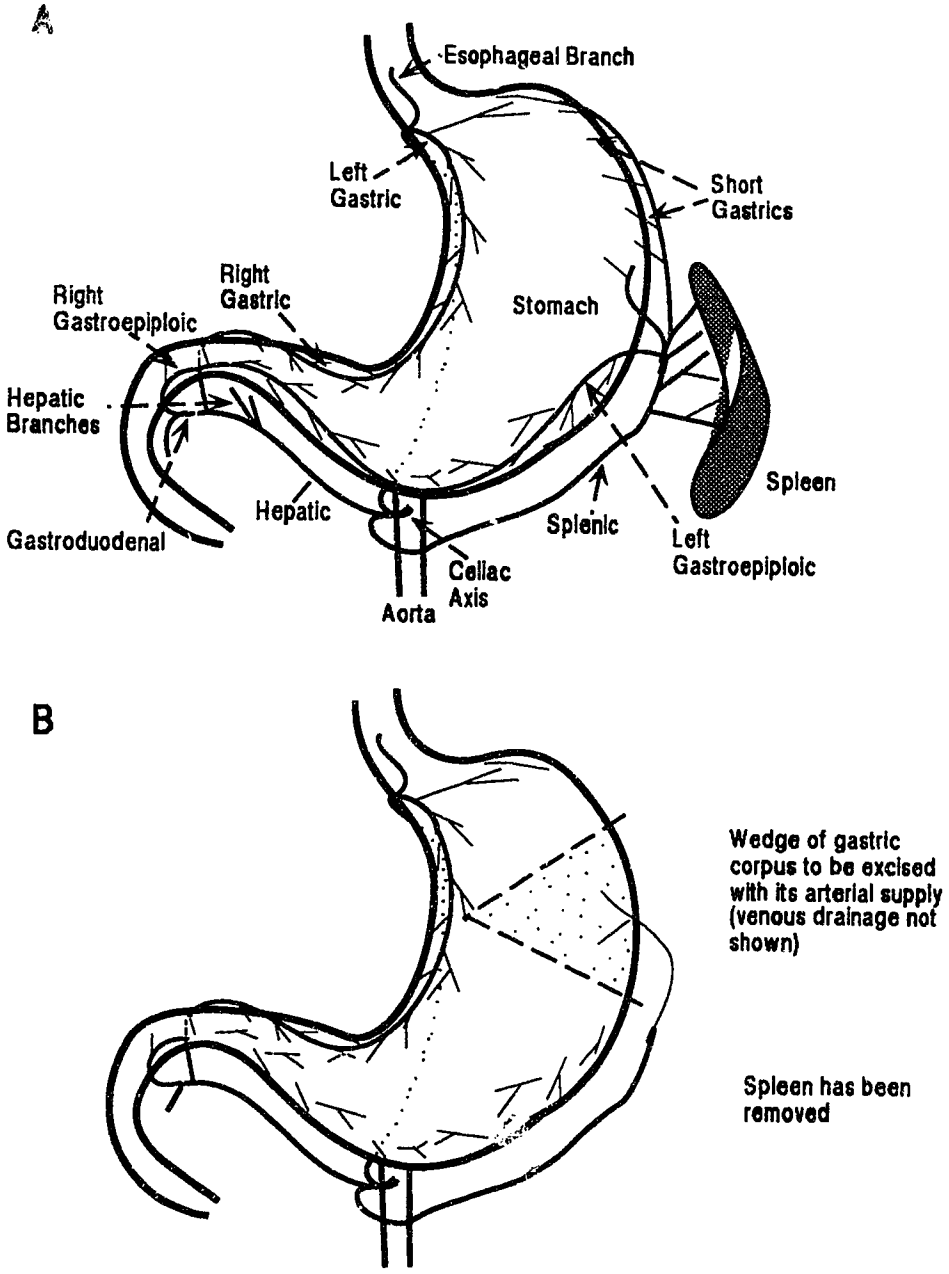

Figure I-1. **A:** Schematic diagram of the main arterial blood supply of the stomach and spleen of the dog from the celiac axis and the abdominal aorta. Venous blood supply is not shown. **B:** Stomach of the dog after removal of the spleen with the demarcated wedge of the gastric corpus and its isolated arterial supply.

and hemostasis is secured. The duodenal stump is closed using a continuous inverting 2-layer Cornell catgut suture and several interrupted silk Lembert sutures as described by Lawrence.[151] Two de Petz clamps are then placed across the stomach at the proximal margin of the wedge of the corpus (Figure 1B). The stomach is transected between the clamps with a scalpel. The crushed edge of the proximal gastric stump is excised and hemostasis is secured. An open suture closure is employed as described above for the distal duodenal stump.

Use of the stapling technique is a faster alternative. This can be performed with minor modifications according to the method for partial gastrectomy in dogs described by Belleger and Archibald.[152] Briefly, ligate and divide the vessels in the greater omentum and the fat using the LDS. Place a TA90 across the stomach just distal to the distal margin of the demarcated wedge of the corpus. The pin is tightened and the staples inserted. A crush clamp (de Petz) is then placed across the stomach proximal to the TA90 and the stomach between them is transected using the TA90 as a guide. The latter is then removed. Depending on the size of the canine distal stomach, a TA55 stapler may be used. Similarly, place a TA90 stapler across the stomach just proximal to the proximal margin of the demarcated wedge of the corpus. Tighten the pin and insert the staples. Place a crush clamp across the stomach distal to the TA90 and transect the stomach between them using the latter as a guide. The TA90 is then removed.

The excised gastric segment is brought out of the abdominal cavity, opened and sandwiched between a lucite ring (Figure 2C) on its serosal side and a lucite chamber (Figures 2A, 2B & 3A) on its mucosal side. The chamber model has two variants, single- and double-lumen (Figure 2: A & B) types, both of which were used in this study. The single-lumen chamber provides a mucosal surface of approximately 36 cm^2. The chamber has input and output ports for the instillation and recovery of fluids, drugs, etc. In the case of the double-lumen chamber, each side is supplied by a separate vascular pedicle, equipped with an input and output port, and the two mucosal surfaces are approximately 17 cm^2 each. The surgical preparation of this variant of the chambered segment model is the same as for the single-lumen chamber except

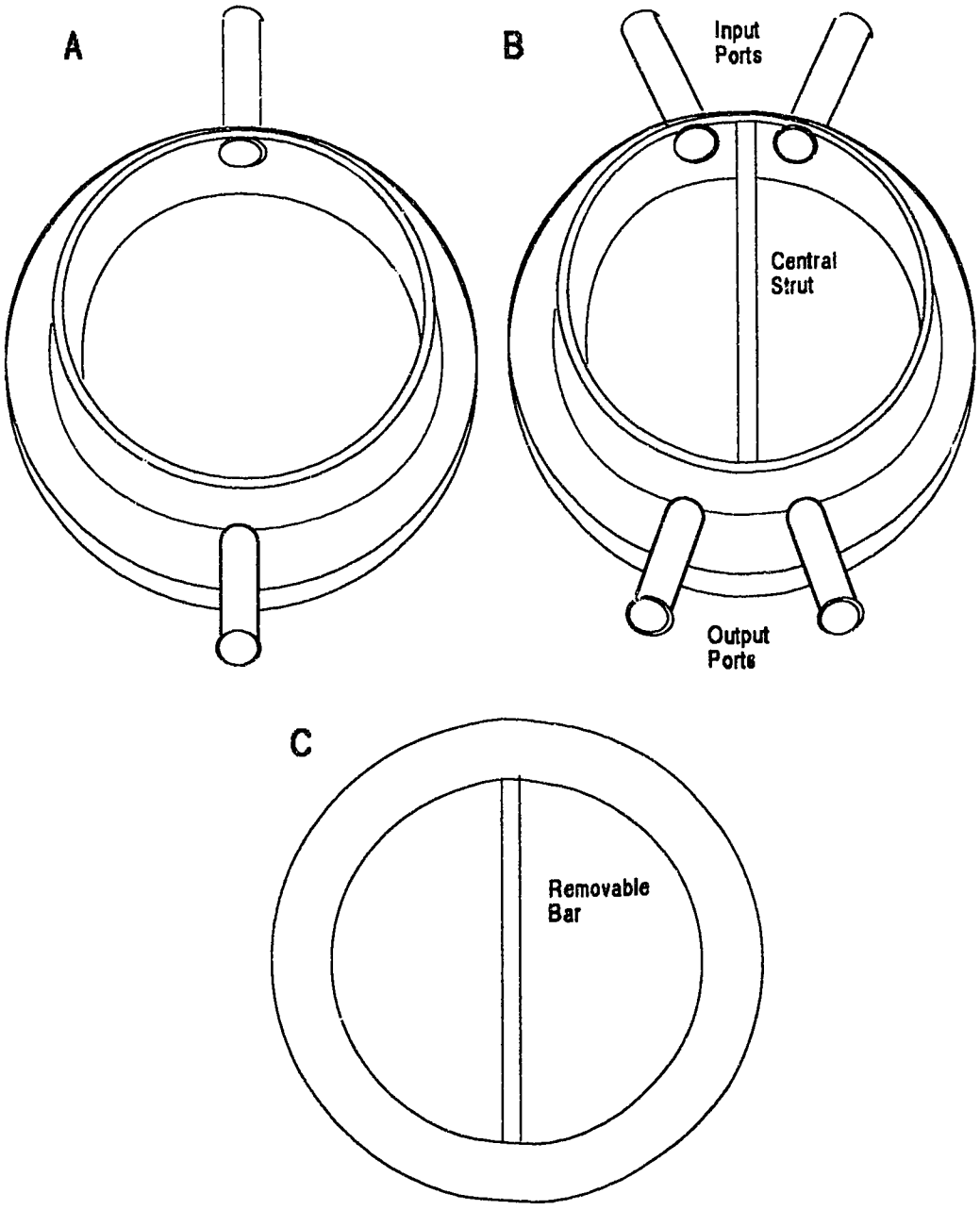

Figure I-2. Diagram of the single-lumen (A) and double-lumen (B) transparent lucite chambers used for mounting the excised canine gastric segment. The transparent lucite ring (C) with its central bar which is removed when used with the single-lumen chamber is also shown.

in choosing a vascular pedicle for the wedge of gastric corpus, a separate artery (which arises from the splenic artery) with its corresponding vein are selected for each half of the chamber; and a central strut divides the chamber into two equal halves. The chambered segment is then

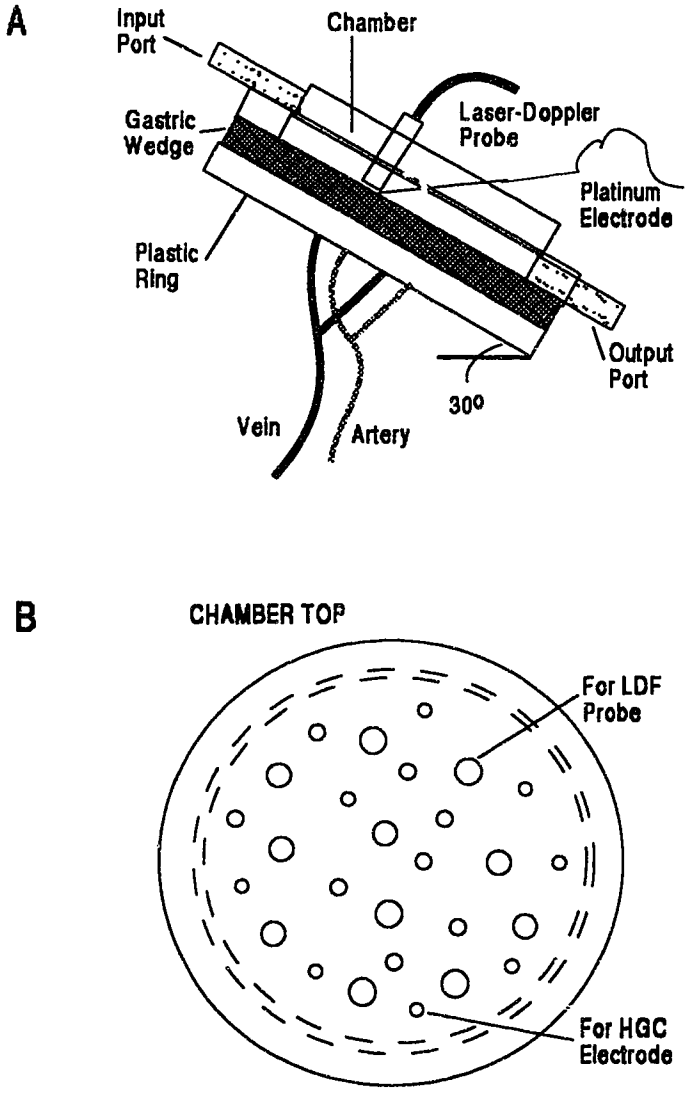

Figure I-3. **A:** Schematic of the chambered gastric segment with its vascular pedicle illustrating its angulation with the horizontal to allow gravity drainage of chamber contents. **B:** The detachable and tranparent lucite top of the chamber.

secured over the animal's anterior abdominal wall with clamps at a fixed angle of between 30-45^{o} and with the mucosal surface facing up (Figure 3A). This allows the instillation and recovery, by gravitational drainage, of mucosal bathing solutions with a high degree of accuracy. The transparent top cover of the lucite chamber was modified so that it is detachable and has holes made in it to allow the LDF and HGC measuring probes to fit snugly and remain anchored onto the mucosa without any movement throughout the experiments (Figure 3B). The surgical preparation of this experimental model along with the insertion of the arterial and venous catheters takes about 3 hours. Use of the stapling technique cuts down the time to about 2 hours.

The advantages of this experimental model are: (i) It permits direct access and visualization of the mucosa (through the transparent lucite) throughout the experiment. This allows direct observation and positioning of the measuring probes and assessment of gross morphological mucosal damage and/or changes. (ii) By allowing the instillation and recovery of mucosal bathing solutions into and out of the gastric chamber, ionic fluxes can be simultaneously studied with mucosal blood flow, drugs can be administered topically, and gastric ionic fluxes can be used as a quantitative index of mucosal damage[153]; and (iii) the double-lumen variant allows the use of one side of the chamber as the test side and the other as control (Figure 2B).

2) **Rat.** In one experiment (Chapter 4), measurement of GMBF was performed in the intact stomach of anesthetized rats. Details of the surgical procedure are included in the section on methodology in that chapter. This became necessary at the stages of this study when the issue of an improved HGC signal using 3% hydrogen gas (concentrations of over 4% hydrogen gas with air are potentially explosive) was addressed. With 3% hydrogen gas, the HGC signal in this species is relatively smallest and has a low signal-to-noise ratio. An improved quality of the small and unstable HGC signal in the rat will facilitate application of the technique in the

human subject where a similarly unstable signal with significant baseline drifts has been obtained in preliminary endoscopic application in our laboratory.[96]

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Chapter 2

Preliminary In Vitro Calibration of the Hydrogen Gas Clearance Electrodes

INTRODUCTION

The hydrogen gas clearance (HGC) electrodes used in the experiments reported in the following chapters were commercially obtained. Hence, these calibration experiments were performed in the manner described by Aukland et al[1] to: (a) obtain a % H_2 concentration-current plot that ensures that the electrodes respond linearly to increasing concentrations of H_2; and (b) determine the voltage range in which the electrode current is diffusion-limited.

MATERIALS AND METHODS

1. **Circuit:** This consisted of a platinum electrode (Unique Medical Co. Ltd, Tokyo, Japan) and a reference Ag/AgCl electrode (Red Dot, 3M Canada Inc., London, Ontario, Canada) whose tips were submerged in stirred, deoxygenated HCl, pH 1.0 or 0.3M phosphate buffer, pH 7.4, inside an air tight test chamber (Figure 1A). The platinum electrode was connected to a 615 Digital Electrometer multimeter (Keithley Instruments Inc., Cleveland, Ohio) which is capable of measuring voltage, current, charge and resistance. The Digital Electrometer was then connected to one of the terminals (terminal 3) of a micropotentiometer (Borg Micropot Potentiometer, Model 205, The George W. Borg Corp., Janesville, Wisconsin). A mercury dry cell (1.35 V) was connected across terminals 1 and 2 of the potentiometer. The circuit was completed by connecting the Ag/AgCl electrode to terminal 2 of the potentiometer. By adjusting the potentiometer and using the voltmeter of the Digital Electrometer multimeter, varying known voltages could be

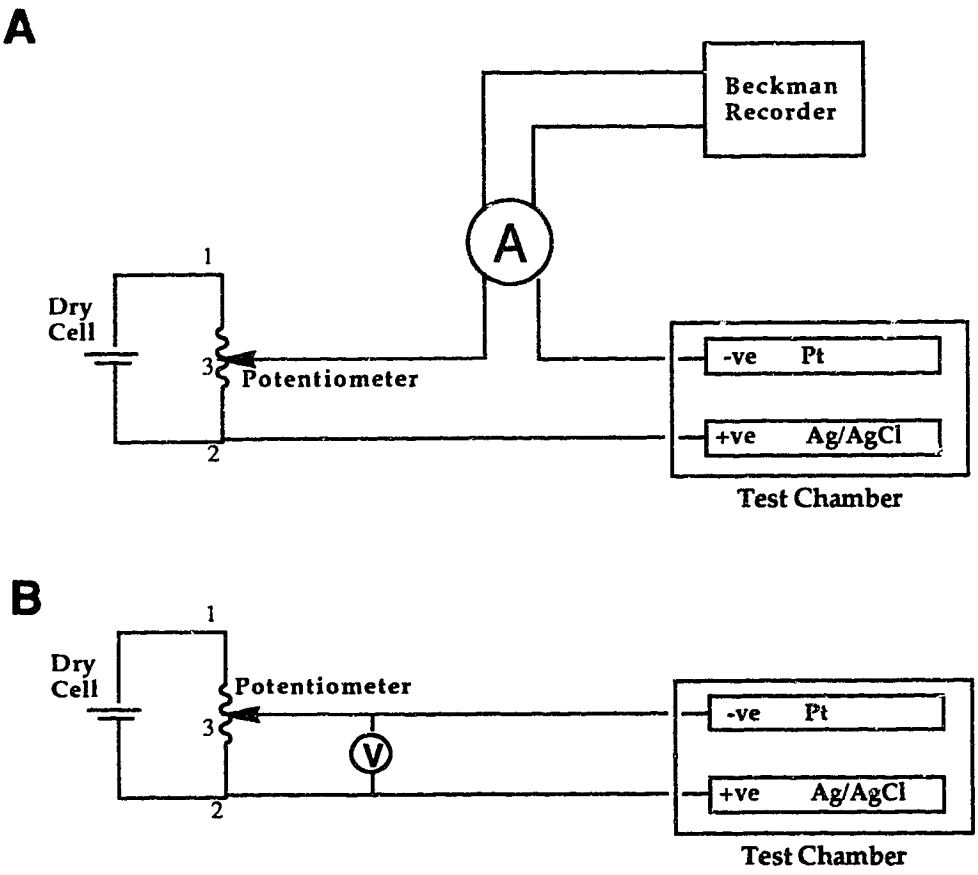

Figure II-1. Schematics of the calibration setup used for testing linearity of the response of the platinum electrodes to increasing concentrations of hydrogen gas, and for determining the voltage range in which electrode current is diffusion-limited. Diagram (A) was the setup used for measuring the current generated on the surface of the platinum electrode; (B) was used for applying different polarization voltages to the platinum electrode by adjusting the resistance of the potentiometer (rheostat). A = Ammeter; 1,2,3 - are the three terminals of the potentiometer; Pt = platinum electrode; Ag/AgCl = the silver/silver chloride electrode; V = Voltmeter.

applied to the platinum electrode (Figure 1B). The output of the Digital Electrometer was connected to a Beckman recorder (Type R Dynograph, Beckman Instruments Inc., Schiller Park, Illinois) to obtain a simultaneous permanent analog record of the digital records of the current measured by the ammeter of the Electrometer.

2. **The Chamber:** The test chamber was designed by me and made by the Glass Blowing shop (Technical Services) of the University of Alberta. A 300 ml capacity, angled, 3-neck, distilling flask (Pyrex Brand, Corning; Fisher Scientific) was modified by: (a) flattening the bottom to enable the flask to sit on a stirrer; (b) making two longitudinal dents each on the front and back of the bulb of the flask; and (c) shortening the joints of the flask to facilitate the immersion of the platinum and reference electrodes inside the chamber solution.

3. **Platinum Electrode:** The commercially obtained platinum electrode (Unique Medical Co. Ltd, Tokyo, Japan) was made from a 0.3 mm diameter epoxy-coated platinum wire. It is 50 mm long with a tapered 1 mm exposed tip which is platinized with platinum black. To the other end of the electrode is attached a 300 mm length vinyl-coated copper wire to facilitate connection to a recording system.

In Vitro Tests of the Electrodes

(i) **Electrode Current Versus H_2 Concentration:** Known amounts of H_2-equilibrated (100%) HCl, pH 1.0, were added to the test chamber containing stirred, deoxygenated HCl solution, pH 1.0, to obtain increasing concentrations (2%, 4%, 6%, 8%, and 10%) of H_2-equilibrated solutions inside the chamber. The HCl solution inside the chamber was deoxygenated by bubbling nitrogen gas into it via a polyethylene tubing for 15 min. The electrode current recorded by the digital ammeter was noted for each % H_2 concentration of the chamber solution. The experiment was performed during platinum electrode polarization with different voltages: 0.1, 0.2, 0.3, 0.4, and 0.5 volts (Figure 1B). The experiment was repeated with the chamber filled with stirred, deoxygenated 0.3M phosphate buffer, pH 7.4, and electrode polarization voltage of 0.1 volts.

(ii) **Current-Voltage Response:** The voltage range in which the electrode current produced by a given concentration of H_2 is independent of the electrode polarization voltage was determined. The test chamber was filled with stirred, deoxygenated HCl solution, pH 1.0. Known amounts of 100% H_2-equilibrated HCl solutions, pH 1.0, were added to the chamber to give different H_2-concentrated solutions (3%, 4%, and 5%) inside the chamber. Platinum electrode polarization voltage was varied by adjusting the potentiometer (Figure 1B). Each time, the corresponding electrode current was measured by the digital ammeter. To apply a negative polarization voltage to the platinum electrode, the mercury dry cell terminals on the potentiometer were reversed.

A water stirrer was used in these experiments to reduce the electrical interference from the motor of an electric stirrer. As reported previously, stirring increased the magnitude of the electrode current[1].

Data Analysis

All electrode current values are expressed as means. Linear regression analysis of electrode current versus % H_2 concentration was performed for the mean of the values obtained for each electrode polarization voltage for HCl and 0.3M phosphate buffer. A plot of electrode current versus polarization voltage was made to visually illustrate the current-voltage plateau for each % H_2 concentration within the test chamber. Data analysis for statistical significance at the 5% level was performed by simple linear regression analysis.

RESULTS

Each time H_2 was added to the test chamber throughout these experiments, a negative electrode current was observed.

Electrode Current versus H_2 Concentration

There was a highly significant linear correlation between electrode current and % H_2 concentration (Table 1; Figures 2 & 3) for each electrode polarization voltage during the presence of HCl and 0.3M phosphate buffer ($r = 0.99$, and $p < 0.01$ in each case) inside the chamber.

The slopes of the regression lines obtained for the plots of current versus % H_2 concentration during the presence of HCl in the test chamber increased with electrode polarization voltage (Table 1 & Figure 2) from 0.1 to 0.4 volts (slopes: 1.63, 1.70, 3.30, and 4.99 respectively). This finding is in agreement with the findings of Young,[2] who reported an increase in linear slope values with polarization voltage in in vitro studies.

Table II-1. RESULTS OF REGRESSION ANALYSES OF ELECTRODE CURRENT VERSUS % H_2 CONCENTRATION FOR DIFFERENT POLARIZATION VOLTAGES

HCl, pH 1.0				
POLARIZATION VOLTAGE (volts)	r	p-VALUE	INTERCEPT	SLOPE
0.1	0.9925	<0.003	0.1000	1.6250
0.2	0.9985	0.009	4.5000	1.7000
0.3	0.9980	<0.002	0.6000	3.3000
0.4	0.9960	0.002	3.0410	4.9935
0.5	0.9980	<0.002	0.4000	3.4000
0.3M PHOSPHATE BUFFER, pH 7.4				
0.1	0.9995	<0.001	-0.4730	3.9875

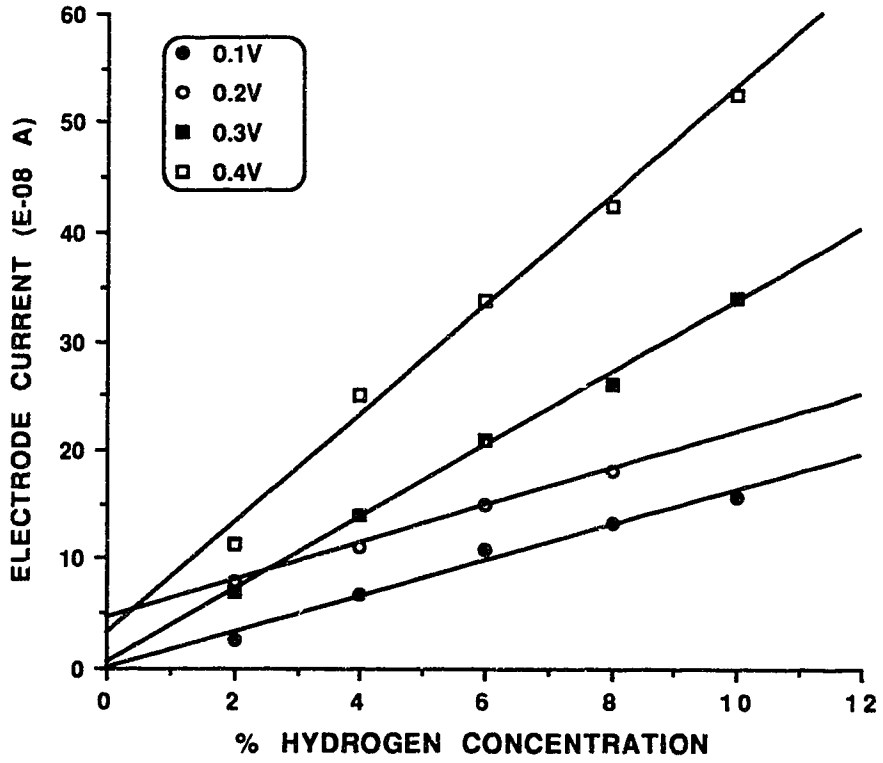

Figure II-2. Regression lines of electrode current versus % H_2 concentration during the presence of HCl, pH 1.0, inside the test chamber and application of increasing polarization voltage. There was a highly significant linear correlation for each polarization voltage ($r = 0.99$; $p < 0.01$ in each case) and the slopes of the regression lines increased with applied voltage. V = volts.

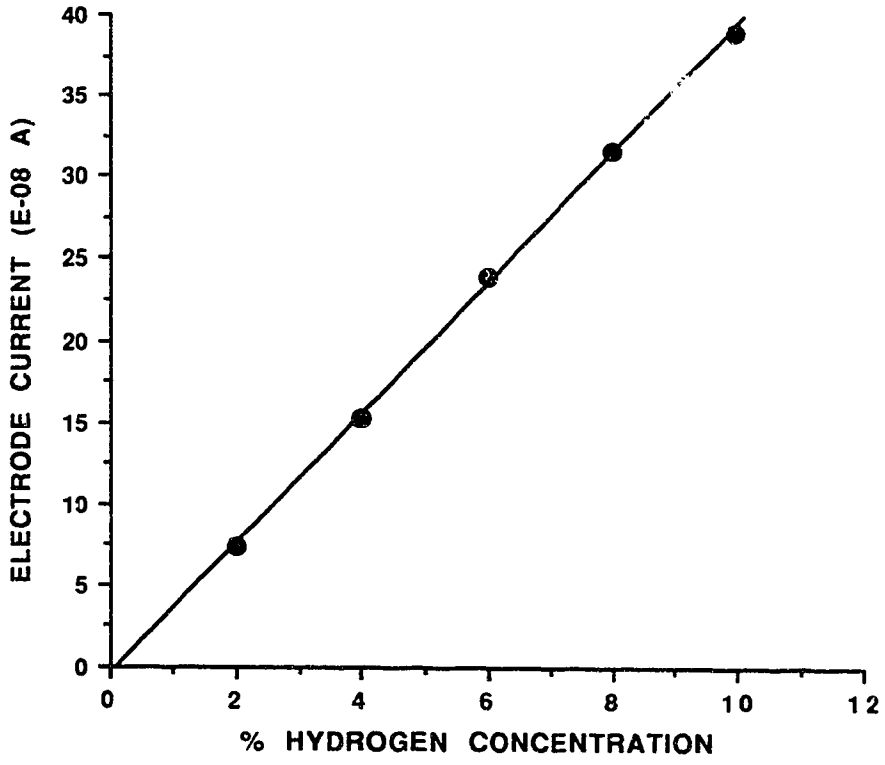

Figure II-3. Regression line of electrode current versus % H_2 concentration during the presence of 0.3M phosphate buffer, pH 7.4, inside the test chamber and application of a polarization voltage of 0.1 volt. There was a highly significant linear correlation ($r = 0.99$; $p < 0.001$).

Current-Voltage Response

There was an increase in the magnitude of the negative electrode current obtained with increase in polarization voltage from -0.3 to +0.0 volts. From +0.1 volts, the response reached a plateau and subsequent increase in the polarization voltage did not result in an increase in the magnitude of the electrode current. These findings were consistent for 3%, 4%, and 5% H_2-equilibrated 0.3M phosphate buffer and 5% H_2-equilibrated HCl solutions (Figures 4 & 5).

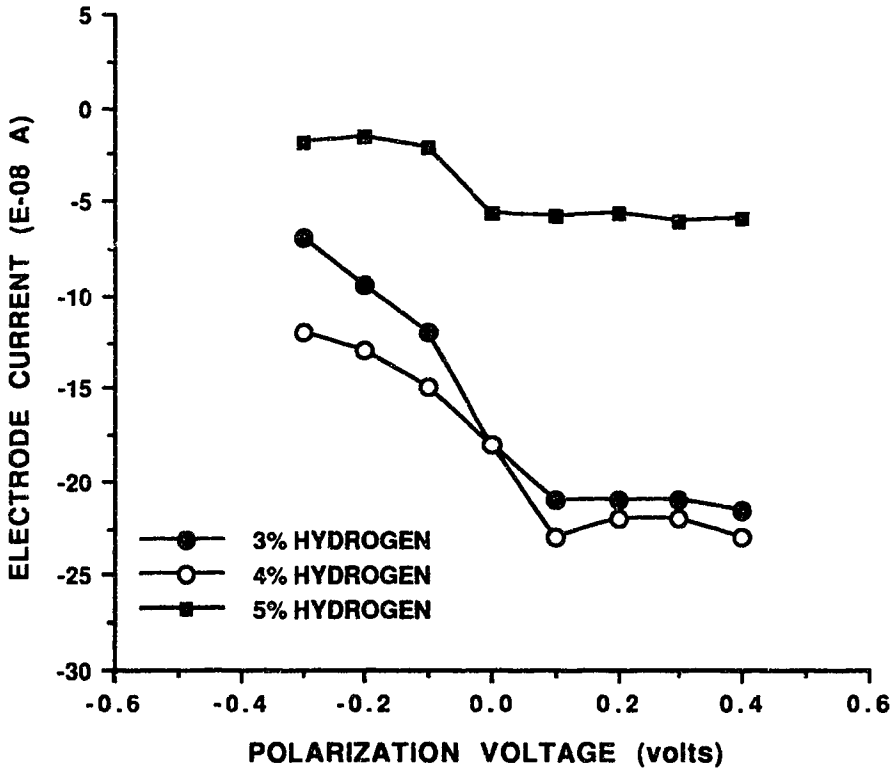

Figure II-4. Electrode current-polarization voltage plots of the response of the platinum electrodes to different concentrations of hydrogen gas. Between +0.1 to +0.4 volts, the electrode current plateaued, i.e., was diffusion-limited, for 3, 4 and 5% hydrogen gas concentrations in the test chamber containing 0.3M phosphate buffer, pH 7.4. The solution in the test chamber was deoxygenated by bubbling pure nitrogen gas into it for 15 min.

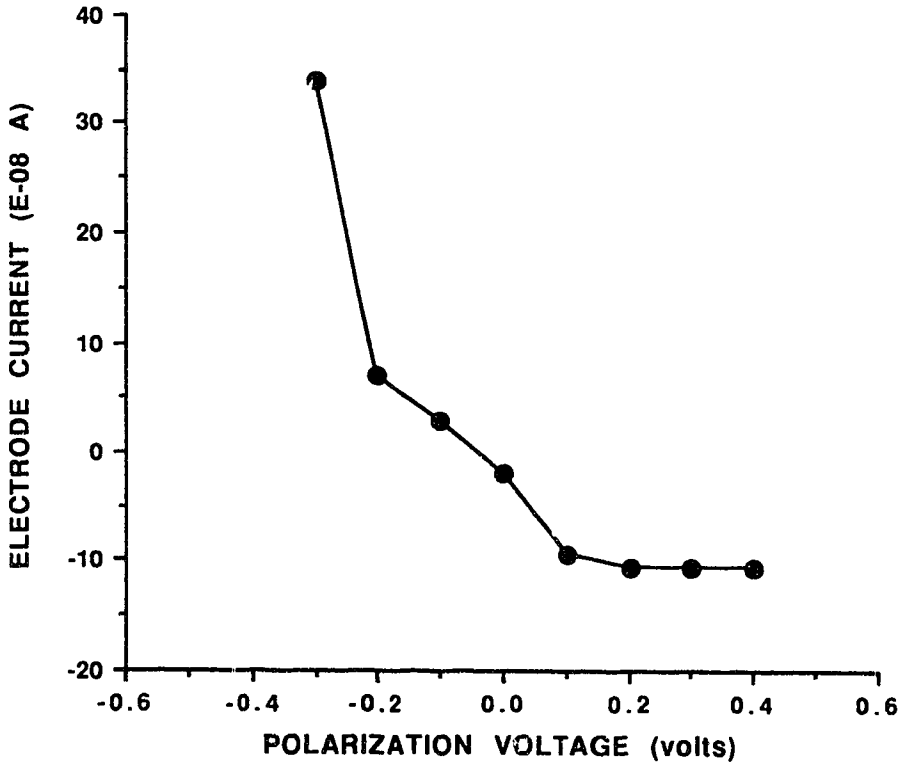

Figure II-5. Electrode current-polarization voltage plot of the response of the platinum electrodes to 5% hydrogen gas. Similarly, between +0.1 to +0.4 volts, the electrode current plateaued, i.e., was diffusion-limited, for 5% hydrogen gas in the test chamber containing HCl, pH 1.0. The solution in the test chamber was deoxygenated by bubbling pure nitrogen gas into it for 15 min.

DISCUSSION

Electrode Current versus H_2 Concentration

One of the basic assumptions of the HGC technique is that the current generated on the surface of a platinum electrode is linearly correlated with H_2 concentration.[1,3] Although the platinum electrodes used in this study were commercially obtained, it was necessary to ascertain the linearity of the responses of these electrodes to increasing concentrations of H_2. Furthermore, since the sets of studies reported in the following chapters, using these electrodes, were performed in the stomach with its unique pH, it was necessary to determine the influence of HCl, pH 1.0, and 0.3M phosphate buffer, pH 7.4, which respectively simulate the normal pH in the gastric lumen and interstitial fluid, on this important electrode response. The above results show that these commercially available electrodes respond linearly to increasing H_2 concentration and do so at the extremes of pH that may be encountered in body tissues, particularly in the gastrointestinal tract.

The reason for the higher slope of the regression line obtained with 0.3M phosphate buffer (pH 7.4) in the test chamber compared to that obtained with HCl (pH 1.0) is not known. However, results of previous in vivo and in vitro studies show that different luminal pH values (pH range 2-8) have no effect on the current generated on the surface of the platinum electrode in the stomach.[4,5]

Current-Voltage Response

The need to use a platinum electrode to make measurements of GMBF at polarization voltages in the diffusion-limited range (the plateau region of the current-voltage plot) is because in that range, hydrogen gas concentration is directly proportional to electrode current and is independent of the potential difference between the platinum electrode and the reference electrode. Simply, the electrode current is dependent on the rate of diffusion of hydrogen gas in the solution to the electrode surface.[2] Electrode polarization is also used to decrease the

sensitivity of the platinum electrode to oxygen and other reducing agents like ascorbic acid. However, the polarization voltage of an electrode also determines in part the intensity of the chemical reaction that occurs on its surface and the time taken to reach steady state current response.[2,6] Thus, knowledge of the voltage range in which an electrode current is diffusion-limited is necessary to determine the polarization voltage to apply to that electrode. The results obtained in this experiment reveal that the electrode current of these commercially available platinum electrodes is diffusion-limited in the voltage range +0.1 to +0.4 volts. Since previous studies have reported that the time required to reach a steady state current response increases with polarization voltage,[2] a polarization voltage of +0.1 volts may be the most ideal for these electrodes. This may allow for a shorter HGC measurement time.

The negative current recorded in the test chamber during the application of a negative polarization voltage to the platinum electrode is unlikely to be due to the reduction of oxygen since the air tight chamber fluid was deoxygenated at the onset of the experiments and secondly, the presence of oxygen in solution tends to generate a positive rather than negative current at the surface of the platinum electrode. In the in vivo situation, the effect of oxygen on the platinum electrode is expected to be minimal if the electrode is operated with a polarization voltage in the range +0.1 to +0.4 volts. Electrolytic reduction of oxygen gas on the surface of the platinum electrode occurs at negative electrode polarization voltage (about -0.2 volts), the current-voltage plateau occuring at more negative polarization voltage (-0.6 to -0.8 volts).[7] Furthermore, any current generated by oxygen gas on the surface of the platinum electrode can be attenuated or suppressed by zeroing the pen of the bioelectric recorder before the administration of hydrogen gas to the subject.

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Chapter 3

Focal Gastric Mucosal Blood Flow by Laser-Doppler and Hydrogen Gas Clearance: A Comparative Study[†]

INTRODUCTION

The increasing importance of focal gastrointestinal microcirculation in health and disease has prompted the need to develop techniques capable of focal gastrointestinal blood flow measurements. Amongst the newest techniques that are being developed and evaluated in the measurement of focal gastrointestinal microcirculation,[1-8] hydrogen gas clearance (HGC) and laser-Doppler flowmetry (LDF) are highly promising, simple, noninvasive, and have potential clinical application. The principles of HGC[9-11] and LDF[5,6,12] are well established. Though Semb[7] first applied HGC to the measurement of gastric mucosal blood flow (GMBF), it is the development of the contact electrode by Murakami and his co-investigators[4] and its subsequent modification by other investigators increasing its sensitivity and enabling the use of 3% hydrogen-20% oxygen gas mixtures[13,14] that has given the technique impetus.

LDF has the unique advantage of being able to offer instantaneous and continuous blood flow measurements. Problems that have been reported with its use and which constitute its main drawbacks are difficulty in maintaining probe-mucosal contact, spatial resolution and the lack of calibration in absolute flow values.[15-20] The future of this technique is no doubt dependent on the ultimate resolution of these problems. We therefore designed this study to compare LDF and HGC and to investigate the feasibility of obtaining a conversion factor for LDF signal to absolute flow values.

[†]A version of this chapter has been published as:
Gana TJ, Huhlewych R, Koo J. Focal gastric mucosal blood flow by laser-Doppler and hydrogen gas clearance: a comparative study. J Surg Res 1987; 43: 337-343.

MATERIALS AND METHODS

Surgical Preparation. The study was performed with nine mongrel dogs weighing 20-30 kg. The dogs were fasted for 24 hr before each experiment (allowed free access to water only). They were anesthetized with sodium pentobarbital (25 mg/kg) and ventilated artificially by a Harvard respirator throughout each experiment. A polyethylene catheter (ID 0.055 in) was placed in one of the femoral veins or a hind-leg vein for infusion of 0.9% normal saline to maintain hydration and a stable blood pressure. The arterial blood pressure was directly monitored throughout each experiment by a saline-filled polyethylene catheter (ID 0.055 in) placed in the left femoral artery (Gould Statham transducer P23Db). Body temperature of the dog was maintained at 37°C by an electric blanket. A midline laparotomy and splenectomy were performed and a chambered segment model of the gastric corpus with an isolated vascular pedicle was prepared according to the method of Moody and Durbin.[21] The top of the lucite chamber was modified to allow stable placement of the laser-Doppler optic probe and the hydrogen gas clearance electrodes. The animals were allowed to stabilize for 30 minutes after the surgical preparation.

Experimental Protocol. Simultaneous LDF and HGC measurements of GMBF were then carried out with the two probes in contact with the mucosa at the same focal point. Two sets of experiments were performed, the first (Experiment 1) involving five dogs and in which the mucosal surface of the gastric chamber preparation was bathed in 20 ml of 0.9% normal saline. The other set of experiments (Experiment 2) involved four dogs (aspirin-induced ulceration experiments) and the chamber mucosa was bathed in 20 ml of 150 mM HCl or 20 ml of 150 mM HCl plus 20 mM aspirin. A permanent analog record of all the monitored variables (BP, LDF, and HGC) was obtained on a multichannel chart recorder (Beckman Dynograph).

Laser-Doppler Flowmetry. LDF measurements were performed with a periflux laser-Doppler flowmeter (Perimed, Stockholm, Sweden). LDF flow values (volts) were obtained from the analog chart recordings by dividing the area under the curve (obtained by the trapezoidal rule) by the total time of the recording. The data from the curve were fed into a personal computer

(IBM) in which we had written a program to automatically give the flow values. In Experiment 1, the Doppler signal upper frequency limit was 4 kHz and the gain setting was X10 throughout the individual experiments while in Experiment 2 the flowmeter settings were 12 kHz and X30, respectively.

Hydrogen Gas Clearance. HGC was performed using standard technique.[4] In summary, the terminals of the platinum electrode (Unique Medical Co., Ltd, Tokyo, Japan) in contact with the mucosa and the reference Ag/AgCl skin electrode (Red Dot, 3M) were connected to the Beckman recorder and the pen was centered on the baseline. Pure hydrogen gas was then administered at the rate of 1-2 liters/min for 60 seconds through the input port of the Harvard respirator, allowing some mixing of the gas with room air. Thus, the actual concentration and rate of H_2 administered is not precisely known. A hydrogen washin-washout curve was obtained. Data from the washout part of the curve were then fed into an IBM personal computer in which we had written a program to obtain semilogarithm plots. The program rejects plots which are not monoexponential at the 5% level of significance. The computer automatically calculates the $t_{1/2}$ of accepted plots and gives GMBF values expressed in ml/min/100g of tissue.

To investigate the reproducibility of the HGC flow values, two platinum electrodes were placed in contact with the mucosa at different points in the same gastric chamber and simultaneous consecutive flow measurements were obtained for 30 min in four dogs.

Statistical Methods. The data obtained in Experiments 1 and 2 were analyzed separately. All flow values were expressed as mean ± SE. Data analysis for statistical significance was obtained by linear regression analysis or a paired Student's *t* test. The slopes of the individual regression lines and the combined data in Experiments 1 and 2 were tested for equality and a common regression line, respectively, by the analysis of covariance. Reproducibility of HGC blood flow values was assessed using the coefficient of variation and was deemed significant at values $\leq 10\%$.

RESULTS

Relationship Between LDF and HGC

Table 1 shows the means ± SE flow values obtained by HGC and LDF in Experiments 1 and 2. Means ± SE HGC flow values of 64.88 ± 5.80 and 52.45 ± 2.21 (ml/min/100g) were obtained for the combined data in Experiments 1 and 2, respectively. Similarly, simultaneous mean ± SE

Table III-1. MEAN (± SE) LDF AND HGC FOCAL GMBF VALUES AND RESULTS OF CORRELATION ANALYSES IN EXPERIMENTS 1 AND 2

Experiment 1 (Dogs)	HGC (ml/min/100g)	LDF (volts)	n	r	p value
1	63.28 ± 8.08	1.70 ± 0.14	3	0.9984	<0.04
2	102.22 ± 20.25	4.42 ± 1.01	5	0.9839	<0.003
3	70.79 ± 14.12	1.49 ± 0.31	9	0.9469	<0.0002
4	40.87 ± 14.22	1.02 ± 0.17	5	0.9821	<0.003
5	57.21 ± 5.65	3.02 ± 0.18	15	0.7499	<0.002
(1-5)	64.88 ± 5.80	2.46 ± 0.24	37	0.7132	<0.0001
Experiment 2 (Dogs)					
1	53.39 ± 2.75	4.51 ± 0.34	20	0.6686	<0.002
2	36.18 ± 3.00	6.00 ± 0.91	11	0.8071	<0.003
3	63.62 ± 2.36	5.87 ± 0.58	17	0.6642	<0.004
4	50.16 ± 6.89	4.03 ± 0.70	13	0.8894	<0.0001
(1-4)	52.45 ± 2.21	5.06 ± 0.31	61	0.5660	<0.0001

LDF flow values for the combined data were 2.46 ± 0.24 and 5.06 ± 0.31 (volts) in Experiments 1 and 2, respectively. Regression analysis revealed a significant linear correlation between LDF and HGC flow values within individual dogs in both experiments.

Figures 1 and 2 illustrate the correlation between the combined LDF and HGC flow values in Experiments 1 and 2, respectively. There was a highly significant linear correlation

between the combined flow values in Experiments 1 ($r = 0.7132$, $n = 37$, $p < 0.0001$) and 2 ($r = 0.5660$, $n = 61$, $p < 0.0001$). A positive LDF signal was present when flow by HGC was zero in both experiments.

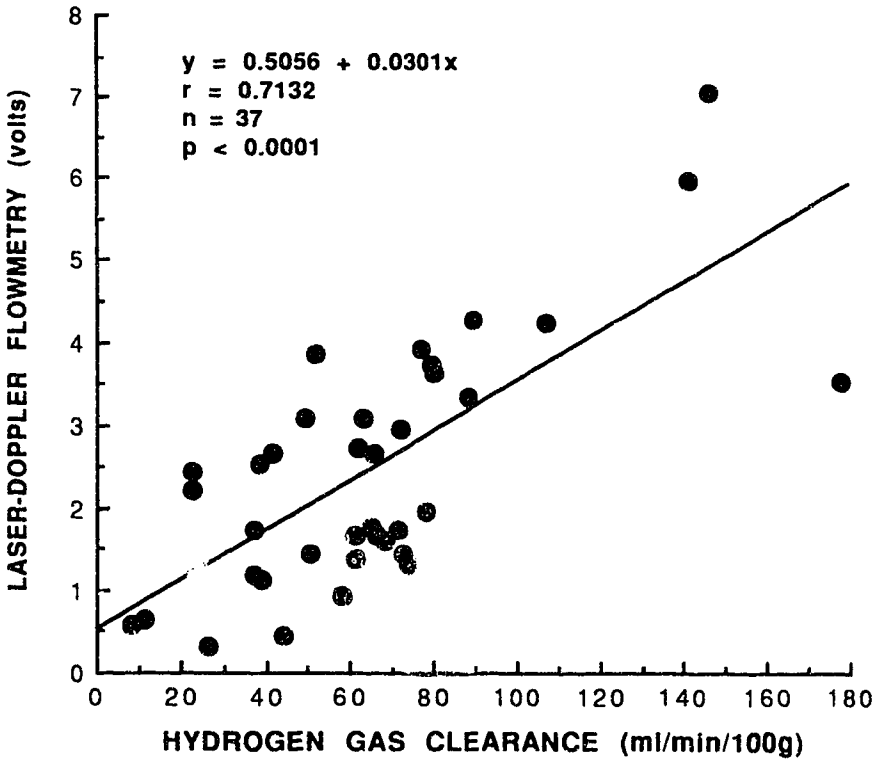

Figure III-1. Regression analysis of LDF versus HGC for the combined basal GMBF values obtained for the 5 dogs in Experiment 1.

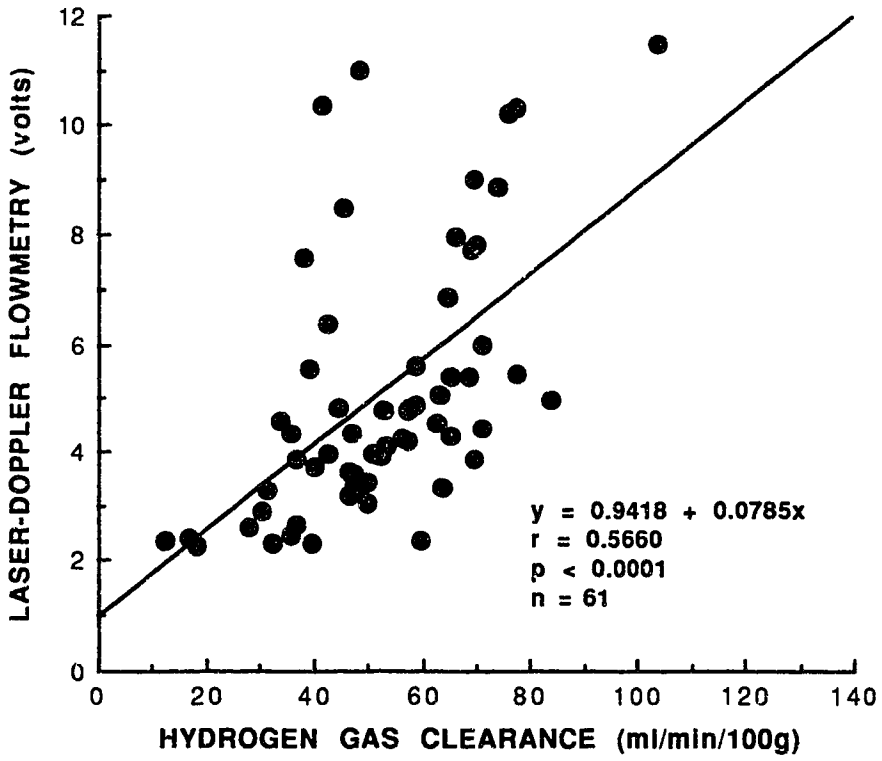

Figure III-2. Regression analysis of LDF versus HGC for the combined basal GMBF values obtained for the 4 dogs in Experiment 2.

Calibration of LDF Signal

Figures 3 and 4 show the 5 and 4 linear regression lines and their slopes, obtained from linear regression analysis of the focal GMBF data in individual dogs in Experiments 1 and 2, respectively. The data did not corroborate the claim that the linear regression lines were coincident in Experiments 1 ($F = 6.59$ [critical $F = 2.29$], $p < 0.0005$) and 2 ($F = 10.57$ [critical $F = 2.31$], $p < 0.0005$). Also, there were statistically significant differences between the slopes of the 5 and 4 linear regression lines obtained in Experiments 1 ($F = 13.15$ [critical $F = 2.79$], $p < 0.0005$) and 2 ($F = 21.14$ [critical $F = 2.73$], $p < 0.0005$), respectively (analysis of covariance). The high F ratios indicate a higher between than within dog variation in the GMBF values obtained in this study by both techniques. This finding is in agreement with earlier observations on the variation of HGC focal GMBF values in rats.[4,22]

Stability of LDF Signal

Only very brief loss of optical coupling of the laser-Doppler flowmeter probe was observed due to occasional gastric contractions. Thus probe contact was not a problem with this experimental model. The LDF signal was stable throughout the experiments except for occasional rhythmic oscillations which were not in unison with the respiratory fluctuations noticed in the simultaneous arterial pressure recordings. Similar oscillations have been reported by other investigators.[15,16,18,20,23] In a recent report, Kiel and his co-investigators[18] had noted that these oscillations occurred at the same frequency with the electrical activity in the muscularis layer of the stomach. We, however, did not monitor the electrical activity of the muscle layer in this study.

Reproducibility of HGC Flow Values

Consecutive HGC measurements of GMBF at the same focal point within a 30-min period showed a coefficient of variation of 10% or less in each of four dogs. Between four dogs, consecutive HGC flow measurements had a coefficient of variation of 18.5%. Simultaneous HGC

flow measurements from two different points on the mucosa in four dogs gave means ± SE flow values of 63.44 ± 3.36 and 60.24 ± 2.58 (ml/min/100g), respectively. There was no statistically significant difference between the two mean flow values ($p = 0.4$, $n = 16$; paired Student's t test).

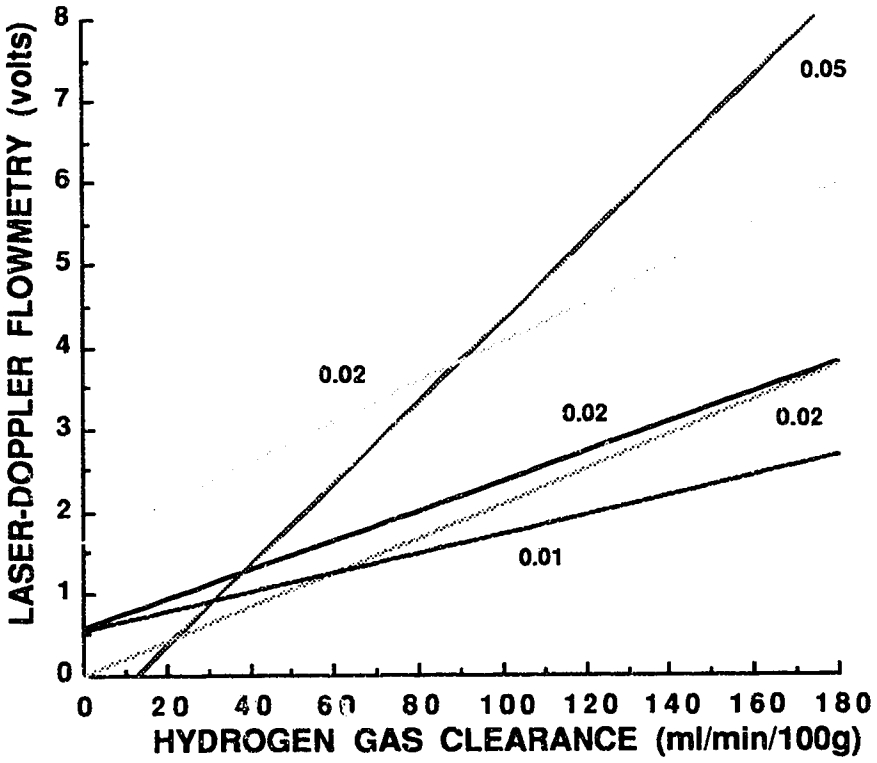

Figure III-3. Linear regression lines and their slopes for the five dogs in Experiment 1. The regression lines were not coincident ($F = 6.59$ [critical $F = 2.29$], $p < 0.0005$) and their slopes were statistically significantly different ($F = 13.15$ [critical $F = 2.79$], $p < 0.0005$).

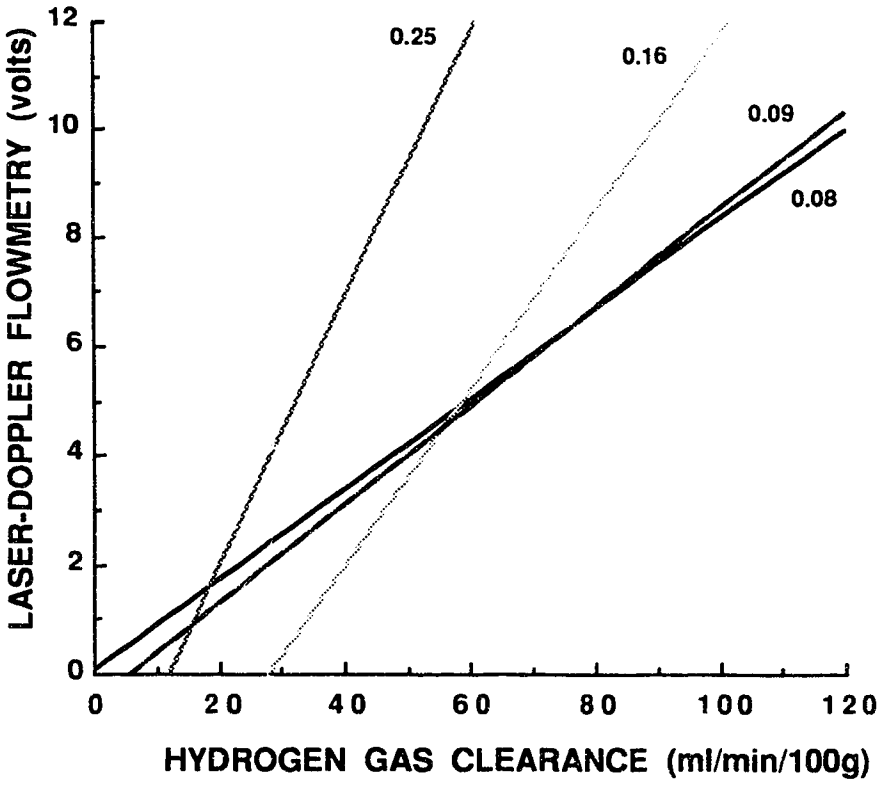

Figure III-4. Linear regression lines and their slopes for the four dogs in Experiment 2. The regression lines were not coincident ($F = 10.57$ [critical $F = 2.31$], $p < 0.0005$) and their slopes were statistically significantly different ($F = 21.14$ [critical $F = 2.73$], $p < 0.0005$).

DISCUSSION

In the last few years, we have witnessed the emergence of four new techniques capable of focal gastrointestinal mucosal blood flow estimates: the hydrogen gas clearance technique,[4,7] the iodoantipyrine clearance technique,[1,8] the computer-equipped reflectance spectrophotometry method,[2,3] and the laser-Doppler flowmetry technique.[5,6,12] Of these, the iodoantipyrine clearance technique cannot give accurate flow measurements in the presence of luminal contents and requires the taking of mucosal tissue biopsy specimens. The computer-equipped reflectance spectrophotometry method is expensive and measures the relative absorbance due to an unchanging hemoglobin concentration. Hydrogen gas clearance and laser-Doppler flowmetry are, however, two simple and safe techniques which fulfill more of the criteria laid down by Jacobson for an ideal blood flow measurement method.[24]

The validity of the HGC technique for the assessment of focal GMBF has been established against other previously existing methods.[4,13,14,22,25] Notable limitations which remain a threat to its widespread use are its inability to offer instantaneous and continuous flow measurements and the difficulty of maintaining electrode-mucosa contact, especially in clinical settings. LDF has been used and validated in intestinal mucosal blood flow measurements in animals[15,16,19,20,26] and humans[27] but only a few reports have appeared on its use in the measurement of GMBF in animals[18,28] and humans.[29] Furthermore, in the stomach its validity has been established only against total blood flow obtained by electromagnetic flowmetry[18] which is not an ideal method for comparison. We therefore designed this study to test the hypothesis that there exists a common conversion factor for LDF signal to absolute flow values which is applicable from experiment to experiment and also to further establish its validity in the measurement of focal GMBF.

In previous studies, a 1-V change in the LDF signal has been observed to approximately equal a change in blood flow of 10 ml/min/100g in skin[12] and intestinal blood flow measurements.[15,19] Our results revealed that a 1-V change in the LDF signal equals a change in flow by HGC of 4-11 ml/min/100g and 12.5 ml/min/100g for individual dogs and the combined

data, respectively, in Experiment 2. Statistical analysis of the results, however, did not corroborate a common regression line hypothesis in both experiments. Similarly, there was a statistically significant difference between the slopes of the individual regression lines in both experiments. This finding suggests that, first, a common conversion factor for LDF signal to absolute flow values cannot be obtained from experiment to experiment. Second, for absolute flow unit measurements of focal blood flow to be feasible with LDF, a comparable technique has to be used simultaneously and conversion must be made within individual experiments. This will be cumbersome and may prove to be unnecessary. We do not know the reasons for this shortcoming but vascular bed geometry, tissue pigmentation, tissue hemoglobin concentration, and fiber separation in the optical probe may all be important factors.

Our results also show highly significant correlation between focal GMBF values obtained by the two techniques in both experiments. This lends credence to reports of previous comparative studies in which LDF flow values correlated well with HGC-measured flow values in the gastric corpus,[28] total flow (electromagnetic flowmetry) in the stomach,[18] total flow (^{85}Kr washout) in the small intestine,[15] total flow (electromagnetic flowmetry) in the small intestine,[20] total flow (venous outflow) and regional mucosal flow (microspheres) in the stomach,[26] and with HGC and microsphere techniques in the small intestine.[19] This result reaffirms the credibility of LDF as an accurate method in the qualitative assessment of focal GMBF. Studies are currently in progress in our laboratory to further evaluate the performance of LDF in drug-induced changes in blood flow along with other techniques. The observation of a LDF signal when flow by other techniques is zero (Figs. 1 and 2) has been a regular observation in previous studies.[15,19,20] This may be related to the extreme sensitivity of LDF technique to motion artefacts in the measured tissue.[15]

A coefficient of variation of 10% and less observed with consecutive measurements of focal GMBF by HGC shows that the technique is highly reproducible for within dog measurements. The between dog estimates of GMBF gave a coefficient of variation of 18.5%. This may be due to inherent biological variations. There was no statistically significant

difference between simultaneous HGC flow measurements from different points in the same gastric chamber. This latter finding suggests that there are no significant differences in GMBF at different points simultaneously within the same gastric chamber.

In summary, we have presented evidence that does not suggest that it is possible to obtain a calibration factor for LDF signal to absolute flow values valid from experiment to experiment. This shortcoming may seriously limit the usefulness of LDF, particularly with respect to its clinical application. The ongoing controversy over its spatial resolution and the problem of probe contact in clinical use require resolution. HGC flow values are reliable and highly reproducible within dog measurements but the time required to complete each measurement, especially in low flow states, is a significant limitation of this technique.

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Chapter 4

Improved Hydrogen Gas Clearance Technique for Measurement of Focal Gastric Mucosal Blood Flow†

INTRODUCTION

The use of hydrogen gas clearance (HGC) to measure local blood flow in the myocardium, renal cortex and skeletal muscle, based on Kety's theory[1,2] was first reported by Aukland and co-workers.[3] However, it was Mackie and Turner,[4,5] who first applied the technique to measure submucosal blood flow in the stomach of rats. Subsequently, Semb applied HGC to measure gastric mucosal blood flow (GMBF) under various experimental conditions in anesthetized and in conscious animals.[6-9] The method received impetus from the development of a contact, coiled-spring, platinum electrode by Murakami and co-investigators.[10] This innovation provided a wider surface area of electrode-mucosal contact and monoexponential HGC curves in contrast to multiexponential curves obtained by previous investigators who inserted the measuring electrodes into the submucosa. More recently, the contact electrode has been made more sensitive enabling the use of a 3% hydrogen-20% oxygen gas mixture, which is non-hypoxic, non-explosive, and safe in clinical use.[11,12]

Using other techniques, HGC has been validated as an accurate tool for estimating focal gastrointestinal mucosal blood flow,[10-12,14] but the technique has not enjoyed widespread use. This may be due to its limitations, some of which include: (i) inability to provide instantaneous and continuous blood flow measurements, (ii) depending on the concentration of hydrogen gas inhaled, it takes up to 30 minutes to complete one HGC measurement,[12-14] thus rapid changes in blood flow can be missed;[13] an assumption of the technique dictates that blood flow should remain constant during the entire measurement period, and (iii) the current generated on the surface of the platinum electrode with a 3%

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†A version of this chapter has been submitted for publication as:
Gana TJ, Kingma YJ, Koo J, Bowes KL. Improved hydrogen gas clearance technique for measurement of focal gastric mucosal blood flow. Clin Invest Med 1991 - submitted.

hydrogen-20% oxygen gas mixture is small and often corrupted by noise and motion artifacts[11,15] frequently resulting in significant baseline drifts, distortion of the hydrogen washout curve and even lack of decline during washout. These may result in significant errors in the ultimate blood flow value or in some cases invalidate the particular flow measurement.

In this study we determined by digital computer analysis the noise and motion artifacts that corrupt the HGC-measured signal, designed a current pre-amplifier and filter which improved the magnitude and quality of the HGC signal, and compared the signals obtained with a current amplifier to those obtained with a voltage amplifier. We further tested the following hypotheses: (a) that GMBF values determined from the washin portion of the hydrogen washin-washout curve are not significantly different from the corresponding values obtained conventionally from the washout portion of the curve, and (b) that brief 1 minute inhalation of hydrogen gas offers blood flow values comparable to those obtained following 5 minutes of inhalation and washin to equilibrium.

MATERIALS AND METHODS

THEORETICAL CONSIDERATIONS

Principle of Hydrogen Gas Clearance

The principle of HGC is based on Kety's theory of blood-tissue exchange of inert gases[1,2] which itself was founded on the Fick principle. Details of the principle of HGC were published previously.[3,10] The experimental animal or subject is made to inhale hydrogen gas. The concentration of hydrogen gas in the blood and perfused tissues rises to an equilibrium (washin) and inhalation of the gas is stopped. A washin-washout curve of the current generated on the surface of a platinum electrode (placed in the measuring tissue) by the oxidation of hydrogen gas to hydrogen ions and electrons is obtained. Assuming that the tissue is homogeneously perfused, and that for a biologically inert and highly diffusible gas (hydrogen) the tissue is in

instantaneous diffusion equilibrium with the venous blood from that particular tissue,[3,10] the waveform obtained by measuring current from the platinum electrode during washout has the form,

$$i(t) = I_{initial}\, exp\, \frac{-Ft}{\lambda} \qquad (1)$$

F is blood flow in units of ml/min/g of tissue, and λ, is the tissue-blood partition coefficient in units of ml/g which is assumed to be 1 for hydrogen gas.[3] F is usually expressed as ml/min/100g.

Current Amplifier for Measurement of HGC

i) Design: A complete circuit schematic of the amplifier and low pass filter designed and built for HGC measurements of focal GMBF is shown in Figure 1. It consists of five stages. In Stage 1, the platinum electrode bias potential can be adjusted from 0 to 190 milliVolts. The positive potential makes the platinum electrode less sensitive to oxygen.[16] Our amplifier had this stage set to +100 milliVolts. In Stage 2, current is measured and converted to voltage with a gain of one million (1 Volt/microAmp). This active circuit presents a very low resistance to the electrode circuit, thus it acts as an ideal current meter. Stage 3 provides further adjustable voltage gain ranging from 6.9 to 59.8, making the overall current to voltage gain 6.9 million to 59.8 million. Significant amplification is necessary since the measured current in 3% HGC is very small (about 10^{-8} to 10^{-7} Amperes). Stage 4 provides an offset adjustment to cancel out any unwanted offsets and to facilitate positioning of a connected recorder pen. The output from this stage can be connected directly to the recorder without filtering, or passed on to the next (filter) stage. Stage 5 is a first order low pass filter with a time constant of 20 seconds. This stage effectively removes or greatly attenuates most noise and motion artifacts. The advantages of using this filter are: (a) the observer can directly assess the effectiveness of the filter by

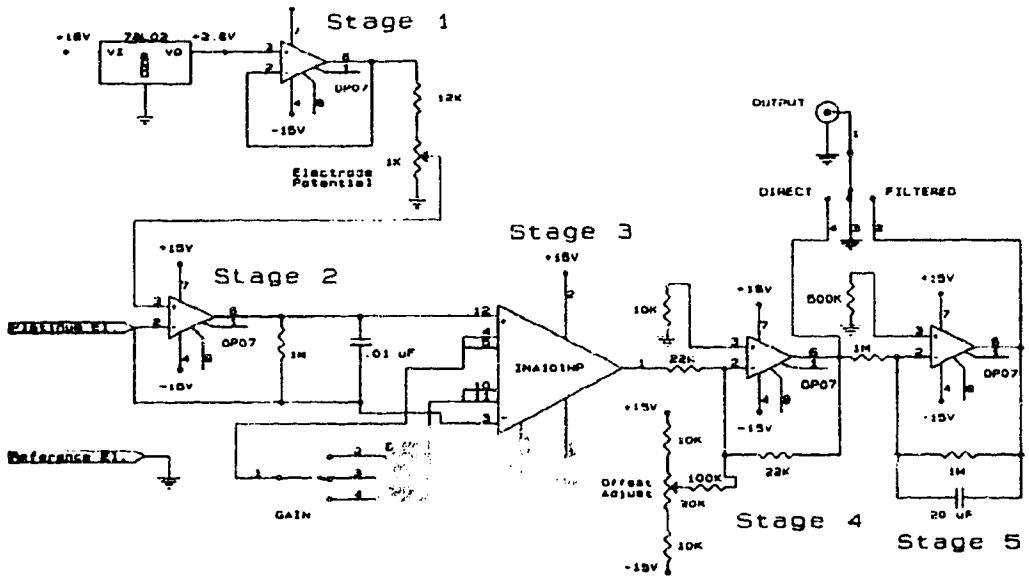

Figure IV-1. A circuit schematic of the current amplifier with adjustable gain and optional low pass filtering suitable for measuring hydrogen gas-induced current from a platinum electrode.

visualizing the recorder chart; and (b) by removal of the higher frequency noise, a relatively low sampling rate may be employed. The output of this stage is connected to the recorder.

ii) Amplifier Analysis: Since the amplifier is to be used to determine blood flow it is important to determine what effect the filtering has on the HGC curves. We will consider the situation where the HGC curve is a monoexponential with time constant τ_{HGC}. When measuring GMBF, τ_{HGC} is usually in the range of 40 seconds to a few minutes (corresponding to flow rates of 150 to 50 ml/min/100g, respectively). The low pass filter also has a time constant, τ_{FIL}, which we

have designed to be 20 seconds. In the time domain, the equation for the HGC curve is a monoexponential:

$$i_i(t) = I_{initial}\, exp\, \frac{-t}{\tau_{HGC}} \qquad (2)$$

Taking the Laplace transform of (2) and operating on this signal by the Laplace transformed low pass filter with time constant τ_{FIL}, we obtain for the output:

$$V_o(s) = \frac{I_{initial}}{(s\tau_{HGC} + 1)(s\tau_{FIL} + 1)} \qquad (3)$$

Taking partial fractions and inverse Laplace transforming we obtain:

$$V_o(t) = I_{initial}\left[\left(\frac{\tau_{HGC}}{\tau_{HGC} - \tau_{FIL}}\right) \cdot exp\left(\frac{-t}{\tau_{HGC}}\right) + \left(\frac{\tau_{FIL}}{\tau_{FIL} - \tau_{HGC}}\right) \cdot exp\left(\frac{-t}{\tau_{FIL}}\right)\right] \qquad (4)$$

Thus the result is the sum of two weighted monoexponential curves. One exponential has the same time constant as the original clearance curve while the second exponential has a time constant equal to the low pass filter time constant. If the low pass filter time constant is not short relative to the HGC time constant, then it alters the original HGC curve. The top of Figure 2 shows four clearance curves for four hypothetical filter time constants while the bottom of Figure 2 shows the same curves on a semi-logarithmic plot. We also analyzed, using the fast Fourier Transform method, the noise that was present in the HGC signal before filtering to determine more precisely what information the filter was removing.

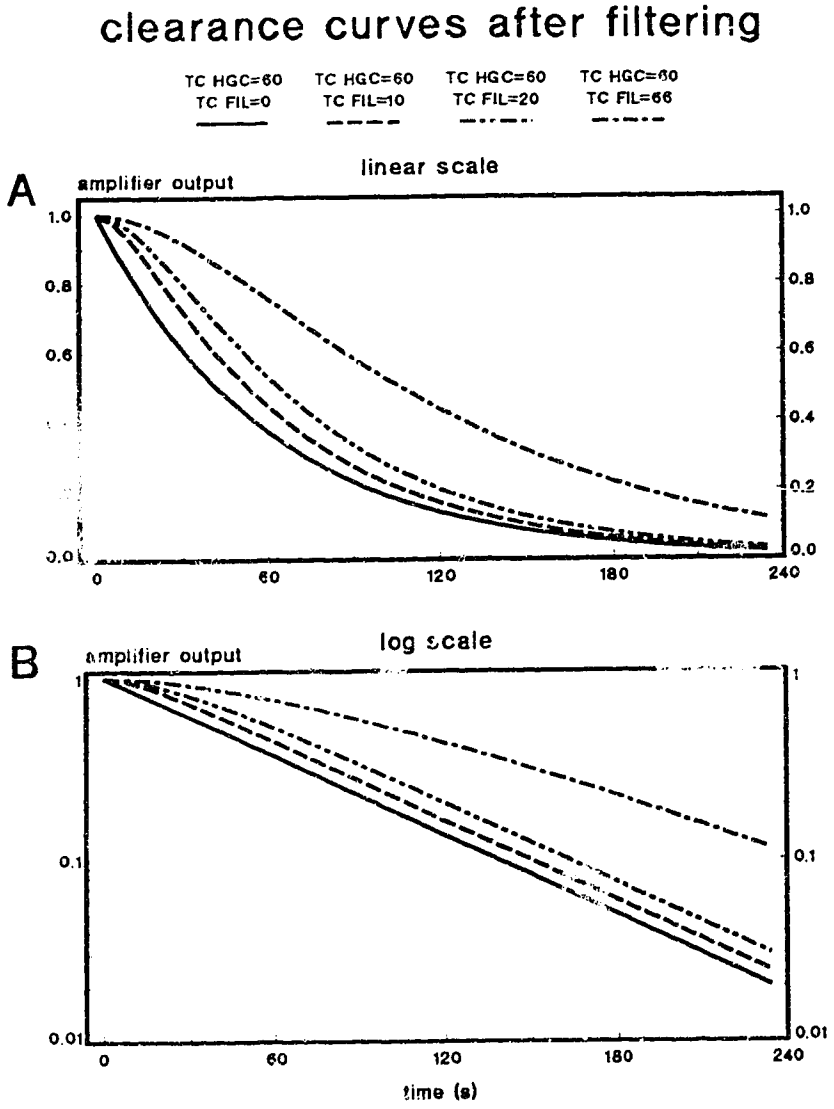

Figure 2. Plots showing the effect of filtering a monoexponential clearance curve with several different filter settings. Blood flow is inversely proportional to the HGC curve's time constant (TC HGC). The filter's cutoff frequency is also inversely proportional to its time constant (TC FIL). In the Linear Scale panel (A), as the filter's time constant is increased with respect to the HGC curve's time constant, the curve becomes delayed and the initial portion becomes more rounded. The Log Scale panel (B) shows that an increased filter time constant causes a delay but the tail portion of the filtered curve is monoexponential with the same time constant as the unfiltered clearance curve. HGC = hydrogen gas clearance.

Analysis of the HGC Curves

The current amplifier we developed introduces an added exponential term to the clearance curve which must be taken into consideration when calculating blood flow. This is effectively done by using an iterative Gauss-Newton curve fitting routine which has an extra term to account for the filter's time constant. We developed our own computer-based system for the acquisition and analysis of the HGC curves. For example, if the unfiltered washout curve were monoexponential we would fit a biexponential of the form:

$$A\,exp^{-Ft} + B\,exp^{-t/20} + C$$

to the filtered curve, where F is blood flow and 20 is the filter time constant. A computer-generated test curve that: (a) decayed from 1 to 0, (b) had a flow rate of 120 ml/min/100g, and (c) included the filter exponential term, was fitted perfectly by the iterative routine. Attempting to fit the same curve with a monoexponential yielded errors in the flow rate which were always low, up to 50% lower, depending on which portion of the curve was fitted.

According to the theory discussed above, if the unfiltered curve were monoexponential then the coefficients A and B of the filtered curve must be of opposite sign. If in the resulting curve fitting they are not, then the original curve was not really monoexponential and the apparent blood flow will be in error. This provides a useful check on the validity of the calculated result.

Determining Blood Flow During Washin and Washout

Blood flow is conventionally determined from the washout portion of the H_2 concentration curve since blood flow determines the rate at which H_2 is cleared from the tissue. Clearly, the rate at which H_2 is accumulated in the tissues is also a function of blood flow and therefore the washin portion of the H_2 concentration curve could also be used to determine a blood flow value. Since determination of blood flow from the washout portion of the clearance curve has been

validated against other techniques previously,[10-12,14] we used the former flow values as the reference against which the values obtained from the corresponding washin portion of the curve were compared. Aukland et al.[3] provided a derivation that yielded an exponential equation for the washout curve that was a function of blood flow. We will provide a very similar but more general derivation based on identical assumptions that shows that the washin curve is also an exponential equation that is a function of blood flow.

The first few steps are the same as those presented by Aukland et al.,[3] i.e., from the Fick principle, gas accumulated (washin) dQ_i or given off (washout) by a homogeneously perfused tissue (i) in time dt is:

$$dQ_i = (C_a - Cv_i)F dt$$

where F is blood flow, C_a is arterial gas concentration and Cv_i is venous gas concentration. If C_i is the concentration of gas in the tissue with volume W then:

$$W dC_i = (C_a - Cv_i)F dt$$

Introducing the tissue-blood partition coefficient, λ, and assuming instantaneous diffusion equilibrium we obtain:

$$W dC_i = (C_a - C_i/\lambda)F dt$$

At this point we depart from the derivation by Aukland et al.[3] They solved for the particular case where the initial arterial concentration of test gas, C_a, was zero (C_a is assumed to have the same concentration of test gas as that present in the inspired air which is zero during washout). Instead we will solve for the case where C_a could be any value, that is, inspired air may contain H_2 gas. Rearranging the previous equation we obtain:

$$\frac{dC_i}{dt} + \frac{FC_i}{\lambda W} = \frac{FC_a}{W}$$

which is a first order differential equation. We can solve this equation to determine how C_i, the concentration of gas in tissue, varies with time to obtain:

$$C_i(t) = (C_{i0} - \lambda C_a) exp^{-Ft/\lambda W} + \lambda C_a$$

where C_{i0} is the initial concentration of gas in the tissue. It should be noted that if C_a is set to zero this equation reduces to that obtained by Aukland et al.[3] This equation makes two predictions which we evaluated in this study: (a) that blood flow can be determined from the washin portion of the hydrogen concentration curve and, (b) blood flow can be determined equivalently from curves that do not necessarily start from zero H_2 concentration (complete washout) and that do not necessarily washin to equilibrium. Restated in different terms, all changes in the concentration of H_2 in the tissue are a function of blood flow (F) and they will follow an exponential time course. If curve fitting analysis that takes into consideration the time constant of the filter is applied, then the accurate blood flow value can be determined.

EXPERIMENTAL CONSIDERATIONS

Animal Preparation

Male Sprague-Dawley rats weighing 200 to 300 g were fasted for 18 to 24 hours before each experiment (allowed only water *ad libitum*). They were anesthetized with 40 mg/kg of intraperitoneal sodium pentobarbital. A tracheostomy was performed and the trachea was intubated with a polyethylene tubing (PE 240; OD 0.095 in) to maintain a patent airway and for delivering hydrogen gas. A midline laparotomy was performed, the stomach was exposed, and mobilized by ligating the gastrohepatic ligaments. A longitudinal incision was made into the anterior aspect of the forestomach to expose the mucosa of the distal glandular stomach. Three platinum electrodes (Unique Medical Company Ltd., Tokyo) were placed in contact with the glandular mucosa within 2 to 4 mm of each other. Electrodes were not relocated once positioned on the mucosa. A reference Ag/AgCl skin electrode (3M Canada) was placed over an abraded area of abdominal wall skin in the right flank. The latter electrode provided three leads for completing the measuring circuits of the three platinum electrodes. Two of the platinum electrodes were connected to our dual current amplifier (one channel filtered, the other

unfiltered) and then to the chart recorder, and the third was connected directly (direct-connect or voltage-amplified) to a multi-channel chart recorder (Beckman Type R511A). The outputs from the recorder were digitized and stored on a PC-AT personal computer (IBM Corp.) using a Lab Master analog-to-digital converter board (Scientific Solutions, Solon, Ohio). The digitized data were then subjected to computerized Gauss-Newton iterative curve fitting[15] and fast Fourier Transform analysis.[17]

Experimental Protocol

HGC was performed as described previously,[18,19] except that instead of using pure hydrogen gas, 3% hydrogen gas in air was administered through an inverted funnel placed 2 to 4 cm above the tracheostomy tube. Blood flow measurements were performed with the 3 platinum electrodes simultaneously.

Study 1: We utilized a protocol of prolonged H_2 administration, for 5 minutes, alternating with brief H_2 administrations for 50, 35, and 15 seconds. The aim was to compare the waveforms and blood flow values obtained during prolonged H_2 administration (5 minutes) or washin to equilibrium, washout (5 minutes), and washout after brief H_2 administration, in the direct-connect (voltage-amplified), filtered and unfiltered current-amplified channels.

Study 2: Based on the results obtained in study 1, the aim was to demonstrate protocols that achieved rapid measurements of blood flow. The protocols were as follows: 5 minutes of H_2 administration followed by 5 minutes of washout, and 1 minute of H_2 administration followed by 5 minutes of washout, the sequence being repeated several times. This produced a blood flow measurement every 5 or 6 minutes respectively.

Data Analysis

Individual blood flow values are expressed as mean ± SE. Statistical analysis of data to obtain levels of significance (p-values) were performed using a two-tailed paired Student's *t*-test, Pearson's correlation analysis, and linear regression analysis. P-values less than 0.05 were considered significant.

Drift, defined here as a lack of return to the original zero baseline after washout of hydrogen gas from the tissue, was compared between the voltage-amplified and the current-amplified channels. Because of the different sensitivities used in the different channels of the strip chart recorder and the biological variation in voltage/current values measured in different animals, drift was expressed as a function of the HGC signal, i.e., as a percentage of the maximum voltage/current reached in each HGC curve before washout.

RESULTS

Following the administration of H_2 to the animals each time throughout the experiments, the platinum electrodes connected to the current amplifier responded to the presence of H_2 with a rise from the baseline (on the chart recorder) about 15 seconds earlier than the voltage amplifier electrode. Also, whenever H_2 administration was halted after brief or prolonged administration, the current amplifier electrode responded almost instantaneously with a decline while the voltage amplifier electrode continued to rise for a short while thereafter before beginning to decline.

Comparison of Current Versus Voltage Signals

Figure 3 shows four sets of curves comparing direct-connect (Trace V), and both the unfiltered (Trace C) and filtered (Trace CF) current-amplified signals. Considerable noise was present in the unfiltered channel. The frequency spectrums of this noise in two animals are shown in

Figure 4. In one animal (A) the noise was dominated by respiration artifacts occurring at a frequency of around 120 cycles per minute. In the second animal (B) the noise was dominated by heart activity that occurred at a frequency of around 400 cycles per minute. Other sources of noise artifacts included 60 Hertz interference (3600 cycles per minute) shown in Panel B, gastric motility artifacts and a signal of about 30 cycles per minute, also shown in Panel A, whose origin we do not know. In Trace CF (Figure 3) the filtering very effectively eliminated the noise artifacts.

When H_2 was administered for a longer period of time, the signal-to-noise ratio was larger (Figure 3). However, with filtering, "clean" curves were obtained for periods of hydrogen administration as short as 35 seconds. Below this time, the limiting factor appears to be other artifacts, occurring at a frequency of less than 1 cycle per minute, which corrupt the washout curves (Figure 3). We do not know what this artifact represents. A second problem that was more pronounced when hydrogen administration was short was baseline drift. Careful analysis of Figure 3 reveals that the baseline level before and after H_2 administration was not always the same, especially in the direct-connect channel. Baseline drift in the direct-connect channel ($11.7 \pm 4.7\%$) was significantly larger ($n = 20$; $p = 0.028$; *t*-test) than that observed in the current-amplified channel ($2.4 \pm 1.2\%$). In the current-amplified channel, baseline drift was reduced by about 80% when compared to the drift in the direct-connect channel.

Blood Flow Values

Blood flow was not analyzed in the noisy unfiltered channel. In the direct-connect channel the blood flow values for the washout curves were 80 ± 7 ($n = 9$) and 35 ± 2 ($n = 10$) ml/min/100g in Experiments 1 and 2 respectively. Similarly, in the filtered current-amplified channel the blood flow values were, respectively, 112 ± 12 ($n = 9$) and 108 ± 7 ($n = 10$) ml/min/100g. The direct-connect blood flow values were statistically significantly lower ($p = 0.0001$ and $p < 10^{-6}$,

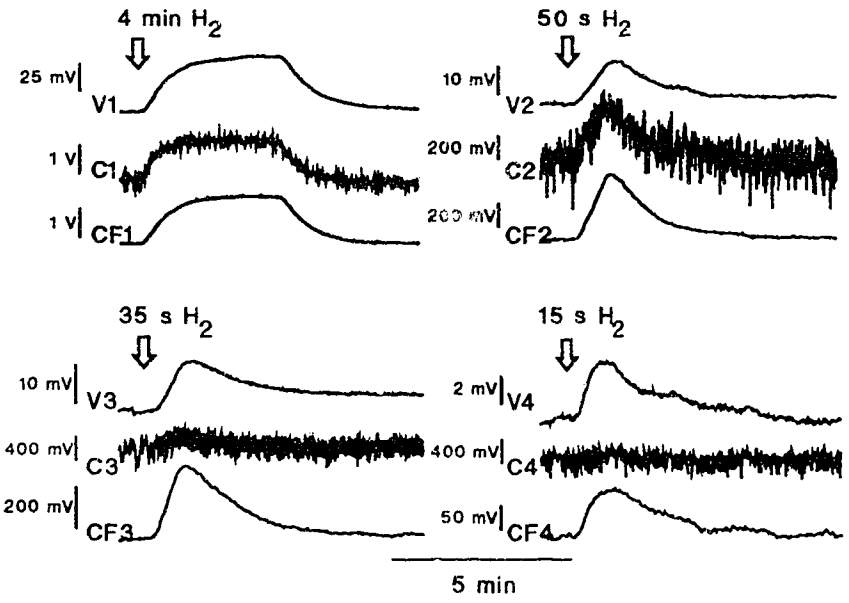

Figure IV-3. HGC curves obtained following different periods of hydrogen gas administration. Trace V was from an electrode that was directly connected to the chart recorder's input (voltage amplifier). Trace C was from a current-amplified channel without filtering. Trace CF was from a current-amplified channel with low pass filtering. The filter effectively removes most noise artifacts. Other low frequency artifacts corrupted the clearance curves when H_2 administration was reduced to below 35 seconds. Trace V shows a significant drift in baseline after 50 and 35 seconds of H_2 administration. Apparent blood flow was lower in Trace V than in Trace CF. The magnitude of the signal in Traces CF were up to 40 times higher than that in Traces V.

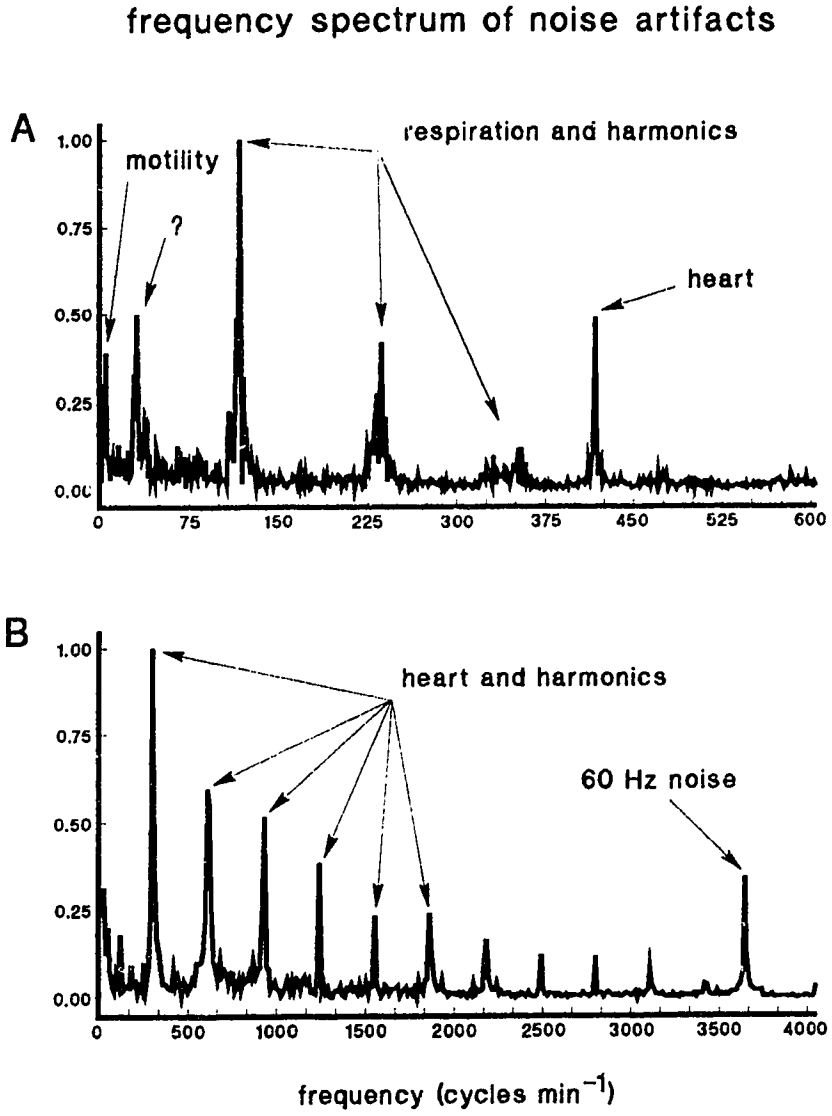

Figure IV-4. Frequency spectrum analysis of the noise artifacts that corrupt the HGC curves obtained in measurements of GMBF. Panels A and B are from different animals. Respiration and heart activity along with their associated spectral harmonics were the predominant sources of noise artifacts. Noise was also caused by interference from the 60 Hertz main power lines, gastric motility activity of around 6 cycles per minute, and an unknown source of about 30 cycles per minute that is indicated in Panel A.

respectively; *t*-test), in both experiments. The direct-connect blood flow values also demonstrated a larger inter-animal variation.

Using the filtered current-amplified HGC curves, the washout flow value for a short 1 minute administration of hydrogen gas was significantly higher than the corresponding value obtained for administration of H_2 for 5 minutes (111 ± 6 versus 101 ± 5 ml/min/100g; n = 7; p = 0.0026; *t*-test). Although the p-value was significant, the absolute difference was less than 10%.

An important hypothesis in this paper was the proposal that the H_2 washin curve could also be used to obtain a measure of blood flow. However, when hydrogen gas was administered for shorter periods in this animal model, about 3 minutes or less, the washin curves could not be reliably fitted using our computerized methods. Therefore, washin curves were only analyzed when hydrogen gas was administered for 4 minutes or longer (Figures 3, 6 and 7) and only curves obtained from the filtered current-amplified channel were used. Washin GMBF values were not significantly different from the corresponding washout values (Experiment 1: 110 ± 7 versus 101 ± 6 ml/min/g, n = 8; and Experiment 2: 108 ± 12 versus 106 ± 11 ml/min/g, n = 8; p > 0.05, *t*-test). Regression analysis of washin versus washout flow values (Figure 5) yielded a highly significant linear correlation (y = 1.013x + 4.127; r = 0.95, p < 0.000001). These findings and a slope value of nearly 1.0 indicate a strong relationship between washin and washout flow values and confirm our theoretical derivation that the washin curve can also be analyzed to determine blood flow.

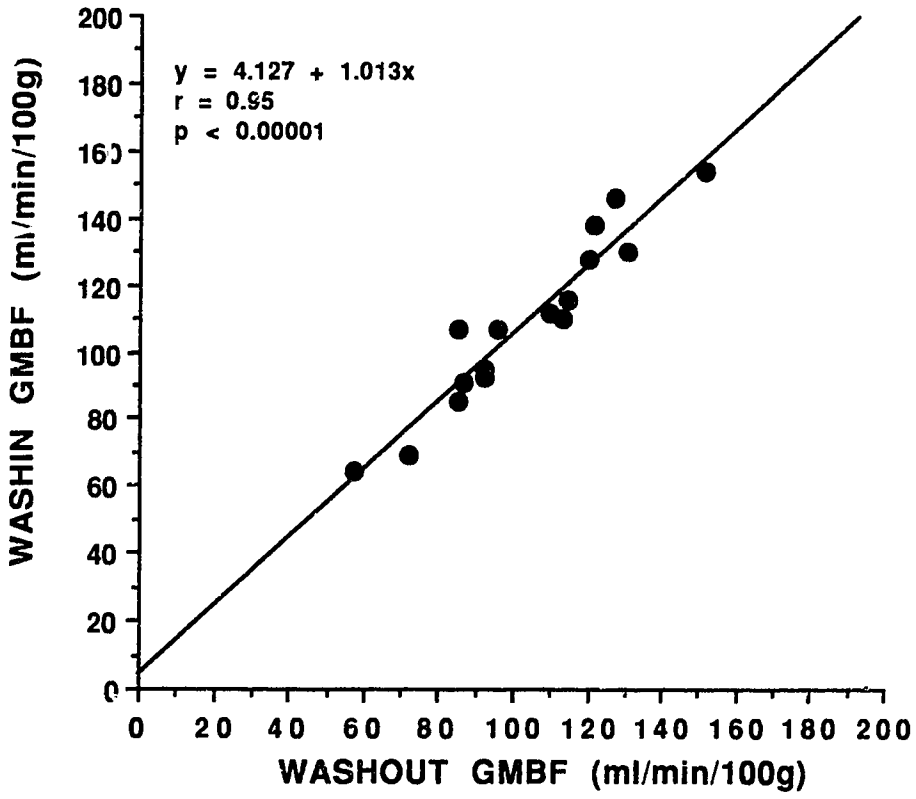

Figure IV-5. Regression analysis of blood flow values obtained from the washin portion of the HGC curves versus those obtained from the corresponding washout portion. The data were obtained from the filtered current-amplified clearance curves. There was a highly significant linear correlation between washin and washout. The slope of the regression line is almost one.

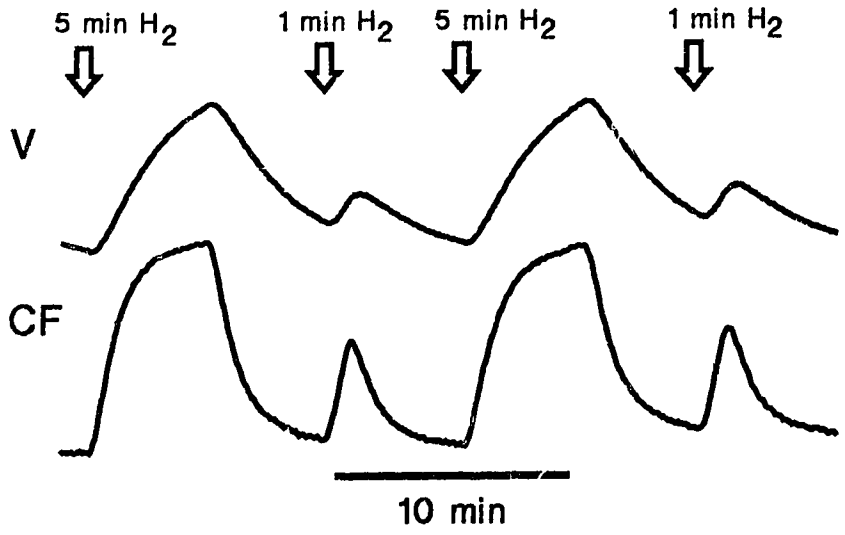

Figure IV-6. Tracings from a repeated hydrogen gas administration protocol of 5 minutes of inhalation followed by 5 minutes of washout, and then 1 minute of inhalation followed by 5 minutes of washout. Trace V was from an electrode directly connected to the chart recorder (direct-connect). Trace CF was from a current-amplified and filtered channel. This protocol permitted comparison of apparent blood flow after short (1 minute) and longer (5 minutes) H_2 inhalation. This protocol also demonstrated the feasibility of obtaining a reliable clearance curve within 6 minutes (1 minute of inhalation followed by 5 minutes of washout). Apparent blood flow value in Trace V was lower than in Trace CF.

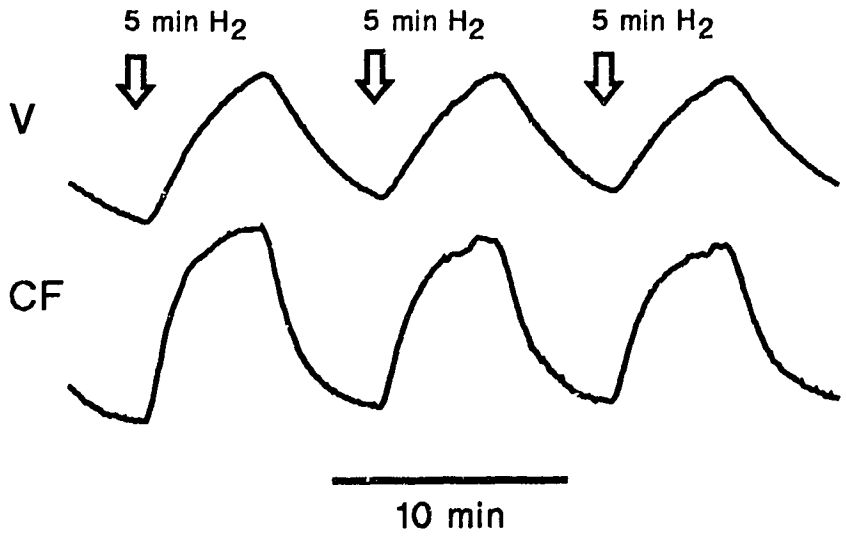

Figure IV-7. Tracings from a rapid hydrogen gas administration protocol that provided one washin and one washout blood flow measurement, in alternation, every 5 minutes. This protocol was repeated continuously. The apparent blood flow value in Trace V was lower than in Trace CF.

Protocols for Rapid Blood Flow Measurements

Both the protocol of 1 minute of H_2 administration followed by 5 minutes of washout (Figure 6) and the protocol of 5 minutes of H_2 administration followed by 5 minutes of washout yielded excellent curves (Figure 7). The former yielded a blood flow measurement every 6 minutes (washout flow) while the latter yielded a blood flow measurement every 5 minutes (washin and washout flow values). Occasionally, a "bumpy" distortion occurred on the plateau portion of the washin curve as is evident in the second curve of Trace CF in Figure 7. The appearance of this type of distortion was not consistent and its origin is not known.

DISCUSSION

Filtering the Clearance Curves

This study has shown that a very low frequency low pass filter together with a suitable amplifier can be used to greatly alleviate the noise and motion artifacts encountered when using the HGC technique to measure mucosal blood flow in the stomach. Artifacts were reduced to a level where they were no longer a concern when analyzing the HGC clearance curves. Murakami et al. presented an amplifier previously,[10] however, it is difficult to compare the latter to our amplifier since their schematic diagram was incomplete and was not discussed.

It is important to reiterate that such a filter alters the HGC curves and therefore the apparent blood flow value. It was therefore, necessary to input the data into a computer to allow computerized curve fitting analysis to determine the correct blood flow value by using a curve fitting algorithm that accounted for the changes caused by the filter.

Using a Voltage Amplifier to Measure HGC

In the original work by Aukland and co-workers,[3] they showed that current in the platinum electrode is the quantity that should be measured when determining blood flow. To measure

current one should ideally have an external resistance of 0 Ω, which our current amplifier approaches. In contrast, if the external resistance is very large one measures the platinum electrode's potential relative to the reference electrode's potential, which varies inversely as the logarithm of H_2 concentration, and which is non-linear and not appropriate for HGC determination. Typical voltage amplifiers have a high input resistance; including the input amplifiers found in chart recorders which are commonly used to measure the "current" from the platinum electrode. A further problem is that the input resistance of the voltage amplifiers from different manufacturers' chart recorders will not be the same.

Electrode polarization in HGC has been used by investigators to reduce the sensitivity of the platinum electrode to oxygen gas in the brain and in other organs outside of the gastrointestinal tract.[3,16] The polarization voltage of +100 milliVolts used in our current amplifier, while reducing electrode sensitivity to oxygen, ensures a most rapid electrode response time, a quality that will be especially useful in clinical application. An increase in the polarization voltage reduces the sensitivity of the electrode to oxygen, however, it increases the electrode response time.[16] Voltage amplifiers, in particular those in the usual chart recorders, do not have the capacity to apply a polarization voltage to the platinum electrode. Secondly, the voltage at the platinum electrode will vary as the hydrogen gas concentration, as a result of both the electrode half-cell potential changes and the current that arises from the oxidation of H_2. For example, a current of 10^{-7} Amperes flowing into the Beckman recorder used in this study will result in a change in electrode potential of 225 milliVolts. Thirdly, in this study we also found that drift was larger in the voltage-amplified channel. This may result in part from polarization of the platinum electrode[20] causing a change in electrode potential and therefore a change in the voltage measured by the voltage amplifier. The current amplifier on the other hand, is less sensitive to electrode polarization since it measures current. All these effects may contribute to the nonlinearity between voltage and H_2 concentration when a voltage amplifier is used for the measurements. For these reasons

we point out that it is not appropriate to measure current from the platinum electrode using a voltage amplifier, even though clearance curves that appear "normal" can be obtained.

Blood Flow Values

Our analysis of the theory upon which the HGC technique is based allowed us to implement new protocols that reduced blood flow measurement time. Our theoretical analysis and experimental results showed that blood flow can be determined during both washin and washout of hydrogen gas from the tissues, and the flow values obtained from both parts of the HGC curve are not significantly different. It is important to note however, that this may be true as long as blood flow remains constant, since flow was not manipulated in any way in this study. This is an accession to the previous practice of using only the washout portion of the HGC curve for blood flow determinations. The theoretical analysis also suggested that washin to equilibrium and complete washout were not necessary. This latter theoretical assumption was used to further reduce blood flow measurement time. Reliable measurements of blood flow were made within 5 minutes with 3% H_2, a time period which is significantly lower than the periods of over 15 to 30 minutes[12,14] reported previously for one HGC measurement of GMBF with the same concentration of H_2. This relatively short measurement time should facilitate new applications of the safer 3% HGC technique and make the latter more compliant to the requirement that blood flow remains constant throughout the entire measurement period of an inert gas technique.

Previous studies[11,12] contained conflicting reports about blood flow values obtained after brief hydrogen gas administration when compared to values obtained after prolonged administration or administration to equilibrium. The theoretical analysis presented above suggests that blood flow values obtained after brief H_2 administration and administration to equilibrium should be identical. However, our experimental results did not corroborate that claim. Instead, our finding that there is a small but significant increase in the flow values

obtained after 1 minute of H_2 administration over that obtained after 5 minutes of administration confirmed the previous findings of Leung et al.[12] We cannot offer any explanation for the lack of concordance between these theoretical and experimental findings. However, Halsey et al.[21] measuring cerebral blood flow with HGC in animals, have suggested that the higher flow values they observed following brief inhalation of H_2 may be due to the intercompartmental diffusibility of hydrogen gas in the brain.

In conclusion, we have presented a current amplifier that significantly improves the magnitude and more importantly, the quality of the HGC signal obtained in the measurement of focal GMBF. The amplifier significantly reduces baseline drifts. Theoretically and empirically, GMBF values determined from the washin portion of the HGC curve are similar and not significantly different from the corresponding flow values obtained conventionally from the washout portion of the curve in steady-state conditions. Washin curve blood flow values and the use of rapid protocols with the current amplifier reduces one HGC measurement time and allows closer compliance to a significant theoretical requirement of this technique.

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Chapter 5

The Dose-Response Of Canine Focal Gastric Mucosal Blood Flow to Misoprostol†

INTRODUCTION

Prostaglandins (PGs) at doses as small as 1% of their antisecretory doses have been reported to protect the gastric mucosa against injury by necrotizing agents.[1] The mechanism(s) of this gastric 'cytoprotection' is not known. However, an increase in gastric mucosal blood flow (GMBF) by PGs has been proposed as a possible mechanism.[2-4] This increase in GMBF is hypothesized to: (i) maintain adequate tissue oxygenation and supply of nutrients, (ii) buffer H^+ that back-diffuses into the lamina propria, and (iii) dilute and remove both back-diffused H^+ and the damaging agent which have gained access into the lamina propria.[5,6] So far, studies of the effects of PGs on gastric blood flow have yielded conflicting results. Investigators have reported an increase in resting gastric blood flow by PGs,[3,7,8], a decrease,[9-11], or no effect.[2,3,12]

Gastric cytoprotection by PGs has been shown to be dose-dependent in laboratory animals[13,14] and humans.[15,16] This suggests that the vasoactive effects of PGs, if fundamental to cytoprotection, may also be dose-dependent. There are no reports in the literature of such dose-response studies of focal GMBF to PGs. The purpose of this study was to determine the dose-response of focal GMBF to misoprostol, a synthetic PGE_1 analog with proven potent antisecretory and cytoprotective properties[3,13,16-19] in canine chambered gastric segment model. We also compared the sensitivity of laser-Doppler flowmetry (LDF) and hydrogen gas clearance (HGC) in the detection of these vascular responses.

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MATERIALS AND METHODS

Surgical Preparation

Five adult mongrel dogs of either sex (weighing 24 - 27 kg) were fasted for 24 hours but allowed free access to water before each experiment. The dogs were anesthetized with sodium pentobarbital (25 mg/kg) and maintained on room air with a Harvard respirator (Harvard Apparatus, South Natick, MA) throughout each experiment. A polyethylene catheter (ID 0.055 in) was placed in a hind leg vein for the infusion of 0.9% NaCl to maintain hydration and a stable blood pressure. Another saline-filled polyethylene catheter (ID 0.055 in) was placed in the left femoral artery for direct monitoring of the arterial blood pressure throughout each experiment via a P 23 Db Gould Statham transducer (Gould Statham Instruments Inc., Hato Ray, Puerto Rico). A midline laparotomy and splenectomy were then performed.

Experimental Model

A chambered segment model of the gastric corpus with an isolated vascular pedicle was prepared by the method of Moody and Durbin.[20] We have previously compared LDF and HGC in this experimental model and shown that probe/electrode-mucosa contact is not a problem.[21] After surgical preparation the animals were allowed to stabilize for 60 minutes. The temperature of the preparation and the body of the dog were maintained at 37°C by infra-red lamps and an electric blanket.

Experimental Design

The protocol involved 15-min periods as follows: basal periods (60 min), during which 10 ml of 150 mM NaCl was instilled into the gastric chamber at the beginning and recovered at the end of each period, and misoprostol periods (150 min), during which increasing doses (0.1, 1, 10, 100 and 1000 μg) of misoprostol (G.D. Searle) were added to the solutions, instilled into the chamber, and recovered at the rate of two periods per dose. The GMBF was measured simultaneously by LDF and HGC for every 15-minute period throughout the experiments. The

measurement of GMBF by both techniques was commenced immediately after dosing at the beginning of each 15-min period.

Measurement of Gastric Mucosal Blood Flow

Hydrogen gas clearance. Hydrogen gas clearance was performed as described previously.[21,22] In this study, HGC was measured with four platinum electrodes (Unique Medical Co. Ltd., Tokyo, Japan) placed in contact with the mucosa at the centre of each of the four quadrants of the chamber. There were no electrode relocations throughout the experiments. The HGC measurement was performed within each 15-minute period.

Laser-Doppler flowmetry. Details of the measurement of GMBF by this technique were as described previously.[21] The LDF probe (Periflux Pf 1d, Perimed, Stockholm, Sweden) was paired with one of the HGC electrodes in each experiment in rotation. The laser-Doppler flowmeter settings for all the experiments were as follows: Doppler shift frequency, 12KHz; and gain, X10.

To obtain simultaneous measurements of GMBF by both techniques at the same focal point, the platinum HGC electrode was first placed in contact with a preselected mucosal spot in the middle of a quadrant, without pressure. The electrode rested on the side wall of the holes provided in the modified top of the transparent lucite chamber at an angle of 45^{o}. This permitted the LDF probe to be placed perpendicularly at a distance of less than 6 mm over the point of mucosal contact of the HGC electrode by centering the narrow beam of laser light on that point. A permanent analog record of all the monitored variables (blood pressure, LDF, HGC) was obtained with a multichannel recorder (Beckman R Dynograph, Beckman Instruments Inc., Schiller Park, IL). LDF and HGC flow values were obtained from an IBM PC (IBM, Boca Raton, FL) by feeding in the raw data from the analog chart records.[21,22]

Data Analysis

All data were expressed as mean ± SE. Analysis for statistical significance at the 5% level was by the paired Student's *t* test and simple linear regression analysis. Computer-assisted analysis of the dose-response curve for LDF was performed with the program developed by Cook and Bielkiewicz[23] to obtain the ED_{50}, E_{max}, and so forth. The GMBF data obtained for the basal periods by both techniques were grouped into 30-min periods to achieve consistency with the misoprostol periods, in which flow data were also grouped into 30-min periods on the basis of dosage.

RESULTS

Arterial blood pressure before and during misoprostol administration remained stable throughout the experiments even during inhalation of hydrogen gas by the dogs.

Macroscopic Appearance

Gross swelling of the gastric mucosa became prominent immediately after the administration of the first dose of misoprostol in each experiment. The swelling increased with each dose, and at the end of each experiment the gastric segment appeared like a piece of brain tissue. There was also a macroscopic increase in gastric mucus adherent to the mucosa. The mucosal surface appeared normal throughout each experiment.

Gastric Mucosal Blood Flow

Basal Flow. The mean (± SE) GMBF values obtained by LDF in the first and second 30-min basal periods were 7.5 ± 1.0 and 6.9 ± 0.7 volts, respectively. There were no statistically significant differences between these means ($p > 0.05$, *t* test). The mean (± SE) GMBF values obtained by HGC in the first and second 30-min basal periods were 51.6 ± 2.4 and 47.6 ± 2.4 ml/min/100g of tissue, respectively. Similarly, these were not significantly different from each other ($p > 0.05$, *t* test). Regression analysis showed a highly significant linear correlation (r =

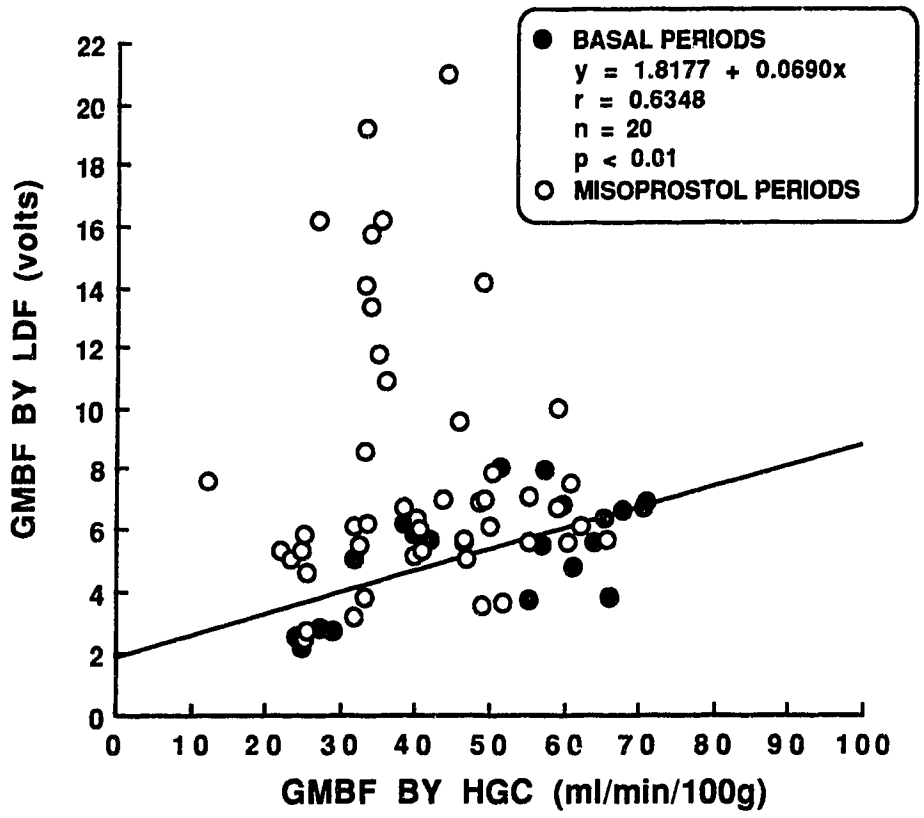

Figure V-1. Regression analysis and scattergram of the simultaneous LDF and HGC GMBF values obtained from all dogs during the experiments. Regression analysis showed a highly significant linear correlation between the GMBF values in the basal periods (●). The GMBF values obtained in the misoprostol periods (O) were not correlated. Regression line for GMBF values in the basal periods is shown.

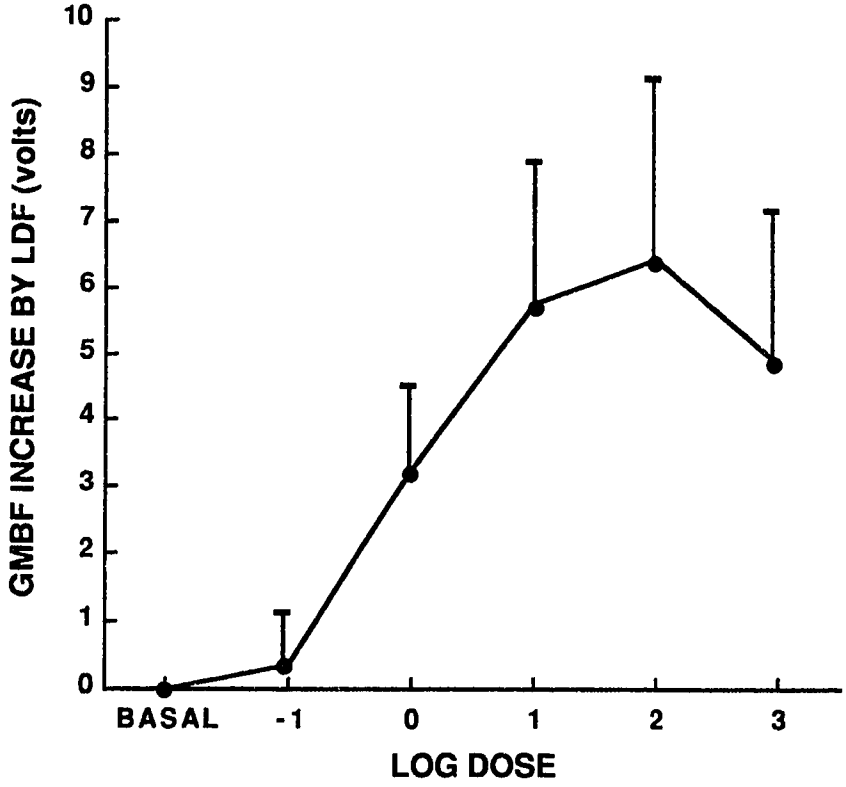

Figure V-2. The dose-response curve of focal GMBF measured by LDF in response to topical misoprostol.

0.63, n = 20, $p < 0.01$) between the simultaneous GMBF values obtained by the two techniques in the basal periods for all the experiments (Fig. 1).

Blood Flow During Topical Misoprostol. (i) Laser-Doppler flowmetry: LDF measured a dose-dependent increase in GMBF during the administration of misoprostol (Fig. 2). The increase was observed with the first dose of misoprostol (0.1 μg). E_{max}, the maximum increase above the basal period mean (± SE) GMBF, was 6.4 ± 2.8 volts, corresponding to a 92.8% increase at the 100 μg dose.The ED_{50} was 1.0 μg. The mean (± SE) time of onset of GMBF increase, after dosing with misoprostol, was 1.8 ± 0.5 min (n = 48) for all the doses administered. With each individual dose the time of onset of the increase in GMBF increased with increasing dose of misoprostol up to a maximum at the 100 μg dose (Table 1). The peak increase in GMBF was

attained in 6.1 ± 0.7 min (n = 48) and was maintained for 1.9 ± 0.3 min after each dose of misoprostol.

Table V-1. MEAN (± SE) TIME OF ONSET OF GMBF INCREASE MEASURED BY LDF AFTER DOSING WITH MISOPROSTOL

Doses (μg)	n*	Time of Onset (min)
0.1	8	0.81 ± 0.81
1	10	0.60 ± 0.34
10	10	1.50 ± 0.79
100	10	3.40 ± 1.80
1000	10	2.60 ± 1.13
Total	48	1.82 ± 0.50

n* = Number of GMBF measurements.

(ii) Hydrogen gas clearance: Mean (± SE) GMBF values obtained by HGC showed a graded drop from the basal period mean flow value after the administration of misoprostol (Fig. 3). The mean (± SE) duration of each HGC measurement was 13.1 ± 0.1 min (n = 271) from the time of dosing with misoprostol, i.e., the beginning of each 15-min period.

The simultaneous LDF and HGC GMBF values obtained during the misoprostol periods were not correlated in each dog or in all dogs combined (Fig. 1).

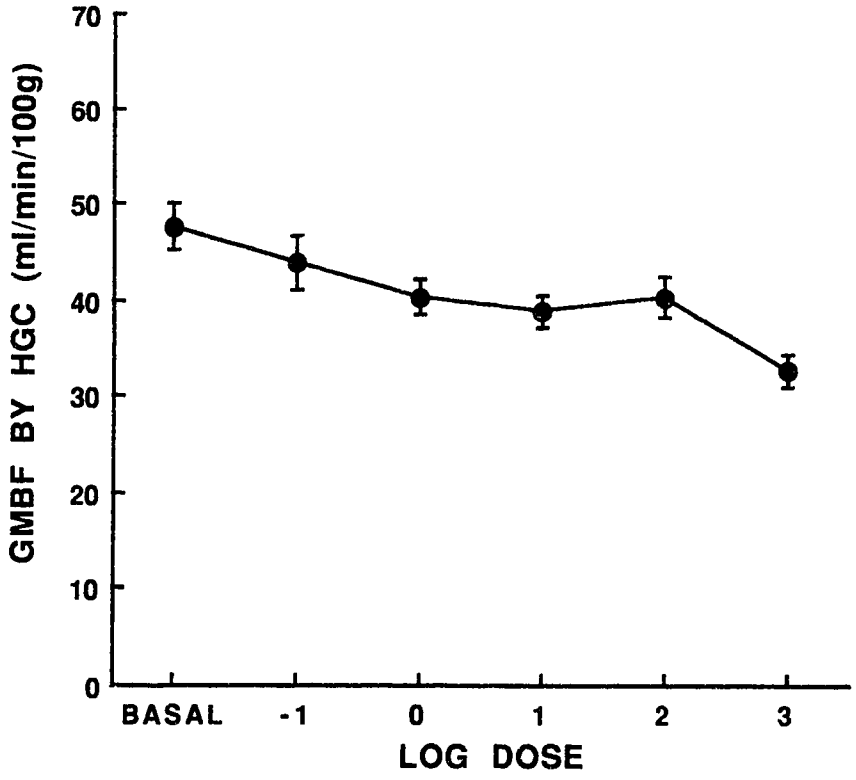

Figure V-3. The dose-response of focal GMBF measured by HGC in response to topical misoprostol. There was no dose-dependent increase in focal GMBF. Basal denotes the final basal 30-min period.

DISCUSSION

Cytoprotective PGs that are known vasodilators have not been shown to consistently increase resting gastric blood flow. With regard to misoprostol, an increase in total blood flow by venous outflow of about 400% after topical administration has been reported in the canine chambered gastric segment model.[3] Similarly, Sato et al.[24] have reported an increase of 10-25% in gastric mucosal blood volume obtained by reflectance spectrophotometry at different sites in the corpus and antrum of healthy human male volunteers after an oral antisecretory dose of misoprostol. In contrast, Leung et al.[12] reported that at both cytoprotective and antisecretory doses, misoprostol had no effect on resting focal GMBF as measured by HGC in rats. The reason(s) for the lack of a consistent finding in vascular responses to PGs may be (i) different routes of PG administration, (ii) different methods of blood flow measurement, (iii) different animal models used, (iv) state of the gastric mucosa, whether resting or stimulated, (v) different experimental protocols, and (vi) the use of different damaging agents with varied effects on gastric blood flow along with PGs in studies of gastric cytoprotection.

The results of our study explain to some extent the inability of previously used techniques to document a consistent increase in GMBF by vasoactive PGs. We utilized two, recently available, noninvasive techniques, LDF and HGC,[25-27] which are capable of focal GMBF measurements. LDF has the unique advantage of measuring flow instantaneously and continuously. The dose-dependent increase in GMBF spanning over both cytoprotective and antisecretory doses detected by this technique is obviously related to this unique ability. HGC did not register an increase in GMBF because the duration of each measurement exceeded the duration of the peak flow increase induced by misoprostol. This may explain why Leung et al.[12], who commenced HGC measurement 15 minutes after dosing with misoprostol were unable to demonstrate any increase in blood flow in their study. Another possibility that may account for the differences in the response to misoprostol-induced GMBF changes by the two techniques is the differences in spatial resolution. Reports in the literature suggest that LDF has a depth

sensitivity corresponding to the full thickness of the gastrointestinal wall,[28-30] whereas HGC is believed to measure flow in the superficial layer (mucosa) even though this has not been verified experimentally in the gastrointestinal tract. We have shown histologically that the misoprostol-induced gross mucosal swelling is due to marked oedema of the mucosa and submucosa.[31,32] Vasodilatation and congestion of the vessels in both layers were particularly prominent in the mucosa. These histologic findings indicate that misoprostol affected both mucosa and submucosa; therefore differences in the depth sensitivity of the techniques cannot explain the lack of response of HGC. On the other hand, the gross mucosal swelling due to oedema may have influenced HGC measurements by reducing the tissue-blood partition coefficient for hydrogen gas (1 ml/g), and hence the flow values. We do not, however, have any data to verify this possibility.

The ED_{50} of 1.0 μg (2.6×10^{-7} M) obtained in this study was 100 times less than the E_{max} dose of 100 μg (2.6×10^{-5} M). This ratio is much less than that of Holm-Rutili and Obrink (between 0.1 and 1), which we calculated from their data for topical PGE_1.[33] Histologic and *in vivo* microscopy studies have shown that in animals and humans, mucosal blood flow originates from submucosal arterioles, which break up into capillaries that run perpendicularly to reach the honeycomb capillary network situated just beneath the surface epithelium.[34-37] *In vivo* microscopy studies have further demonstrated that the dilatation or constriction of these submucosal arterioles is responsible for the local control of GMBF.[36] It is therefore conceivable that the dose-dependent increase in the mean time of onset of GMBF increase after dosing observed in this study is related to the time required for misoprostol to be absorbed across the mucosa/submucosa. In the study by Holm-Rutili and Obrink,[33] increase in blood flow in single superficial gastric microvascular vessels measured by *in vivo* microscopy occurred within a few minutes and reached a maximum in 15 min after topical application of PGE_1 to a mucosal preparation. The differences in results might be due to the fact that they measured blood flow in single microvascular vessels, whereas we observed changes in the relative flux of RBCs by LDF.

Previous reports have shown that oral PGs reduce the number and severity of gastric lesions by as much as 80% when given 1 min before topical absolute ethanol, and the protection is complete when given 2 min before injury. This protective action lasts approximately 2 hours.[1,38] We have previously shown that in misoprostol-pretreated aspirin-induced injury, misoprostol induces only a transient increase in GMBF and does not prevent the subsequent decrease in GMBF by aspirin even though mucosal protection was observed.[32] The results of this study confirm our former findings[32] that PG-induced changes in GMBF are transient and further strengthens our suggestion that increase in or maintenance of GMBF *per se* may not be the mechanism of gastric cytoprotection by PGs. This does not exclude the possibility that increase in GMBF may be related to other processes that lead to eventual cytoprotection.

The significant correlation between LDF- and HGC-measured GMBF values in the basal periods but not during misoprostol dosing indicates that when blood flow was stable, HGC was sensitive enough to measure blood flow, but during rapid changes in flow, as induced in this case by misoprostol, HGC was not sensitive enough to detect these changes. This finding constitutes a significant limitation of HGC in all situations in which steady blood flow states are not maintained.

In conclusion, we have demonstrated a dose-dependent increase in focal GMBF by a synthetic PGE_1 analog, misoprostol. Owing to the transient nature of the change in GMBF, however, only LDF is sensitive enough to detect it. The duration required for each blood flow measurement by HGC is a major limitation of this technique. Thus HGC only measures blood flow in steady states. In any study of focal GMBF, therefore, the choice of a measurement technique is decisive, and the limitations of each technique should be given due consideration.

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Chapter 6

Focal Gastric Mucosal Blood Flow in Aspirin-Induced Ulceration†

INTRODUCTION

There is virtual unanimity among investigators that the one basic pathogenic feature of all stress ulcers is mucosal ischemia.[1] The dearth in methods capable of estimating mucosal blood flow in localized areas of the stomach has hindered the investigation of focal ischemic phenomena. Although drug-induced acute erosive gastritis is often identical in both gross and microscopic appearance to stress ulcers,[1-3] a distinction in pathogenesis has been made between them. Much of this controversy is consequent on contradictory reports on the role of mucosal blood flow in drug-induced acute erosive gastritis.[4-8] Most previous studies either reported an increase in total gastric mucosal blood flow (GMBF)[4,6,7] or inability to demonstrate focal ischemia[8] during acute erosive gastritis induced by aspirin, ethanol, and bile salts.[5]

More recently, hydrogen gas clearance (HGC) has been developed and its validity has been established for the assessment of focal GMBF.[9-13] Ashley et al. had subsequently applied it to the measurement of focal GMBF at the site of aspirin-induced ulceration.[14] They reported a decrease in GMBF at the site of ulceration and a hyperemic response at the nonulcerated sites. This demonstration of focal ischemia at the site of aspirin-induced ulceration has provided the first supportive evidence for ischemia as a major pathogenic feature in a drug-induced gastric mucosal injury. The aim of this study was to further investigate the role of focal GMBF in acute erosive gastritis by simultaneously studying flow at both the nonulcerated and ulcerated sites before, during, and after topical aspirin administration using the HGC technique.

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MATERIALS AND METHODS

Four adult mongrel dogs of either sex (mean weight: 24 kg) were fasted for 24 hours before each experiment and anesthetized with pentobarbital sodium (25 mg/kg). The dogs were ventilated by a Harvard respirator (Harvard Apparatus, South Natick, MA) throughout each experiment. A polyethylene catheter (PE 200) was placed in a femoral vein for infusion of fluid, and arterial blood pressure was directly monitored throughout each experiment by another saline-filled femoral arterial polyethylene catheter (PE 200; Gould Statham transducer P 23 Db) (Gould Statham Instruments Inc., Hato Ray, Puerto Rico). A chambered segment model of the gastric corpus with an isolated vascular pedicle was prepared according to the method of Moody and Durbin.[15] The top of the lucite chamber was modified to allow stable placement of the HGC electrodes. After surgical preparation, the animals were allowed to stabilize for 30 minutes.

The experimental protocol involved 15-min periods during which isotonic HCl or HCl plus aspirin were instilled into and recovered from the gastric chamber at the end of the periods. In the first 1-1.5 hours (basal periods) of each experiment, 20 ml of 150 mM HCl was instilled, then in the next hour (aspirin periods), 20 ml of 150 mM HCl plus 20 mM of aspirin was instilled. In the last 2 hours of each experiment, 20 ml of 150 mM HCl only was again instilled into the chamber in each period. During each 15-min period, simultaneous measurements of focal GMBF were performed at least once using two HGC electrodes (Unique Medical Co. Ltd., Tokyo, Japan) placed in contact with the mucosa at different points within the chamber. It was possible to perform HGC measurements of focal GMBF twice within each 15-min period in the basal periods. Only one of the electrodes was moved during the aspirin periods to an area of focal pallor on the mucosa once such an area became evident. However, in one experiment it was not necessary to move the electrodes because one of the electrodes was located at a site that subsequently became pale and then ulcerated during and after aspirin administration, respectively. Once an electrode had been relocated to a pale area it was never

moved again until the end of the experiment. Simultaneous flow measurements from the pale and nonpale areas were then carried out throughout the remaining periods of the experiment.

HGC was performed with pure hydrogen gas using standard technique.[10] A Ag/AgCl skin electrode (Red Dot, 3M, Canada Inc., London, Canada) attached to a shaved area of groin skin was used as a reference. The terminals of the reference and hydrogen electrodes were connected to a strip chart multichannel recorder (Beckman Dynograph). The recorder pen was centered on the baseline, and hydrogen gas was administered through a funnel to the input port of the Harvard respirator, allowing some mixing with room air, at the rate of 1-2 L/min for 60 seconds. A hydrogen washin-washout curve was obtained and the data from the washout part of the curve was fed into a microcomputer (IBM-PC, IBM, Boca Raton, FL) in which we had written a program to obtain semilogarithm plots of current versus time. The program rejects plots that are not monoexponential at the 1% level of significance. The computer also automatically gives the $t1/2$ of accepted plots and calculates GMBF values expressed in ml/min/100g of tissue from the formula $F = (0.693 \div t1/2)\ (\lambda) \times 100$.[10]

Flow values are expressed as mean ± SE. The data were analyzed for statistical significance with either the Student's *t* test or ANOVA. To allow for more data in testing for statistical significance, the flow values were lumped together into sequential 30-min periods.

RESULTS

Focal mucosal pallor after topical aspirin administration was not evident until the second half hour of aspirin exposure in all experiments. The areas of mucosal pallor became darkened and subsequently ulcerated as reported by Ashley et al.,[14] but we did not observe areas of focal pallor within the first 30 minutes after exposure to aspirin in any of our experiments. Although hemorrhagic areas were seen in the gastric chamber mucosa in some of the experiments, frank bleeding was not seen in this study.

Simultaneous measurements of resting focal GMBF by two HGC electrodes located on different areas of the mucosa gave mean ± SE values of 67.26 ± 3.14 and 61.18 ± 2.16

(ml/min/100g), respectively. A paired *t* test revealed no significant difference between these means ($p > 0.05$). This finding suggests that resting focal GMBF in different areas of the same gastric segment are not significantly different during the same periods.

The sequential changes in the combined focal GMBF measured in the basal periods, nonulcerated and ulcerated areas during and after 20 mM aspirin exposure in all experiments are shown in Figure 1. During the three basal periods, GMBF was stable with mean ± SE values of 62.32 ± 2.40, 65.39 ± 3.10, and 62.03 ± 3.12 (ml/min/100g), respectively. There were no significant differences between these mean flow values ($p > 0.05$; ANOVA). Blood flow subsequently remained stable during the first half hour period of aspirin exposure as the mean ± SE flow value of 61.58 ± 4.01 (ml/min/100g) was also not significantly different from the mean flow value in the final basal period ($p > 0.05$). In the second half hour period of aspirin exposure, however, there was a significant decrease in GMBF (45.92 ± 4.97; $p < 0.05$), which was followed by a highly significant decrease in flow ($p < 0.01$ or $p < 0.001$) in the remaining four post-aspirin exposure 30-min periods. The data did not show a recovery in blood flow to basal levels. The basal mean blood flow values reported here are similar to the resting mean focal GMBF values obtained previously by HGC in the same experimental model by other investigators.[11] Comparable resting focal GMBF values have also been obtained by HGC in the intact stomach of dogs by the same investigators.[13] The time course of events and the decrease in GMBF in both ulcerated and nonulcerated areas combined are in agreement with the studies of O'Brien and Silen[5] in which a severe decrease in total GMBF measured by aminopyrine clearance was reported following aspirin-induced gastric mucosal injury in denervated canine fundic pouches.

Figure 2 demonstrates the changes in focal GMBF in the nonulcerated and ulcerated areas of the mucosa before, during, and after topical administration of 20 mM of aspirin. We observed a graded decrease in GMBF after aspirin exposure in the nonulcerated areas but this was not significant until 90 minutes after exposure ($p < 0.05$). The mean blood flow value in the last half hour period was not significantly different ($p > 0.05$) from the mean flow value in the

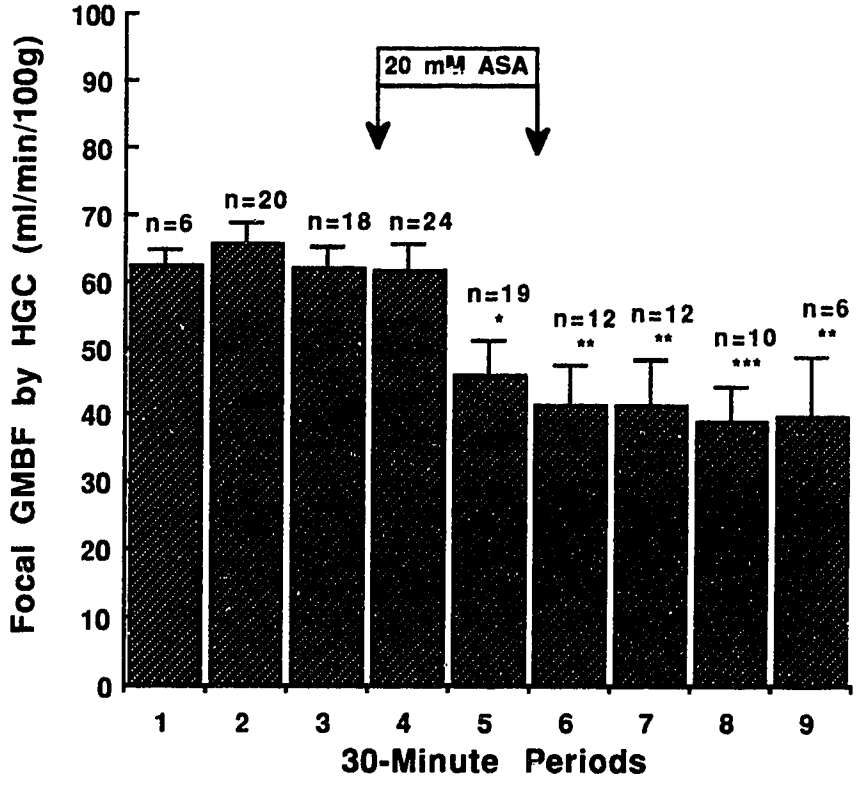

Figure VI-1. Focal GMBF in ml/min/100g (mean ± SE) before, during, and after topical aspirin administration (ASA). Asterisks indicate significant difference from the mean (± SE) basal GMBF in period 3. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. n = number of observations in each period. HGC = hydrogen gas clearance.

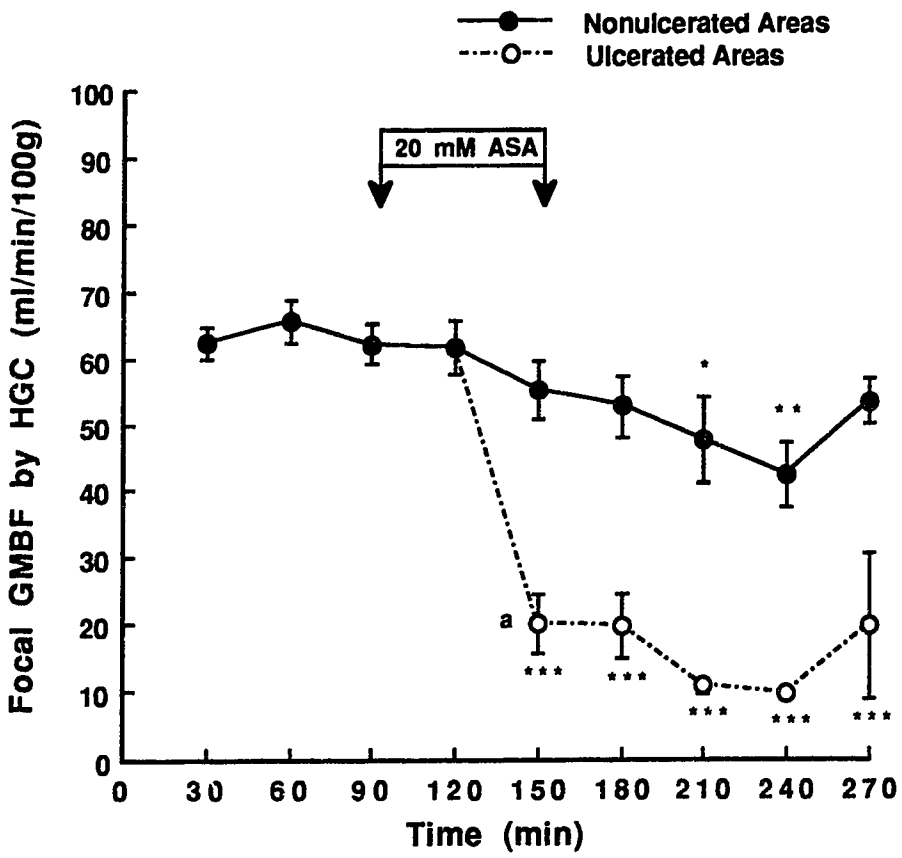

Figure VI-2. Sequential changes in focal GMBF (mean ± SE) in the ulcerated (O) and nonulcerated areas (●) before, during, and after ASA. a - indicates point at which one electrode was relocated to an area of focal mucosal pallor during the ASA periods. Asterisks indicate significant difference from the mean (± SE) basal flow values at 90 min. Since the simultaneous measurements of resting focal GMBF by the two HGC electrodes located on different areas of the mucosa gave mean ± SE values which were not significantly different, the basal flow values were lumped together.

final basal half hour period, indicating a recovery in blood flow. We did not record a hyperemic response in the nonulcerated areas as previously reported.[14] In the ulcerated areas, 30 minutes after aspirin exposure, there was a highly significant decrease in GMBF ($p < 0.001$) that persisted throughout the rest of the half hour periods. There was no recovery in flow in the ulcerated areas to basal values.

Figure 3 illustrates the mean ± SE focal GMBF values simultaneously obtained from the nonulcerated and ulcerated areas in all the experiments. A paired Student's *t* test revealed a highly significant difference between the means ($p < 0.001$). The mean blood flow in the ulcerated areas was about 50% and 30% of the mean flow in the nonulcerated areas and the mean flow in the basal periods, respectively.

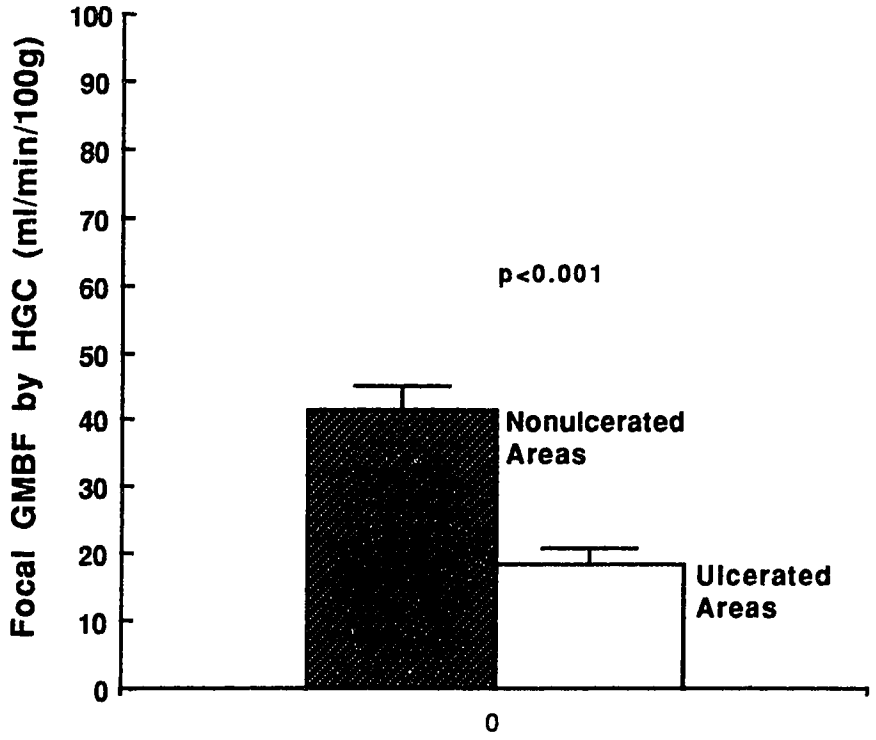

Figure VI-3. Comparison of simultaneous focal GMBF (mean ± SE) in the ulcerated and nonulcerated areas during and after ASA.

Figure 4 shows the pattern of change in focal GMBF in one experiment recorded by an HGC electrode that was positioned in an area of the mucosa that became pale and subsequently ulcerated after exposure to 20 mM of aspirin. There was no necessity for an electrode shift in this experiment. The changes in focal GMBF before, during and after aspirin recorded by this electrode are similar to those recorded in other experiments where an electrode was shifted to a focal area of mucosal pallor.

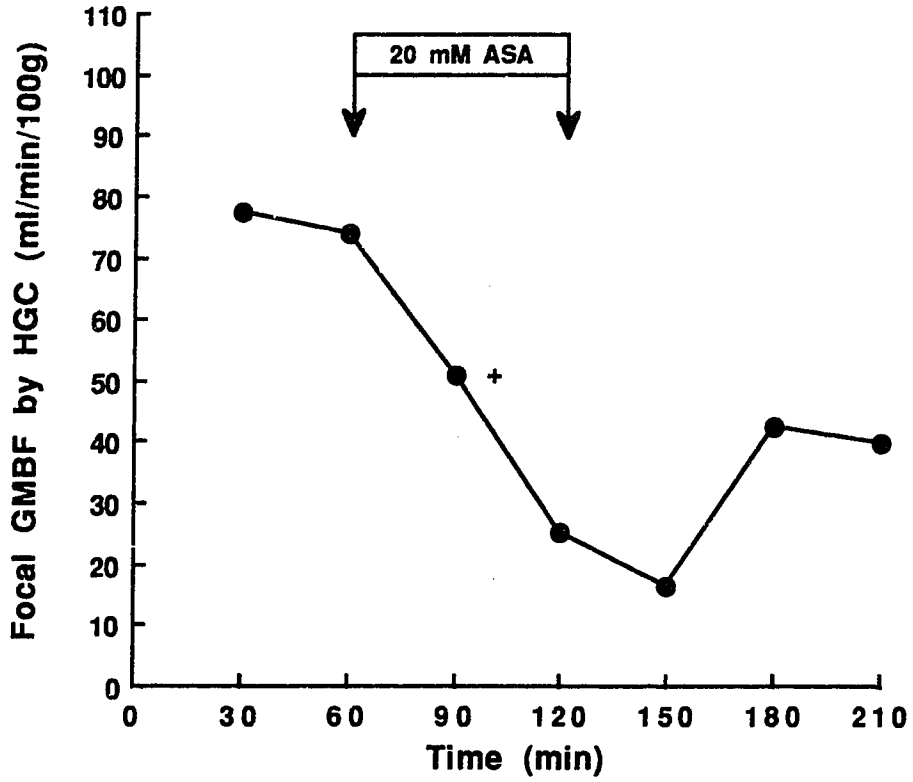

Figure VI-4. Sequential changes of GMBF at one focal point in one experiment where there was no necessity for an electrode shift. Cross (+) denotes the point at which the mucosa became pale and then subsequently hemorrhagic and ulcerated.

DISCUSSION

A few reports have implicated focal mucosal ischemia as a major event in the development of aspirin-induced acute erosive gastritis.[5,14,16,17] Investigators have also reported the localized nature of the lesions in aspirin-induced mucosal injury.[5,7,14,16,17] However, the demonstration of an increase rather than a fall in GMBF after aspirin injury by others,[4,7] has suggested a contradictory role for mucosal blood flow in aspirin-induced acute erosive gastritis. These conflicting results may be due to limitations of the techniques used in estimating GMBF, i.e., aminopyrine clearance and radioactive microspheres. Both of these techniques are limited by their inability to document differences in blood flow in very small areas.[14] In a recent study by Ashley et al., HGC was used to estimate focal GMBF.[14] They found GMBF to be significantly reduced at the site of aspirin-induced ulceration. They also reported a hyperemic response in the nonulcerated areas and speculated that the increase in GMBF in these areas was the result of a compensatory protective response to back-diffusion of hydrogen ions. After the removal of aspirin they noticed a return of GMBF to control levels in both ulcerated and nonulcerated areas. They concluded that the redistribution of GMBF was consistent with the localized nature of aspirin-induced injury. This focal nature of blood flow changes also explained the inability of previous techniques that measure flow of the entire stomach mucosa to demonstrate a consistent response.

Our results confirmed the occurrence of focal ischemia at the site of aspirin-induced injury. The fall in focal GMBF preceded the appearance of grossly visible lesions such as hemorrhagic foci and ulceration. The mean focal GMBF in the ulcerated areas dropped to about 30% of the mean basal GMBF; it was about 50% of the mean flow in the nonulcerated areas measured simultaneously. There was no recovery of focal GMBF in the ulcerated areas to basal levels. This absence of recovery and the appearance of areas of focal mucosal pallor after only 30 min of aspirin exposure are in conflict with the findings of Ashley et al.[14] The reasons for these discrepancies may be due to the rather low basal (control) GMBF values of their preparations (30 - 40 ml/min/100g). These basal values were about 50% of our basal values and

were only slightly above the focal GMBF values at which we observed features of gross mucosal injury. Furthermore, in two other separate studies reported by the same authors and using the same techniques, higher values of resting GMBF comparable to ours were obtained in the chambered canine gastric segment model[11] and in the intact stomach of dogs.[13] Their much lower basal GMBF may offer an explanation for the immediate appearance of focal mucosal pallor after aspirin exposure and the recovery in the ulcerated areas of focal GMBF to its antecedent low basal values. The appearance of grossly visible lesions only 30 min after aspirin exposure in our experiments is in agreement with observations reported by other investigators.[4,8]

In the nonulcerated areas, focal GMBF decreased to about two thirds of the basal levels after aspirin exposure and was accompanied by recovery to basal flow levels after removal of aspirin. We did not record a hyperemic response in the nonulcerated areas immediately after exposure to aspirin as reported by Ashley et al.[14] It is unlikely that a hyperemic response was missed because we did not shift the electrodes on the nonulcerated areas throughout our experiments. Our results are in agreement with those reported by O'Brien and Silen who also did not observe a hyperemic response but a significant decrease in total GMBF after aspirin treatment.[5]

The pattern of change in focal GMBF during and after aspirin injury was consistent throughout our experiments. On exposure to topical aspirin, there is a decrease in focal GMBF of varying degrees. It appears that there is a "critical focal GMBF value" below which flow must drop for the gross features of mucosal injury (focal pallor progressing to hemorrhagic foci and ulceration) to appear. Any decrease in focal GMBF that does not fall below this "critical GMBF value" fails to show visible mucosal injury and is followed by recovery to basal GMBF levels after removal of the aspirin insult. On the other hand, decreases in focal GMBF below this "critical value" are not associated with recovery, at least within 2 hours after injury. From our data, it would appear that this "critical focal GMBF value" is about 20 - 30 ml/min/100g.

We do not know the reason for the selective focal nature of aspirin-induced mucosal injury but it has been suggested that the effect may be a threshold one as might be obtained by a high local absorption of drug caused by the presence of particles.[17] Ultrastructural studies have revealed that the primary effect of aspirin on the gastric mucosa is focal and on the basement membrane of the capillary and postcapillary venules leading to their breakdown before any other cytolytic effects.[17] These studies had led investigators to conclude that localized vascular stasis precede the development of frank gastric erosions in aspirin injury.[16,17] The demonstration of varying degrees of focal ischemia after aspirin exposure at ulcerated and nonulcerated sites in this study is consistent with and may be the functional manifestations of these ultrastructural changes. Our inability to demonstrate recovery in focal GMBF to basal levels in the ulcerated areas may be due to the tissues' inability to achieve complete repair to the frankly damaged blood vessels within 2 hours after aspirin injury.

In conclusion, we have demonstrated focal ischemia at the site of aspirin-induced acute erosive gastritis. Based on our results, a "critical GMBF value" hypothesis has been proposed. It is our proposition that aspirin induces a decrease in focal GMBF of varying degrees that, if severe enough to result in a fall of GMBF below the "critical GMBF value," leads to the appearance of gross mucosal injury without recovery to basal levels. If the reduction in focal GMBF is not below this "critical value" there is no visible mucosal injury but is followed by recovery of GMBF to basal levels after removal of aspirin. The demonstration of the absence of a hyperemic response to aspirin injury in the nonulcerated mucosa using a focal technique is a novel one. Our results are consistent with the view that focal mucosal ischemia is an important pathogenetic mechanism antecedent to aspirin-induced mucosal erosions. Finally, there may be no real difference in the focal ischemic nature of the gastric mucosal injury in drug-induced and stress-related ulceration. The issue of the mechanisms for the selective focal nature of aspirin-induced injury will await further research.

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Chapter 7

Gastric Mucosal Blood Flow in Misoprostol Pretreated Aspirin-Induced Ulceration†

INTRODUCTION

The precise mechanism(s) of gastric cytoprotection by prostaglandins (PG) is not known. The circulatory hypothesis proposes that the cytoprotective action is mediated through an increase in gastric mucosal blood flow (GMBF).[1-7] This hypothesis has, however, been faced with problems including (1) the results of studies of the effects of PG on GMBF have been inconsistent[3]; (2) though a number of the A, E, and I PG increase GMBF,[3,8-10] $PGF_{2\alpha}$, also cytoprotective,[11,12] is a known vasoconstrictor[8,13-16]; and (3) 16,16-dimethyl PGE_2 has been reported to prevent ulcer formation in the absence of arterial perfusion in isolated canine gastric mucosa,[17] in amphibian gastric mucosa *in vitro*,[18] and in gastric cell cultures in rats.[19,20]

Misoprostol is a synthetic PGE_1 analog that has been reported to have both gastric antisecretory and cytoprotective properties.[21-30] There have been few reports in the literature on the effects of misoprostol on gastric blood flow. While some investigators have reported an increase in total gastric blood flow[2] and gastric mucosal blood volume,[31] others have reported either a decrease in total GMBF[23] or no effect.[27] We designed this study to elucidate further the circulatory hypothesis by a controlled investigation of focal GMBF changes using the hydrogen gas clearance (HGC) technique before, during and after misoprostol pretreatment of aspirin-induced mucosal injury in a canine chambered gastric segment model. Specifically, the aim of this study was to test the hypothesis that pretreatment of the gastric mucosa with an antisecretory dose of misoprostol would increase or maintain gastric mucosal blood flow during aspirin injury. Second, the fluid and ionic fluxes were measured to determine any interrelationship between GMBF, fluid/ionic fluxes, and gastric cytoprotection.

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†A version of this chapter has been published as:
Gana TJ, MacPherson BR, Koo J. Gastric mucosal blood flow in misoprostol pretreated aspirin-induced ulceration. Ann Surg 1988; 207: 327-334.

MATERIALS AND METHODS

Surgical Preparation:- Four adult mongrel dogs of either sex, weighing 20-30 kg, were anesthetized with sodium pentobarbital (25 mg/kg) after a 24-hour fast (only water *ad libitum*). The dogs were intubated and maintained on a Harvard respirator (Harvard Apparatus, South Natick, MA) throughout each experiment. A hind leg vein was cannulated with a polyethylene catheter (PE 200) for infusion of 0.9% sodium chloride for the maintenance of hydration and a stable blood pressure. Arterial blood pressure was directly monitored by a saline-filled polyethylene catheter (PE 200) in the left femoral artery via a Statham Gould P 23 Db transducer (Gould Statham Instruments Inc., Hato Ray, Puerto Rico). Following a midline laparotomy and splenectomy, a split chambered segment model of the gastric corpus with an isolated vascular pedicle was prepared according to the method of Moody and Durbin.[32] This model permits the use of one side of the chamber as test, the other as control and provides gastric mucosal surface of 17 cm^2 in each side. We have shown that electrode-mucosa contact is not a problem in this experimental model.[33] Body temperature and the temperature of the preparation were maintained by an electric blanket and infra-red lamps. The preparation was allowed to stabilize for 60 min after surgery.

Experimental Design:- Focal GMBF measurements were performed once every 15-min by two HGC electrodes placed in contact with the mucosa in each lumen of the chamber. One electrode was located in the upper and the other in the lower quadrants of each lumen throughout the experiments. Once the electrodes had been located at the beginning of each experiment there was no relocation throughout the experiments. Bathing solutions (8 ml) were instilled into and recovered from each lumen every 15-min as follows:-

Test Chamber. (1) basal periods (60 min): 100 mmol/L HCl + 50 mmol/L NaCl; (2) misoprostol periods (30 min): 100 mmol/L HCl + 50 mmol/L NaCl + 1 ml misoprostol (200 μg); (3) aspirin

periods (60 min): 100 mmol/L HCl + 50 mmol/L NaCl + 20 mmol/L aspirin; and (4) post-aspirin periods (120 min): same as for the basal periods.

Control Chamber. Solutions instilled were the same as for the test chamber except in (2) where instead of misoprostol, 1 ml of vehicle (0.2 ml absolute ethanol + 0.8 ml phosphate buffer) was added to HCl and NaCl.

Laboratory assays were performed on the recovered bathing solutions to determine the changes in volume, pH, sodium and hydrogen ion concentrations. The pH of the bathing solutions and hydrogen ion concentration (titratable acid) were measured using a pH meter (Radiometer Copenhagen, Bach-Simpson Ltd., London, Ontario, Canada). Titration of the recovered solutions were performed to pH 2, the pH of the instilled solutions, with 0.1 N NaOH. Sodium ion concentration was measured with a Nova 1 Na^+/K^+ analyzer (Nova Biomedical, Newton, MA, USA). The difference between the product of concentration and volume for the instilled and recovered solutions per 15 min periods are the net fluxes expressed for sodium and hydrogen ions in the results below.

Misoprostol Preparation:- Misoprostol was received as neat chemical in dry ice (G.D. Searle and Co. Inc., Skokie, IL). It was dissolved in isotonic 20% ethanol-containing phosphate buffer (pH 7.4). The misoprostol stock-solution was stored in vials at below -20^{o}C when not in use and allowed to thaw to room temperature before use.

Hydrogen Gas Clearance:- This was performed by standard technique as described previously.[33,34] The four HGC electrodes (Unique Medical Co. Ltd., Tokyo) with the four reference Ag/AgCl skin electrodes (Red Dot, 3M Canada Inc., London, Ontario Canada) were connected to a Beckman multichannel recorder (Beckman Type R Dynograph, Beckman Instruments Inc., Schiller Park, IL) to obtain a permanent record of the measurements.

Histology:- Following the termination of each experiment the mucosa and submucosa of tissue from both chambers were quickly dissected from the underlying muscularis and immersed in 10% neutral buffered formalin. The mucosa-submucosa preparation was then spread out and pinned flat on a cork slab within the fixative. After 24 hours fixation the tissue was Swiss-rolled and stored in 70% ethanol. Three random slices from each roll were resected and processed through to either paraffin and stained with hematoxylin and eosin or embedded in glycol methacrylate and stained with methylene blue-basic fuchsin. The slides were coded and examined blindly. Several morphological parameters were noted and/or recorded for each sample including mucosal height, number and spatial relationship of gastric glands, integrity and continuity of the epithelial sheet, and presence of inflammatory cells in the lamina propria, as well as the appearance of mucosal capillaries. The sections were organized into groups based on the morphologic analysis before the code was broken. Comparison of the groupings permitted evaluation of consistent morphologic features within and between each group.

Data Analysis:- All data are expressed as mean ± SE. GMBF data are expressed in ml/min/100 g of tissue. Statistical analysis for significance at the 5% level was performed using either the paired student's *t* test or ANOVA, and linear regression analysis. Because there is a significant difference in GMBF between the upper and lower quadrants of the gastric chamber model, in comparing flow values between the test and control chambers, they were paired upper *versus* upper and lower *versus* lower in the *t* tests.

RESULTS

Gross and Microscopic Appearance

In the test chamber, there was gross mucosal swelling within 5 min of topical application of 200 µg of misoprostol, a feature that persisted throughout the experimental period. No grossly visible lesions were observed either during or after aspirin administration. Mucosal integrity

was maintained throughout the experiment except for an obvious increase in the amount of mucus adherent to the luminal surface. Histologic evaluation of the misoprostol-treated tissue revealed a swollen lamina propria giving the gastric glands a widely spaced appearance and increased mucosal height. There was no increase in cellularity of the mucosa. Vascular channels were easily identifiable, dilated but empty of any cellular components (Fig. 1).

In the control chamber, multiple focal punctate lesions appeared on the mucosa 45 min after aspirin administration. Mucosal swelling did not occur within this chamber. Typical aspirin-induced superficial erosions could be identified in localized areas (Fig. 2). Although a representative sample of the tissue exhibited a normal morphology, there was frequent observation of a widened zone of lamina propria just beneath the surface epithelium (Fig. 3).

Gastric Mucosal Blood Flow

Resting focal GMBF measured by the four HGC electrodes consecutively for 1 hour revealed a positional effect on blood flow in the four quadrants of the double-lumen chamber. In both test and control chambers, there was a highly significant difference between the mean ($\pm$ SE) resting GMBF values in the two upper and lower quadrants. Between the two chambers, there were no significant differences in the mean resting GMBF values recorded in the upper and lower quadrants (upper test: 66.31 $\pm$ 6.86; upper control: 63.66 $\pm$ 6.52; $p > 0.05$; lower test: 48.97 $\pm$ 4.08; lower control: 41.67 $\pm$ 3.45; $p > 0.05$; *t* test). Linear regression analysis revealed a highly significant correlation between consecutive resting GMBF values recorded by the upper and lower electrodes located within each chamber (test: $r = 0.7511$, $n = 13$, $p < 0.01$; control: $r = 0.7151$, $n = 13$, $p < 0.01$), but consecutive resting GMBF values recorded by electrodes located on the same horizontal axis in the two chambers were not correlated.

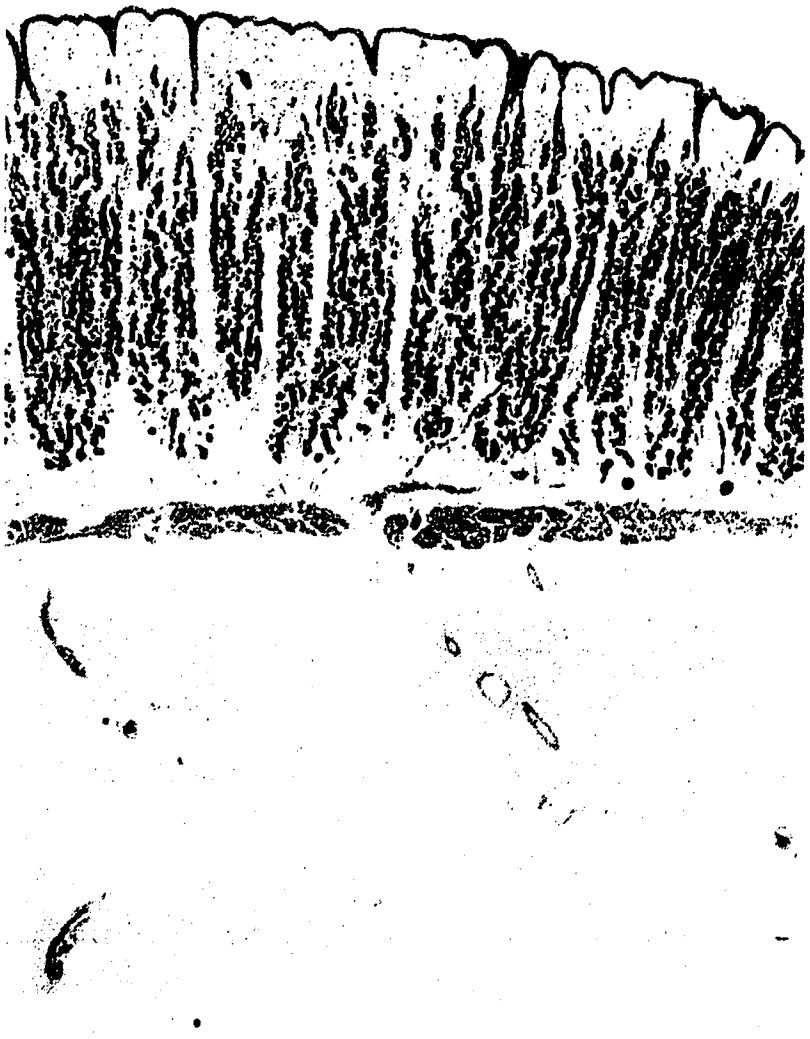

Figure VII-1. Tissue from the test chamber illustrating the swollen, edematous appearance of the mucosa following misoprostol administration. The submucosa exhibits a similar reaction. Note the increased height of the mucosa when compared to Figure 2 (methacrylate, MBBF stain, original magnification X100).

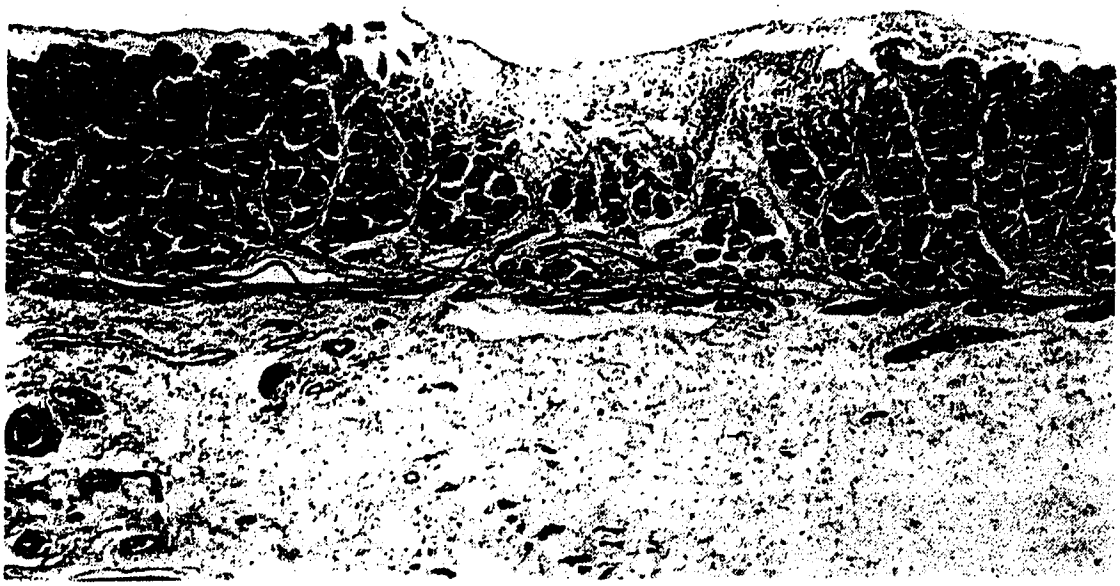

Figure VII-2. A section through an aspirin-induced lesion in tissue from the control chamber. Areas adjacent to the lesion as well as the submucosa exhibit otherwise normal morphology (paraffin, hematoxylin and eosin, original magnification X100).

Figure VII-3. Histologic appearance of a representative sample of tissue from the control chamber. Note the widened zone of lamina propria immediately beneath the surface epithelium (arrow) (paraffin, hematoxylin and eosin, original magnification X160).

Figure 4 depicts the simultaneous sequential changes in mean (± SE) GMBF values measured in the test and control chambers during the basal, misoprostol/control, aspirin and postaspirin periods. In both chambers, the four consecutive mean GMBF values obtained in the basal periods were not significantly different from each other (test: 64.36 ± 10.77, 66.70 ± 9.36, 54.82 ± 7.54 and 55.71 ± 7.80; $p > 0.05$; control: 56.09 ± 6.23, 55.79 ± 7.99, 52.77 ± 9.53, and 48.37 ± 7.58; $p > 0.05$; ANOVA). In the test chamber immediately after the administration of misoprostol, there was a significant and transient increase (18%) in GMBF in the first misoprostol period above the final basal period mean flow value (from 55.71 ± 7.80 to 65.84 ± 6.12; $p < 0.05$; *t* test). In the second misoprostol period, the mean GMBF value of 51.27 ± 6.10 was not significantly different from the final basal period mean flow value, indicating a return of GMBF to premisoprostol (basal) levels within 15 min even though administration of misoprostol was continued for an additional 15-min. After the misoprostol periods focal GMBF in the test chamber showed a graded drop throughout the aspirin periods, became significantly less than the final basal period mean flow value in the second aspirin period, and remained so until the end of the experiments. In the control chamber, there was a graded drop in GMBF throughout the aspirin periods, which became significantly different from the final basal period during the first postaspirin period and remained stable but significantly less than the basal period mean flow value in the remaining periods of the experiments.

Comparison of the simultaneous GMBF values obtained from the two chambers revealed no significant differences except during the first misoprostol/control period (test: 65.84 ± 6.12; control 41.43 ± 6.25; $p < 0.01$; *t* test). In the second misoprostol/control period, though the mean GMBF value in the test chamber remained higher than in the control chamber, the difference was not significant statistically. Thereafter with aspirin, GMBF dropped in both chambers and remained higher though insignificantly so in the test chamber.

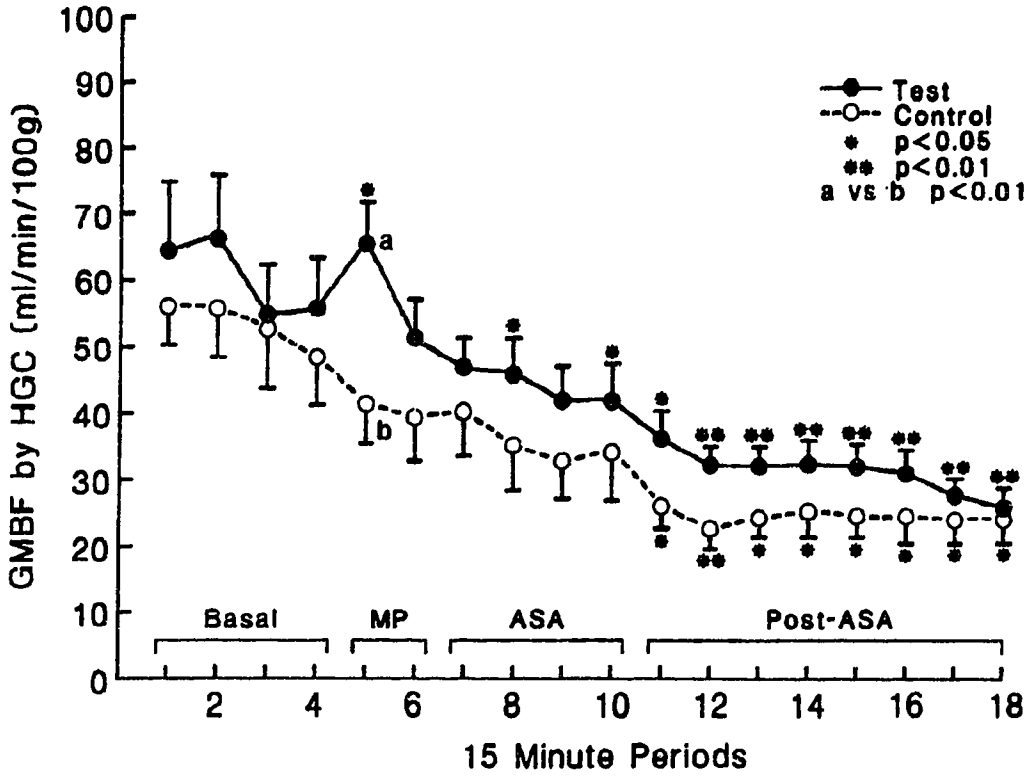

Figure VII-4. The simultaneous sequential changes in mean (± SE) GMBF measured in the test and control chambers during the basal, misoprostol/control, aspirin and postaspirin periods. n = 8 for each point. Asterisks indicate significant differences from the final basal period mean (± SE) flow value within each chamber. MP = misoprostol/control periods; ASA = aspirin periods; HGC = hydrogen gas clearance.

Ionic Fluxes

The results of the net fluxes of ions and fluid are summarized in Table 1. In this text, influx denoted by a minus (-) sign in front of the flux value means net luminal loss while efflux denoted by a plus (+) sign means net luminal gain.

Table VII-1. NET IONIC AND FLUID FLUXES IN THE TEST AND CONTROL GASTRIC CHAMBERS

Periods	Flux Types	Test Chamber	Control Chamber	Test Vs. Control p Value
Basal	Fluid (ml/15 min)	+0.02 ± 0.15	+0.09 ± 0.18	NS
	Na^+ (µmol/15 min)	+79.91 ± 25.29	+95.63 ± 29.35	NS
	H^+ (µmol/15 min)	-64.83 ± 15.69	-69.27 ± 17.38	NS
Misoprostol/control	Fluid (ml/15 min)	+3.36 ± 0.78*	+0.18 ± 0.28	<0.01
	Na^+ (µmol/15 min)	+593.45 ± 103.21*	+171.42 ± 17.94	<0.01
	H^+ (µmol/15 min)	-101.27 ± 27.65	-133.51 ± 35.99	NS
Aspirin	Fluid (ml/15 min)	+1.06 ± 0.29†	-0.49 ± 0.22	<0.01
	Na^+ (µmol/15 min)	+213.52 ± 35.63†	-4.06 ± 11.11†	<0.001
	H^+ (µmol/15 min)	+5.52 ± 12.02†	-20.53 ± 29.33	NS
Postaspirin	Fluid (ml/15 min)	+0.17 ± 0.07	-0.48 ± 0.11†	<0.001
	Na^+ (µmol/15 min)	+84.28 ± 10.97	+26.52 ± 8.02†	<0.01
	H^+ (µmol/15 min)	-45.49 ± 5.63	-88.30 ± 13.90	<0.01

Values are given as mean (± SE). A plus (+) sign denotes a net luminal gain or efflux and a minus (-) sign a net luminal loss or influx.

* Significant difference from the basal period within the same chamber, $p < 0.001$.

†Significant difference from the basal period within the same chamber, $p < 0.01$.

NS = not significant.

Sodium ion flux. In the test chamber, immediately after misoprostol administration, there was a highly significant increase in Na^+ efflux into the lumen that was sustained until the end of the aspirin periods. Na^+ efflux returned to basal levels during the postaspirin periods (Fig. 5). In the control chamber during aspirin administration, there was a significant Na^+ influx from the lumen. This was reversed in the postaspirin periods but the Na^+ efflux remained significantly less than that of the basal period.

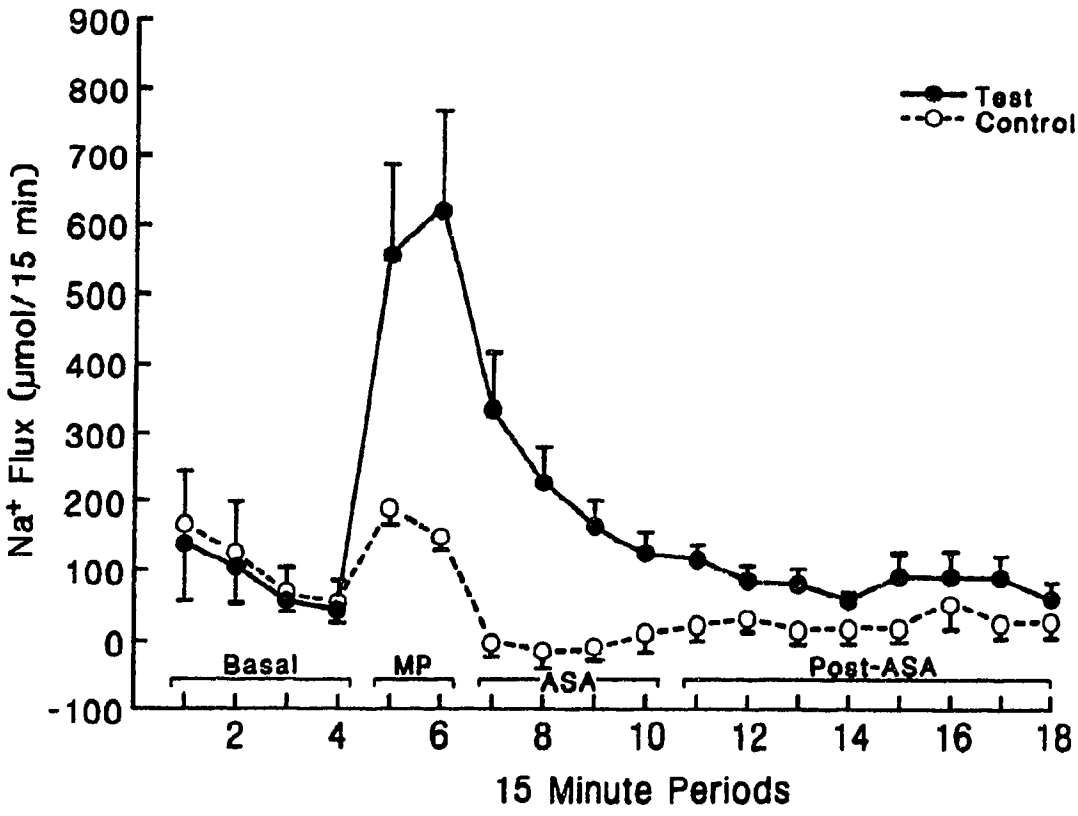

Figure VII-5. The sequential changes in the flux of sodium in the test and control chambers during the basal, misoprostol/control, aspirin, and postaspirin periods.

Fluid flux across the mucosa behaved in exactly the same way as sodium in the test chamber (Fig. 6). In the control chamber, there was an insignificant fluid influx after the control period.

There were highly significant linear correlations between net changes in Na^+ and fluid in the test ($r = 0.9903$, $n = 18$, $p < 0.001$) and control ($r = 0.8652$, $n = 18$, $p < 0.001$) chambers.

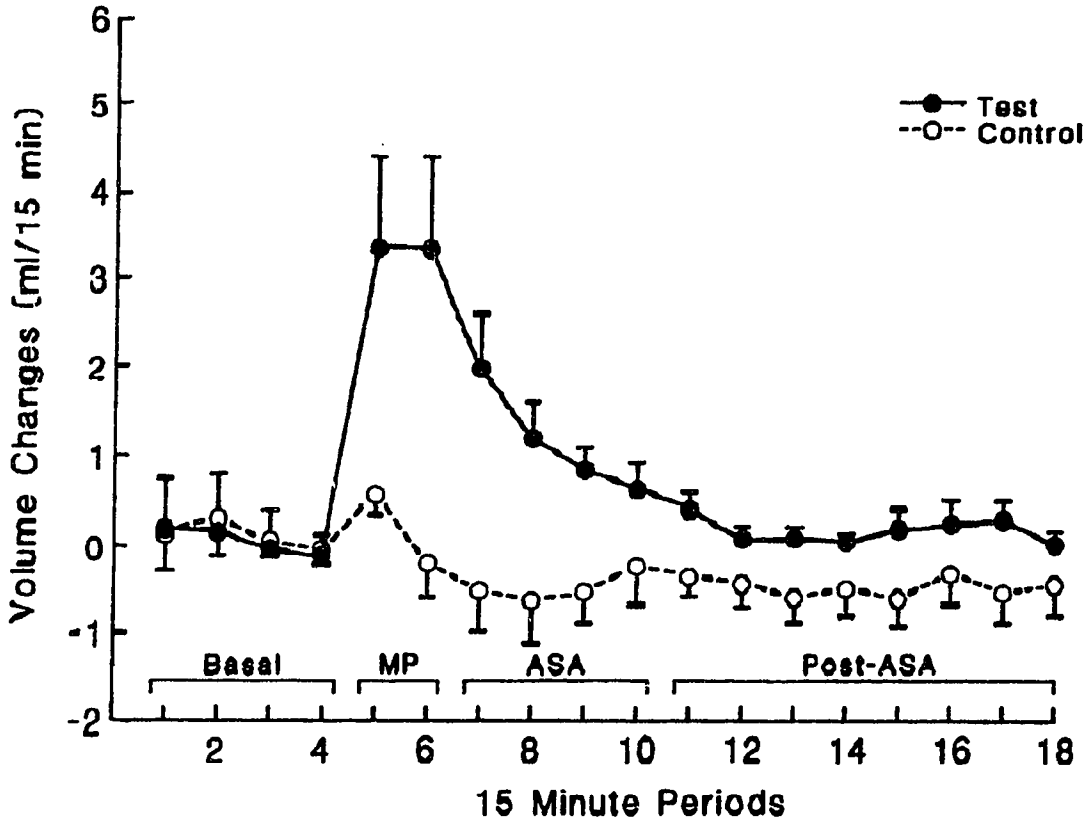

Figure VII-6. The sequential changes in the flux of fluid in the test and control chambers during the basal, misoprostol/control, aspirin, and postaspirin periods. The pattern of the changes are identical to that of sodium.

Hydrogen ion flux. In the test chamber there was a significant ($p < 0.01$, *t* test) H^+ efflux in the aspirin periods. In the control chamber there was no significant change in the net H^+ flux throughout the experiments. The only difference between the test and control chambers was a significantly higher H^+ influx in the latter in the postaspirin periods.

Relationship Between Gastric Mucosal Blood Flow and Fluxes

In the test chamber no correlations were observed between GMBF and the fluxes of Na^+, H^+, and fluid. In the control chamber, there were highly significant linear correlations between GMBF and fluid flux ($r = 0.7364$, $n = 18$, $p < 0.001$), and GMBF and Na^+ flux ($r = 0.5928$, $n = 18$, $p < 0.01$).

DISCUSSION

Though the precise mechanism(s) of gastric cytoprotection is not known, some experimental evidence has emerged in support of the circulatory hypothesis.[1-6] According to the latter, an increase in GMBF is believed to (1) maintain an adequate supply of O_2 and nutrients to the mucosal cells, thereby preventing a deficit in energy metabolism; (2) supply bicarbonate that will buffer back-diffused hydrogen ions, and (c) rapidly eliminate both back-diffused hydrogen ions and the damaging agent that may have gained access into the subepithelial tissues.[35,36] Discrepancies in the results of previous studies on the role of GMBF in cytoprotection may be due to measurement techniques employed, experimental models, experimental designs, and route of administration of the PG.

HGC is a noninvasive method that has been validated for focal GMBF measurements in animals[37-39] and man.[37] Using this technique in our study after topical administration of misoprostol, we observed an immediate and small transient increase (18%) in focal GMBF. The nature of this increase in blood flow is suggestive of a direct vasodilatory effect by misoprostol. The results of this study differ from that of Leung et al.[27] who also (using HGC) reported no effect by misoprostol on resting GMBF and on GMBF during inhibition of stimulated acid

secretion in rats. The differences may be due to the fact that in their study GMBF measurements were commenced 15 min after misoprostol treatment. Similar to our results, Sato et al.[31] reported that misoprostol increased gastric mucosal blood volume by 10-25% at various sites in the fundus and antrum as measured by reflectance spectrophotometry in healthy human male volunteers. Likewise, Larsen et al.[2] observed an increase in total gastric blood flow obtained by venous outflow in the canine chambered gastric segment model after topical misoprostol. Colton et al.[23] observed that misoprostol at doses that inhibited histamine-stimulated acid secretion, reduced GMBF as measured by aminopyrine clearance, but the ratio (R) of GMBF to rate of acid secretion was actually increased. This indicated that misoprostol has a vasodilating effect in the gastric mucosal vascular bed. Similarly, intra-arterial PGE_1 has been reported to significantly increase total (by venous outflow and radioactive microspheres) and mucosal blood flow (by radioactive microspheres) in canine chambered gastric segment model.[40] Also, topical PGE_1 has been shown to dose-dependently increase superficial gastric mucosal microvascular flow measured by *in vivo* microscopy in rats.[41] The findings in this study confirm these earlier observations that misoprostol, a synthetic PGE_1 analog, is a vasodilator in the gastric mucosal vascular bed.

Our results also confirmed previous reports that misoprostol is cytoprotective.[2,24-26,28-30] The degree of mucosal swelling observed after misoprostol administration was surprising. The histology clearly attributed this gross observation to an edematous reaction within the lamina propria causing increased spacing and separation of the gastric glands. A thick mucoid layer overlying the surface epithelium was also evident in these sections. The aspirin-induced lesions were similar in severity and morphology to those described by other authors.[42] The frequent observation of a widened zone of lamina propria immediately below the surface epithelium is reminiscent of the fashion in which this layer is shed in response to necrotizing agents such as absolute ethanol.[43] The fact that many regions of tissue from the control chambers lacked any evidence of mucosal disruption could be attributed to the random nature of

sample site selection as well as the length of the experimental period. It has been shown that epithelial repair under these conditions can begin within 3, and is completed within 60, min.[44]

The extent to which GMBF contributes to cytoprotection is unknown. Larsen et al., in the aforementioned study observed an increase in total gastric blood flow by misoprostol of over 400% that was sustained during subsequent aspirin-shock injury, and concluded that the increase in blood flow was responsible for the cytoprotective effects.[2] Recently, we have shown that topical aspirin induces a reduction in GMBF of varying degrees and that mucosal areas with blood flow reduced to below a *critical value* develop gross damage.[34] In this study we were unable to demonstrate that misoprostol pretreatment reverses aspirin-induced decrease in GMBF or maintains blood flow after aspirin administration. Our results therefore do not suggest that this transient vasoactive effect is an important mechanism of gastric cytoprotection.

In terms of ionic fluxes, Colton et al.[24] found no significant difference in the influx of H^+ and Cl^-, and efflux of Na^+ observed with misoprostol and aspirin when administered into canine Heidenhain pouches separately or together. They concluded that influx of H^+ and efflux of Na^+ from the stomach does not necessarily indicate mucosal damage. On the contrary, Larsen et al.[2] have reported that misoprostol had a negligible effect on the fluxes of Na^+, Cl^-, and H^+ that were not significantly different from the controls. In this study the only consistent flux changes observed were the significant increase in the efflux of Na^+ and fluid into the test chamber lumen immediately after administration of misoprostol. The efflux of Na^+ and fluid could be due to either damage to the gastric mucosal barrier by misoprostol, as was initially attributed to PGE_2,[45-47] or stimulation of a nonparietal cell secretion rich in Na^+.[48,49] Since no reports have shown that misoprostol damages the mucosa grossly or histologically, and the 200 µg dose of misoprostol used in this study has been experimentally and clinically shown to be cytoprotective, we favour the latter explanation for the observed increase in Na^+ and fluid efflux. The highly significant linear correlation observed in the test chamber between the

fluxes of Na^+ and fluid further suggests that these fluxes may be mediated through the same pathway, namely, stimulation of nonparietal secretion rich in Na^+.

We cannot offer any explanation for the findings in H^+ fluxes. It is noteworthy that in the presence of PG, agents that stimulate active secretion of bicarbonate or Na^+-rich fluids,[50,51] disruption of the gastric mucosal barrier by topical damaging agents (e.g., aspirin, alcohol and bile salts) will not result in the classical picture proposed by Davenport, namely, back-diffusion of H^+ and efflux of Na^+ into the lumen.[52] Neutralization of acid by bicarbonate and the large amounts of Na^+ effluxed into the lumen under these circumstances will inevitably alter the net ionic fluxes. This may be the reason for the failure to obtain results consistent with Davenport's hypothesis in this and other studies when an agent that stimulates active secretion of Na^+ bicarbonate is used. This finding also raises questions about the validity of using the efflux of Na^+, and the luminal loss of H^+ as indicators of gastric mucosal barrier damage under these circumstances. The linear relationship between GMBF and the fluxes of Na^+ and fluid in the control but not the test chamber may be accounted for by misoprostol since it is the only different variable between the two chambers.

In conclusion, though our results did show a significant and transient increase in GMBF by misoprostol pretreatment, it did not prevent subsequent decrease in GMBF by aspirin. The sustained efflux of Na^+-rich fluids (i.e., nonparietal cell secretion) induced by this synthetic PGE_1 analog may be important in gastric cytoprotection. Our results confirm that misoprostol is vasodilatory and cytoprotective, and it stimulates nonparietal cell secretion. Further studies of the vasoactive effects of PG as an important mechanism of cytoprotection are required.

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Chapter 8

General Discussion and Conclusions

A. TECHNIQUES

Of the techniques that have been developed for the measurement of focal gastrointestinal mucosal blood flow in the last decade, laser-Doppler flowmetry (LDF), hydrogen gas clearance (HGC), iodoantipyrine clearance and reflectance spectrophotometry can be applied clinically through flexible endoscopy. However, the iodoantipyrine clearance technique[1,2] is associated with the use of radioactivity, has a requirement for tissue biopsy specimens in humans and is inaccurate in the presence of liquid lumenal contents in the gastrointestinal tract. Reflectance spectrophotometry[3,4] is expensive, is not calibrated in absolute blood flow units and it relates a static tissue hemoglobin concentration to blood volume rather than to blood flow. On the other hand, LDF[5-7] and HGC[8,9] constitute two of the most promising techniques for clinical use in the measurement of gastrointestinal mucosal blood flow and possess more of the qualities of an ideal blood flow measurement technique proposed by Jacobson.[10] The validity of both techniques in the assessment of focal gastric mucosal blood flow (GMBF) has been firmly established.[11] The capacity for noninvasive endoscopic application in human subjects, the relative ease of performance of measurements, and their safety are obvious advantages of these techniques that supersede those of other techniques. Despite these advantages, however, limitations still exist which need resolution. LDF is not calibrated in absolute flow units, it is sensitive to movement artifacts and the depth of measurement of the technique is still a subject of controversy.[11,12] It is also speculated that probe contact may be a problem especially in clinical endoscopic use. HGC, when performed with 3% hydrogen gas mixed with air, takes about 30 min to complete one measurement, is associated with a small and contaminated signal, requires that blood flow remains constant during the entire measurement period, has an ill-defined depth of measurement (e.g., in the brain and spinal cord, where it has been used longer

and more widely) and is unverified experimentally in the gastrointestinal tract. The primary objective of this study was therefore to address some of their limitations, and compare and evaluate LDF and HGC in the measurement of focal GMBF.

(i) Calibration of Laser-Doppler Flowmetry Signal

Although the results of this study show significant linear relationships between simultaneous basal GMBF values obtained by LDF and HGC within each animal, a universal calibration factor for the LDF signal that can be applied between different animals is not feasible (Chapter 3). This result corroborates the findings of other recent studies in which there was variation between calibration factors obtained for the LDF signal following simultaneous measurements of gastric and intestinal mucosal blood flow by LDF and other measurement techniques.[13,14] In those studies the calibration factors varied between different tissue sites in the same animal and between different animals. The results of this and the latter studies seriously calls into question the factory-calibrated laser-Doppler flowmeters currently in the market, whose calibration factors are based on physical-optical theoretical models and which are not necessarily valid for various living tissues.[15] The reason for the inability to obtain a universal calibration factor for the LDF signal is not known. However, movement artifacts, spatial and temporal variations in blood flow, variations in the optical properties of tissues and the influence of the fiber diameter within the optic probe may be important factors. Movement artifacts, an effect unaccounted for in the theory of LDF,[16] could result from tissue movements induced by working muscles, pumping action of the heart or pressure waves in the arterial tree.[15] The LDF signal obtained at such a site often contains contributions indistinguishable from the part of the signal generated from the flux of red blood cells.[15,17] Furthermore, studies by various investigators have shown that fiber diameter is linearly related to motion artifact.[15,18]

In accession to this and the aforementioned studies,[13,14] other suggestions have been made by investigators attempting to resolve the issue of calibration of the LDF signal. Some

investigators have suggested making multiple measurements at different locations and averaging the results or making simultaneous recordings with multiple laser-Doppler instruments, approaches that are tedious, may be subject to time-dependent variations in blood flow or are expensive.[19] Salerud & Nilsson[20] have designed an integrated laser-Doppler probe with seven receiving fibers instead of the normal two for the Periflux flowmeter and showed that it significantly reduced spatial and temporal variations in measurements performed with the standard probe by a factor of the square root of the number of fibers (in this case $\sqrt{7}$). Such an improved LDF signal may be amenable to calibration.

Based on the results of this study, my proposition is that a combined LDF/HGC probe that simultaneously measures GMBF at the same focal mucosal point may provide the solution to this problem of calibration. Such a hybrid probe was recently presented by DiResta et al.[21] Although preliminary evaluation of that probe showed highly significant linear correlations between the flow values obtained by the two techniques, a major disadvantage of the probe is that it utilizes the HGC technique in which H_2 is generated within the tissue by electrolysis. That HGC technique is fraught with a number of unproven assumptions, nonconvectional losses of H_2 from the measurement site have to be determined by killing the animal at the end of the experiment, hence, has no clinical potential; and the potential for tissue damage has not been excluded.[22] Theoretically, a combined LDF/HGC probe that utilizes the standard inhalational HGC technique will be a valid alternative and it should have a capacity for endoscopic clinical application.

GMBF values obtained simultaneously by LDF and HGC were significantly correlated in the basal (Chapters 3 & 5) but not misoprostol periods (Chapter 5), when rapid changes in flow were induced. The reason for this finding is not known? However, it is possible that the gross mucosal swelling induced by misoprostol and which was shown to be due to massive subepithelial and submucosal edema in this (Chapter 7) and other studies performed in our laboratory,[23,24] may have decreased the tissue-blood partition coefficient for hydrogen gas. This remains a subject of further investigation.

(ii) HGC Signal and the Duration of Measurement

A current amplifier with a low pass filter that significantly improves the magnitude and quality of the HGC signal has been developed (Chapter 4). Using this amplifier/filter, there was a significant reduction in baseline drift and the sensitivity of the HGC platinum electrode to the presence of hydrogen in the measuring volume of tissue was increased. Also, using the amplifier/filter, it was possible to use rapid protocols of H_2 administration to reduce the duration of HGC measurement. The finding that GMBF values obtained from the washin part of the washin-washout curve are not significantly different from those obtained conventionally from the corresponding washout part during basal flow measurements, makes it feasible to obtain two instead of the usual one (washout) measurement during the period of acquisition of the curve. This, apart from reducing the duration of one HGC measurement, also makes the method more compliant to the significant requirement of all inert gas washout techniques that blood flow should remain constant during the entire measurement period.[25]

(iii) Probe Contact

These studies reveal that with both LDF and HGC, probe contact is not a problem in laboratory studies in the models used. Recently, a clinical, controlled, endoscopic study of focal GMBF by LDF performed in our laboratory[26] has further revealed that probe contact is not a problem with the application of LDF endoscopically in humans. Future studies of human focal GMBF by endoscopic HGC in our laboratory, using the improvements reported in this study, will determine if indeed probe contact constitutes a problem in the clinical application of HGC through flexible endoscopy.

The issue of a universal calibration factor for the LDF signal to absolute flow units remains unresolved. It is hoped that the improvements to the quality of the LDF signal being reported by others and discussed above will ultimately result in a signal that will be amenable

to calibration. The measurement depth of LDF and the sensitivity to movement artifacts are two problems of LDF which were not addressed in this study. However, they have received attention elsewhere. In a recent study, Johansson et al[27] have shown that the measuring depth of LDF in the gastrointestinal tract is highly dependent on fiber diameter and geometry of the measuring probe. They further showed that by manipulating both factors, it is possible to vary the measuring depth of a probe. The results of that study will have a significant impact on the resolution of the depth sensitivity of LDF. Movement artifacts on the other hand, have been avoided by designing laser-Doppler instruments which do not require optical fibers to transmit laser light to and from the tissue being assessed[28,29] and by electronic identification and rejection, the latter, an innovation used by Perimed (Perimed, Stockholm, Sweden) in their flowmeters.[15] Even with these unresolved problems, it is worth emphasizing here that LDF remains the best of the techniques currently available for estimating focal gastrointestinal blood flow.

With the improvements reported in this study, HGC has moved closer to both LDF and the ideal technique. Compared to LDF, reduction in one measurement time may be the closest HGC may get to being able to assess blood flow continuously. Verification of the measurement depth of HGC, also an unresolved problem, can be addressed in future studies with the improved technique developed in this study. Furthermore, it may now be possible to apply the technique successfully in endoscopic studies of human focal GMBF in health and disease in our laboratory. To date there is only one report on the application of endoscopic HGC to measure focal GMBF in humans.[9] Subsequent attempts by the same group[30] to measure human focal GMBF by endoscopic HGC in a comparative study with endoscopic LDF was unsuccessful, as has also been reported from our laboratory.[31]

Since measurement of gastrointestinal blood flow has become routine in laboratories devoted to the study of gastrointestinal dysfunction,[32] reports of studies aimed at further refinement and evaluation of LDF and HGC are to be eagerly awaited in the years ahead.

B. APPLICATIONS

Studies of the response of GMBF to prostaglandins have been fraught with conflicting results.[33] Similarly, the effects of aspirin and other topical necrotizing agents like alcohol and bile salts, on GMBF have yielded conflicting results.[33-36] Many of these conflicting reports have been attributed to a multitude of factors which include differences in: blood flow measurement techniques; administered dosages; route of administration; experimental models; experimental conditions; and the concurrent administration of [illegible] have opposing effects on GMBF. The mechanism(s) of prostaglandin cytoprotection, [illegible]ently as protection against gross and histological gastric mucosal injury by a mecha[illegible]ther than inhibition or neutralization of gastric acid secretion,[37] remains unknown. However, the circulatory hypothesis proposes that an increase in GMBF provides adequate oxygenation and supply of nutrients to cells making them more resistant to injury.[33,38,39] There are both concordant and discordant evidences for this hypothesis as with others proposed for the mechanism(s) of gastric cytoprotection.[33,38] Specifically, experimental and clinical gastric mucosal lesions induced by aspirin and which are shown to be prevented or markedly reduced by prostaglandin pretreatment are focal in nature.[40-42] This fact coupled with the fact that most previous studies of the role of GMBF in aspirin-induced mucosal injury and in prostaglandin cytoprotection of such injuries have utilized measurement techniques that lack the capacity for focal estimates of blood flow, indicated the need for and the importance of this study. The second objective of this study was therefore to apply LDF and HGC to define the response of focal GMBF to prostaglandins, study the role of GMBF in aspirin-induced gastric mucosal injury and in prostaglandin cytoprotection against such injury.

(i) Response of Focal Gastric Mucosal Blood Flow to Prostaglandins

The results of this study shows that misoprostol, a synthetic PGE_1 analog, increases GMBF in the gastric mucosal vascular bed. The observation of dilated gastric mucosal and submucosal

blood vessels in histologic sections of tissue treated with misoprostol (Chapter 7) corroborates the vasodilatory effect. The dose-response nature of the vasodilatory action of misoprostol observed in this study (Chapter 5) is also corroborated by a recent gross and histologic study of its topical effects on the resting canine gastric mucosa. Gana et al,[23,24] utilizing identical doses of misoprostol to the ones used in this study, have reported a dose dependent increase in the dilatation of mucosal and submucosal blood vessels. Subepithelial edema, increased mucosal height and submucosal edema were also induced in a dose dependent fashion. The results of the present study (Chapters 5 & 7) also shows that the vasoactive effect of misoprostol is transient, and both the choice of a measurement technique and the time of measurement are absolutely important in the demonstration of the flow increase. This latter point reveals why in a previous study by Leung et al,[43] no increase in GMBF was measured by HGC after topical application of misoprostol to the gastric mucosa.

In the experiment where the gastric mucosa was pretreated with 200 μg of misoprostol (Chapter 7), a transient increase in GMBF was measured by HGC while in the dose-response study (Chapter 5), HGC did not measure an increase in GMBF at all the doses administered. The reason for this discrepancy is not clear. However, it is possible that the transient increase in GMBF following 200 μg of misoprostol was detected by HGC because it is close to the E_{max} dose (100 μg) for the response of GMBF to misoprostol measured by LDF (Chapter 5).

(ii) Gastric Mucosal Blood Flow in Aspirin-Induced Ulceration

The finding of a decrease in GMBF simultaneously in both grossly ulcerated and grossly nonulcerated areas of the mucosa using a focal technique is novel (Chapter 6). Hitherto, the only study in which HGC was used to measure focal GMBF in aspirin-induced gastric mucosal injury also reported a decrease at the site of gross mucosal injury but showed a hyperemic response at the grossly nonulcerated areas.[44] The increase in mucosal blood flow in the grossly nonulcerated areas has been proposed to be a secondary compensatory response of the gastric mucosa to damage.[34,44] However, in contrast to this study, GMBF at the grossly ulcerated and

nonulcerated areas were not obtained simultaneously in the same animal in the latter study.[44] Much of the confusion over the direction of change of GMBF in aspirin-induced gastric mucosal injury may be due in part to the fact that most previous studies have utilized the aminopyrine clearance technique, a now obsolete technique, which is inaccurate in any situation in which the stomach is not actively secreting acid and in gastric mucosal damage.[45-49]

A hyperemic response at the grossly nonulcerated areas of the gastric mucosa in aspirin-induced injury may not be a likely possibility since morphological studies of such injury have revealed that grossly normal areas of mucosa exhibit extensive histological damage,[40] a finding consistent with the decrease in blood flow observed in both grossly ulcerated and nonulcerated mucosa in this study. Also, it is highly unlikely that such a response was missed since GMBF was simultaneously and continuously monitored in the grossly nonulcerated and ulcerated areas of the mucosa throughout all experiments. Furthermore, electron microscopic and microvascular permeability studies of the sequence of events in aspirin-induced gastric mucosal injury[35,42] have shown that the initial injury occurs in the endothelial cells or microvasculature respectively, before subsequent cytosolic injury is observed.

The consistency of the sequential changes in GMBF observed following aspirin administration throughout the experiments in this study (Chapter 6) had led to the proposition of a "critical GMBF value" hypothesis for grossly visible damage. This "critical GMBF value" below which blood flow must be reduced in order to observe the features of gross aspirin-induced gastric mucosal damage is 20-30 ml/min/100g. This represented a fall from the mean ± SE GMBF value of 66%. In the nonulcerated areas, GMBF was reduced below the mean ± SE basal value by 33%, that is, the blood flow in the ulcerated areas was 50% of the corresponding value in the nonulcerated areas measured simultaneously.

The aspirin-induced gastric mucosal lesions in this study were focal grossly and histologically. The reason for the focal distribution of aspirin-induced gastric mucosal lesions will remain a subject of further study.

(iii) Gastric Mucosal Blood Flow in Misoprostol Pretreated Aspirin-Induced Ulceration

The transient 18% increase in GMBF induced by topical misoprostol pretreatment did not prevent the subsequent decrease in GMBF induced by aspirin even though gross and histologic mucosal protection was observed (Chapter 7). This suggests that increase or maintenance of GMBF may not be the mechanism of gastric cytoprotection by this vasoactive prostaglandin. However, it does not preclude the fact that GMBF may be important in the initiation of the events that ultimately lead to cytoprotection. These results lend credence to the view that the mechanism of prostaglandin cytoprotection may indeed be multifactorial.

On the other hand, the sustained alkaline nonparietal secretion induced by misoprostol and the subepithelial edema observed may be important in misoprostol gastric cytoprotection. Recently, the alkaline nonparietal secretion and subepithelial edema induced by topical misoprostol (Chapter 7) have been shown to be dose dependent in flux and histologic studies.[23,24,50] It was hypothesized by Gana et al,[23,24] that the massive misoprostol-induced mucosal and submucosal edema may be important in the mechanism(s) of gastric cytoprotection by (a) increasing the distance of penetration or absorption of a mucosal damaging agent; (b) diluting its concentration intramucosally; and (c) disemminating any focal accumulations of red blood cells. More recently, this hypothesis has received support from reports that have shown that prostaglandins do not prevent the absorption of mucosal damaging agents into the mucosa[51,52] but decrease their penetration into the mucosa[53] and decrease the severity of damage by restricting damage to the superficial epithelium while protecting the deeper and vascular parts of the gastric mucosa.[54]

Gastropathy induced by aspirin and nonsteroidal anti-inflammatory drugs continues to be a significant cause of morbidity and mortality in patients on chronic administration for rheumatoid arthritis. A higher incidence of gastric ulcers, life threatening gastrointestinal bleeding or perforation may be associated.[55] Similarly, acute stress ulcers in the stomach are associated with a high mortality, up to 30% in most series studied, once the complication of hemorrhage sets in. Elucidation of the mechanism(s) of injury in these conditions through

studies utilizing LDF and HGC like the present one, can lead to the development of better prophylactic and treatment regimens to deal with these conditions. LDF and HGC can also be used in the early detection of acute stress ulcers in predisposed subjects and intestinal ischemia, conditions in which early institution of treatment significantly affects outcome.

CONCLUSIONS

In conclusion, LDF and HGC are comparable in the measurement of focal GMBF. However, the slope of this linear relationship differ from animal to animal and a universal conversion factor (slope) is not feasible. A combined LDF/inhalational HGC probe may resolve this problem. An amplifier/filter that measures current and significantly increases the magnitude, quality and sensitivity of the HGC signal is presented. Using this amplifier/filter, there was no significant difference between washin and washout GMBF, thereby reducing the duration of one HGC measurement and making the technique more compliant to a significant theoretical requirement. HGC does not detect a rapid increase in blood flow in non-steady state. Using this improved HGC technique it may now be possible to utilize this method in endoscopic measurements of human focal GMBF in health and disease. Misoprostol dose dependently increases GMBF. In contrast, aspirin induces focal ischemia of varying degrees in the gastric mucosa and areas with blood flow reduced below a "critical value", corresponding to 66% reduction below basal, develop gross damage. The simultaneous demonstration of a reduction in GMBF in the nonulcerated mucosa using a focal technique is novel. Misoprostol pretreatment induced a transient 18% increase in GMBF but it did not prevent the subsequent drop in GMBF induced by aspirin even though cytoprotection was observed. Misoprostol also induced a sustained gastric alkaline nonparietal secretion and massive mucosal and submucosal edema. Increase or maintenance of GMBF may not be the direct mechanism of prostaglandin cytoprotection. The mucosal edema and sustained nonparietal secretion induced may be more important.

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APPENDIX

Measurement of Focal Gastric Mucosal Blood Flow: Methodology

A. Physiological Recorder

1) The physiological recorder (Beckman Recorder) is calibrated as recommended by the manufacturer.

2) The output of the laser-Doppler flowmeter (Pf 1d, Periflux, Perimed, Stockholm, Sweden) is connected to the input (AC/DC Coupler) of one of the channels of the Beckman recorder. Also, the output of the amplifier/filter used for performing hydrogen gas clearance is connected to the input of another channel of the recorder.

3) The output from the respective channels of the recorder are connected to a Lab Master analog-to-digital converter (ADC) board (Scientific Solutions, Solon, Ohio). The output from the ADC is the hooked up to an IBM PC - AT 386 (IBM Corp.).

B. Hydrogen Gas Clearance

1) An area of skin over the groin (dog) or the postero-lateral aspect of the abdomen (rat) is shaved and abraded. Electrode gel is applied and a reference Ag/AgCl skin electrode is attached to the prepared area of skin. The electrode is fixed in place with adhesive tape. Abrasion of the skin helps to reduce artifacts due to the skin potential. Fixing the electrode in place reduces the chances of movement artifacts which can remove the electrode-electrolyte interface from direct contact with the experimental subject and interfere with the electrical stability of the electrode and hence the electrode current.

2) The measuring platinum electrode is placed in contact with the desired point on the gastric mucosa where GMBF is to be measured. We have modified the detachable top of the plastic chamber used for mounting the excised canine gastric segment. Several holes have been made in it to permit stable placement of both the HGC electrode and the LDF probe. When using the intact stomach of rats (Chapter 4), a transverse gastrotomy is performed in the fore-stomach and the electrode is placed in contact with the gastric mucosa at the desired point. The electrode is held in place with a stitch.

3) The reference Ag/AgCl and the measuring platinum electrodes are connected to their respective terminals on HGC amplifier/filter box. The platinum electrode is polarized with a voltage of +100 millivolts.

4) The recorder pen is adjusted back to the zero baseline and the sensitivity of the channel is set to ensure a deflection of up to 80% of the width of the channel when hydrogen gas is administered to equilibrium. This is usually done by an initial test administration of H_2 to

obtain a rise on the chart recorder channel and thereafter accordingly adjusting the sensitivity of the channel.

5) Simultaneously, the software program which we have developed for the acquisition and analysis of the data on the IBM PC - AT 386, is turned on and the channel(s) are also adjusted to the zero baseline [as for the recorder in (4) above] for data acquisition.

6) Hydrogen gas is administered through a funnel placed at the input port of the Harvard respirator in the dog (Chapters 3,5,6 & 7). Pure hydrogen gas is then delivered at a flow rate of 1 - 2 L/min to the input port of the respirator in such a way that it mixes with the room air. Hence the ultimate concentration and flow rate at which hydrogen gas reaches the dog are less than 100% and determined by the Harvard respirator settings respectively. Blood concentrations of hydrogen gas were not monitored. In the experiments involving rats (Chapter 4), 3% H_2 plus air was administered through an inverted funnel placed 2-4 cm above the tracheostomy tube.

7) A hydrogen washin-washout curve is obtained. The pen deflections on the chart recorder and the IBM personal computer are watched as H_2 is administered until equilibrium is reached, indicated by a plateauing of the washin curve. The delivery of hydrogen gas is stopped and the washout part of the curve is obtained.

8) Using the analysis part of the computer program, non-linear regression analysis (iterative curve fitting) of the washin and washout parts of the curves are performed to obtain GMBF values. Precisely, the washout curve is fitted by iterative curve fitting until a perfect fit is obtained. The $t_{1/2}$ is given by the program as well as a flow value. The program also fits curves to the washin curve which mathematically has a different equation from the washout curve.

GMBF has also been calculated manually from the physiological recorder strip charts. This is done by taking several points (about 20) along the entire length of the washout curve. The current at each point is plotted against the time on semilogarithm paper; the beginning of the curve where the electrode current is maximal (hydrogen equilibrium between blood and tissue) corresponds to time zero. A straight line is obtained and the $t_{1/2}$ is obtained. Blood flow is calculated from the formula:

$$F = 0.693/t_{1/2} \times (\lambda) \times 100$$

Where F is flow in ml/min/100 g of tissue; $t_{1/2}$ is the time in minutes taken for the electrode current to be reduced to half its numerical value; and λ is the tissue-blood partition coefficient for hydrogen gas which has been determined to be 1 ml/g. Flow is expressed per 100 gram of tissue for ease of comparison with other results in the literature.

C. Laser-Doppler Flowmetry

1) Laser-Doppler flowmeter settings are adjusted as follows:
Upper Doppler frequency limit - 12 kHz

	- 4 kHz setting was used in some experiments in chapter 3 for comparison with previous results reported in the literature on calibration of the LDF signal
Gain:	X10 or X30
Time Constant:	1.5 or 3 s

2) The Beckman recorder channel is calibrated to the laser-Doppler flowmeter. The sensitivity of the recorder channel is set to obtain a full scale deflection of 10 volts, the maximum output of the flowmeter.

3) The recorder pen is adjusted to the zero baseline.

4) The laser-Doppler flowmeter probe is then positioned on the mucosa at the focal point where blood flow is to be measured. To position the LDF and HGC probes for simultaneous measurements of GMBF by both methods, the platinum HGC electrode is first placed in contact with the mucosa at a preselected point without pressure. The electrode rests on the side wall of the holes provided in the modified top of the transparent lucite chamber at an angle of about 45^{o}. This permits the LDF probe (PF 308; Head: Plastic cylinder, diam. 6 mm, length 30 mm; Fiber line diam. 2.0 mm, length 1.75 m) to be placed perpendicularly at a distance of less than 6 mm over the point of mucosal contact of the HGC electrode by centering the narrow beam of laser light on that point. It is recommended by the manufacturer that the probe tip lightly touches the tissue under study to minimize the influence of movement artifacts, indications on the LDF signal (caused by spontaneous movements, spasms, breathing, peristaltic action, or heart activity) that are not perfusion related.

5) The LDF perfusion signal is instantaneously and continuously recorded simultaneously by the computer program and the recorder.

6) GMBF values are subsequently obtained from the records using the data acquisition menu of the computer program. The program is designed to measure the area under the curve and divide it by the time of the recording to obtain blood flow values in volts. In studies wher LDF and HGC GMBF values were simultaneously obtained, the LDF values were average flow values obtained over the period or duration of the corresponding HGC washin or washout curve.

Similarly, GMBF by LDF has also been calculated manually from the physiological recorder strip charts by determining the area under the curve (using a digitizer) and dividing it by the time of the recording..

VITA

NAME:	Theophilus Jones **GANA**
ADDRESS:	Apt. 607 20 Vanauley Street Toronto, Ontario M5T 2H4 Canada
TELEPHONE:	(416) 864 5513 - Work (416) 365 0910 - Home
FAX:	(416) 864 5870

PROFESSIONAL QUALIFICATIONS:

June 1979	**M.B., B.S.** Ahmadu Bello University, Zaria, Kaduna State, Nigeria

SCHOLARSHIPS/FELLOWSHIPS/AWARDS:

1984/85 - 1985/86	Federal Government of Nigeria Post-graduate Scholarship Award
July 1990 - June 1991	One of two winners of the 2nd Annual Lederle Surgical Infectious Diseases Fellowship Offered by Lederle Laboratories in co-operation with the Canadian Association of General Surgeons to Canadian Surgical trainees
May 1991	Andrew Stewart Memorial Graduate Prize Awarded by the Faculty of Graduate Studies, University of Alberta, Edmonton, Canada, to Ph.D students for outstanding doctoral research

POST-QUALIFICATION EXPERIENCE:

July 1979 - June 1980	House Officer [Intern] Jos University Teaching Hospital, Jos, Plateau State, Nigeria

July 1980 - June 1981	General Duty Medical Officer Nigerian Air Force Medical Centre, Jos, Nigeria [National Youth Service]
July 1981 - June 1982	Senior House Officer Department of Surgery, General Hospital, Minna, Niger State, Nigeria
July 1982 - August 1983	Registrar Department of Surgery, General Hospital, Minna, Niger State, Nigeria
October 1983 - October 1984	Senior House Officer [Resident I - Training Post in General Surgery] Department of Surgery, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria
January 1985 - May 1991	Research Fellow Surgical-Medical Research Institute, Department of Surgery, University of Alberta, Edmonton, Alberta, T6G 2N8
July 1991 - present	Research Associate Division of General Surgery, Department of Surgery University of Toronto and St. Michael's Hospital, Toronto, Ontario, M5B 1W8

RESEARCH SUBJECTS:

Ph.D. (Experimental Surgery)

- Techniques: compare, refine and further evaluate laser-Doppler flowmetry and hydrogen gas clearance in the measurement of focal gastric mucosal blood flow in experimental animals

- Applications: apply these techniques to study the: (i) response of focal gastric mucosal blood flow to prostaglandins; (ii) role of blood flow in drug-induced acute erosive gastritis by aspirin; and (iii) role of blood flow in prostaglandin cytoprotection against aspirin-induced mucosal injury

Research Fellow/Associate

- Defining the role of ionic fluxes and histology in gastric cytoprotection by prostaglandins

- Use of in vivo fluorescent video microscopy to study the microcirculatory changes in shock- and drug-induced acute gastric mucosal injury

- Use of in vivo fluorescent video microscopy to study the role of neutrophils and oxygen-derived free radicals in drug-induced gastric mucosal injury

- Use of in vivo fluorescent video microscopy to study the role of neutrophils and oxygen-derived free radicals in shock-induced gastric mucosal injury

- Endoscopic measurements of human focal gastric mucosal blood flow using laser-Doppler flowmetry and hydrogen gas clearance

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Presentations:

1. **Gana TJ,** Huhlewych R, Koo J. Laser-Doppler flowmetry and hydrogen gas clearance - are they comparable in the measurement of canine gastric mucosal blood flow? 87th Annual Meeting of the American Gastroenterolog- ical Association (Digestive Diseases Week), May 17-23, 1986, San Francisco, California, U.S.A.: poster session.

2. **Gana TJ,** Huhlewych R, Koo J. Focal Gastric mucosal blood flow in aspirin -induced ulceration. Sixth Annual Heritage Medical Research Days, November 6-7, 1986, Edmonton, Alberta, Canada: poster session.

3. **Gana TJ,** MacPherson BR, Koo J. Misoprostol-induced ionic fluxes in resting canine gastric mucosa. 89th Annual Meeting of the American Gastroenterological Association (Digestive Diseases Week), May 15-18, 1988, New Orleans, Louisiana, U.S.A.: poster session.

4. **Gana TJ,** MacPherson BR, Koo J. Mucosal blood flow, ionic fluxes and histology in gastric cytoprotection by prostaglandins. International Conference on Gastroenteric Biology, October 25-28, 1988, Oxnard, California, U.S.A.: poster session.

5. **Gana TJ,** MacPherson BR, Ng D, Koo J. Use of cobalt-EDTA as a gastric juice volume marker. Eighth Annual Heritage Medical Research Days, November 3-4, 1988, Edmonton, Alberta, Canada: poster session.

6. **Gana TJ,** Soenen GM, Koo J. Human gastric mucosal blood flow by endoscopic laser-Doppler flowmetry. Annual Meeting of the American Society for Gastrointestinal Endoscopy (Digestive Diseases Week), May 13-17, 1989, Washington, D.C., U.S.A.: poster session.

7. MacPherson BR, **Gana TJ,** Koo J. The response of the gastric mucosa to a topically applied prostaglandin and its implication in cytoprotection. 32nd Annual Meeting of the Canadian Federation of Biological Societies, Gastrointestinal Physiology and Metabolism Section, June 1989, Calgary. Alberta, Canada: poster session.

8. Sabourin PJ, Kingma YJ, **Gana TJ,** Koo J, Bowes KL. An improved amplifier for measuring hydrogen gas clearance. The Canadian Medical and Biological Engineering Society Conference, June 9-12, 1990, Winnipeg, Manitoba, Canada: poster session.