PHARMACODYNAMIC ANALYSIS AND METHODOLOGICAL ADVANCEMENTS FOR THE STUDY OF VASOACTIVE AGENTS AND THEIR FUNCTIONS IN THE HEPATIC AND MESENTERIC VASCULAR BEDS IN HEALTH AND DISEASE

BY

MARK STEVEN D'ALMEIDA

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

Department of Pharmacology and Therapeutics
Faculty of Medicine, University of Manitoba

(c) February 1993

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This thesis is dedicated to all those who think they "can't". Try it, you just might succeed.

Portions of my research appearing in this thesis have been previously reported in the following publications:

Lautt, W.W., d'Almeida, M.S., McQuaker, J.E., D'Aleo, L. Impact of the hepatic arterial buffer response on splanchnic vascular responses to intravenous adenosine, isoproterenol and glucagon. Can. J. Physiol. Pharmacol. 66: 807-813, 1988.

d'Almeida, M.S., McQuaker, J.E., D'Aleo, L. and Lautt, W.W. Competing effects of intravenously infused dilator agents and raised portal blood flow on hepatic arterial conductance. Proc. West. Pharmacol. Soc. 31: 113-115, 1988.

d'Almeida, M.S. and Lautt, W.W. The effect of glucagon on vasoconstriction and vascular escape from nerve- and norepinephrine-induced constriction of the hepatic artery in the cat. Can. J. Physiol. Pharmacol. 67: 1418-1425, 1989.

d'Almeida, M.S. and Lautt, W.W. The effect of glucagon on autoregulatory escape from hepatic arterial vasoconstriction in the cat. Proc. West. Pharmacol. Soc. 32: 265-267, 1989.

d'Almeida, M.S. and Lautt, W.W. Glucagon pharmacodynamics and modulation of sympathetic nerve- and norepinephrine-induced constrictor responses in the superior mesenteric artery of the cat. J. Pharmacol. Exp. Ther. 259: 118-123, 1991.

d'Almeida, M.S. and Lautt, W.W. Expression of vascular escape: conductance or resistance? Am. J. Physiol. 262: H1191-H1196, 1992.

Published studies not appearing in this thesis are:

Lautt, W.W., Legare, D.J. and d'Almeida, M.S. Adenosine as putative regulator of hepatic arterial flow (The buffer response). Am. J. Physiol. 248: H331-H338, 1985.

d'Almeida, M.S., Zhang, Y. and Lautt, W.W. Determination of the effectiveness of DPSPX as an adenosine receptor antagonist, in vivo. Proc. West. Pharmacol. Soc. 33: 265-270, 1990.

ACKNOWLEDGEMENTS

No man is an island. To have completed my Ph.D without the assistance and support of those to be mentioned would have been virtually impossible.

The members of the Lautt research unit are the best collegues, collaborators and friends one could ever hope to work with. I would like to thank Wayne for his initial confidence in me when he accepted me into his laboratory, for his financial assistance in the early part of my program, his guidance in research, advice on personal matters, and his perpetual expounding on the "philosophy of science". Dallas is a cut above any technician I have ever met. I am grateful to him for the excellence in technical training I received and for keen insights into the running of a productive, efficient laboratory. I would like to thank the rest of our technicians, past (Janet, Lil and Karen B.) and present (Melanie) for their technical assistance and Karen for her incredible secretarial skills and ability to make sense out of the instructions on any application form and "instructions to the authors", upon manuscript submission. A special thanks must be extended to Egon Brockhausen for his assistance over the years and making his lunch a communal event. Above everything else, I thank all the members of the lab, and our post docs and students (Yong, Andres and Hong) for making it a great place to work and for the close friendship through the years.

I would like to thank the members of my thesis committee, Drs. Clive Greenway, Carl Pinsky, Gerry Minuk, and my external reviewer, Dr. Andrew Rankin, for reading this document and for their comments.

There are several members of the faculty to whom I would like to express my gratitude. I would like to thank Dr. Clive Greenway for many insightful discussions about

my research, science in general, and for some of the best lectures and seminars I have attended. Evenings in the department were always an adventure when Dr. Pinsky was working late. I extend my most sincere thanks to Dr. Pinsky for hours upon hours of discussion on everything from anthropology to zoology, with a bit of electromagnetic physics, hockey, politics and super-conductivity thrown in for fun. I have had only one nightmare in my life and Dr. Dan Sitar was playing the lead. While this may appear to be the furthest thing away from an expression of thanks, I feel deeply indebted to Dr. Sitar for making me realize the necessity of being prepared and not to take anything too lightly; you might just get asked about it in class!

I would also like to thank Dr. Kathie McCutcheon and the staff of Central Animal Care for their invaluble assistance especially during my last series of investigations.

Without funding, my work could not have been conducted and therefore acknowledge and thank The Medical Reseach Council of Canada and The Heart and Stroke Foundation of Canada for operating funds and personal support.

I am deeply indebted to my family, Mom and Dad, Vasco and Lynn, Joâo, David and Estelle, Maria and Bill and all the kids, for their unending moral, spiritual and financial support throughout my program, but especially in these last hectic months. I extend these same heartfelt sentiments to the Lynch family, in particular Mike and Robin and to my good friends Tim, Christine, Dan, Mary Jane, Tim, Lynn, Loree, Gordo, Aaron, Chris, Tom, Weimin, Dorothea, Myc-O, Johnnye and Vern. And finally, to Joanna, my pillar of strength that kept me going, and my security blanket when the darkness seemed to never end, Thank you... I never could have done this without you.

ABSTRACT

The studies presented in this thesis were carried out in cats and address 3 main objectives. The first objective was to develop new methods to assess the pharmacology of vasoactive agents in the hepatic artery (HA), with special reference to the effect of the hepatic arterial buffer response (HABR) on HA responses. It was possible to demonstrate the confounding influence of the HABR on responses in the HA upon i.v. and i.a. infusions of glucagon and how to circumvent this effect, thereby fulfilling the first objective. The second primary research objective was to study the pharmacology and function of glucagon in the HA using the techniques developed pursuing the first objective. The pharmacodynamic estimates of Rmax (maximal response) and ED_{50} (dose of drug producing 50% of Rmax) for glucagon in the HA were determined for the first time without the confounding influence of the HABR. Comparative studies in the superior mesenteric artery (SMA) demonstrated that glucagon was 9 times more potent in the HA than in the SMA (ED_{50}) and the dilatory efficacy of this peptide (Rmax) in the SMA was double that of the HA. Functionally, glucagon was tested as an inhibitory modulator of the extrinsic regulatory systems of the HA and SMA. Glucagon had no effect on nerve- and norepinephrine-induced peak constrictor responses in either vessel, which is contrary to previous reports, but did inhibit vascular escape in the HA. It is unlikely that glucagon has major vascular effects at physiological levels, but these findings may help elucidate the mechanism of vascular escape. The third objective was to study the effect of chronic bile duct-ligation (CBDL) in the cat, as a model of portal hypertension (PHT) and to determine the effect of this pathology on the control of portal and intrahepatic pressures over a 3 week period. CBDL did not produce

PHT after 21 days, despite the development of hepatic dysfunction, severe histological disruptions and parenchymal cell injury. A selective impairment of nerve-induced constrictor responses of the portal and hepatic venous resistance vessels did occur, followed by a partial regeneration of the portal venous responses. Vascular responses to norepinephrine were potentiated at 3 weeks. Thus, CBDL is not effective at producing PHT after a 3 week period of biliary obstruction, but does produce some of the cardiovascular complications associated with PHT without the development of a greatly elevated hepatic vascular resistance.

LIST OF FIGURES

Figure 1. Stereogram of liver cell plates 2

Figure 2. Three dimensional depiction of liver structure 11

Figure 3. The hepatic acinus according to Rappaport 14

Figure 4. Example of the hepatic arterial buffer response 23

Figure 5. Microvascular arrangement of hepatic acinus 28

Figure 6. Structure of glucagon 42

Figure 7. Relationship between hepatic venous resistance (HVR) and hepatic venous distending pressure 57

Figure 8. Effect of changing hepatic blood flow on intra-hepatic pressure, in vivo 59

Figure 9. Theoretical data for predicted intrahepatic pressure as total hepatic blood flow changes 60

Figure 10. Percentage transmission of central venous pressure upstream to intrahepatic pressure 62

Figure 11. Relationship between hepatic blood volume and intrahepatic pressure, in the absence and presence of norepinephrine 64

Figure 12. Surgical preparation used to investigate the impact of the HABR on hepatic arterial conductance during an i.v. infusion of glucagon 97

Figure 13. Effect of eliminating the HABR on hepatic arterial conductance 107

Figure 14. Interaction of the hepatic arterial buffer and intravenous glucagon infusion on hepatic arterial blood flow 108

Figure 15. Preparation used to study the pharmacology and modulatory effect of glucagon in nerve-and norepinephrine-induced constrictions of the hepatic artery 119

Figure 16. Intra-arterial and intraportal dose-response curves for glucagon in the hepatic artery 126

Figure 17. Effect of low, mid and high doses of glucagon on nerve-induced peak constrictions in the hepatic artery 127

Figure 18. Effect of low, mid and high doses of glucagon on norepinephrine-induced peak constrictions in the hepatic artery 128

Figure 19. Intra-arterial dose-response curve for glucagon in the superior mesenteric artery 142

Figure 20. Intra-arterial dose-response curve for norepinephrine in the superior mesenteric artery 143

Figure 21. Effect of low, mid and high doses of glucagon on norepinephrine-induced peak constrictions in the superior mesenteric artery 145

Figure 22. Effect of low, mid and high doses of glucagon on nerve-induced peak constrictions in the superior mesenteric artery 146

Figure 23. Plot of the hypothetical relationships between vascular escape calculated using blood flow and vascular escape calculated using conductance and resistance 161

Figure 24. Comparison of linear regressions between superior mesenteric arterial blood flow vascular escape and escape responses calculated using conductance and resistance 163

Figure 25. Nonlinear regression of pooled superior mesenteric arterial flow-escape and resistance-escape responses form norepinephrine and nerve stimulation 165

Figure 26. Inhibitory effect of low, mid and high doses of glucagon on escape responses from nerve- and norepinephrine-induced constrictions in the hepatic artery 178

Figure 27. Tracing depicting the inhibitory effect of glucagon on vascular escape from a 6 Hz nerve-induced constriction in the hepatic artery 180

Figure 28. Tracing of a typical intrahepatic pressure profile in the basal state, taken from a sham-operated animal 207

Figure 29. Basal levels of femoral arterial pressure, portal venous pressure and lobar venous pressure taken from sham-operated, 10, 14 and 21 day CBDL test cats 210

Figure 30. Basal hepatic pressure gradients for the sham, 10, 14 and 21 day CBDL test cats 212

Figure 31. Changes in femoral arterial pressure in response to nerve and norepinephrine stimulation in sham-operated, 10, 14 and 21 day CBDL test cats 217

Figure 32. Changes in portal venous pressure in response to nerve and norepinephrine stimulation in sham-operated, 10, 14 and 21 day CBDL test cats 218

Figure 33. Changes in lobar venous pressure in response to nerve and norepinephrine stimulation in sham-operated, 10, 14 and 21 day CBDL test cats 219

LIST OF TABLES

Table 1. Mean values of distending pressure, hepatic blood flow, percentage hepatic arterial flow, index of contractility values across the liver and total resistance across the liver at low, mid and high hepatic blood flows 58

Table 2. Percent inhibition of nerve- and norepinephrine-induced vasoconstrictions in the hepatic artery by intraportal glucagon 129

Table 3. Percent change in flow, conductance, and resistance at peak vasoconstriction in the superior mesenteric artery 158

Table 4. Hypothetical data predicting resistance-flow escape and conductance-flow relationships 160

Table 5. Vascular escape calculated in terms of flow, conductance and resistance 166

Table 6. Average arterial, portal venous and perfusion pressure in the superior mesenteric artery before and during the escape phase from constrictor responses induced by norepinephrine and nerve stimulation 167

Table 7. Percent escape from initial peak constriction in the presence and absence of glucagon in the superior mesenteric artery 181

Table 8A. Plasma values for sham-operated controls, and after 10, 14 and 21 days of bile duct-ligation 202

Table 8B. Plasma values for sham-operated controls, and after 10, 14 and 21 days of bile duct-ligation 203

Table 8C. Plasma values for sham-operated controls, and after 10, 14 and 21 days of bile duct-ligation 204

Table 9. Peak responses of absolute femoral arterial pressure, portal venous pressure, and lobar venous pressure, to 8 Hz frequency nerve stimulation in sham-operated and chronic bile duct-ligated cats 215

Table 10. Peak responses of absolute femoral arterial pressure, portal venous pressure, and lobar venous pressure, to 0.50 μg/kg/min norepinephrine into the portal vein in sham-operated and chronic bile duct-ligated cats 216

Table 11. Values for the changes in femoral arterial pressure, portal venous pressure and lobar venous pressure in response to 8 Hz frequency nerve stimulation in sham-operated and chronic bile duct-ligated cats 220

Table 12. Values for the changes in femoral arterial pressure, portal venous pressure and lobar venous pressure in response to 0.50 μg/kg/min norepinephrine infusion into the portal vein in sham-operated and chronic bile duct-ligated cats 221

Table 13. Liver weights and percentage of body weight for sham-operated and 10, 14 and 21 day chronic bile duct-ligated cats 225

TABLE OF CONTENTS

Acknowledgements.. I

Abstract.. III

List of Figures.. V

List of Tables.. VIII

Preface.. XIV

Introduction.. 1

Section I. Anatomy of the Hepatic Circulation.. 9

I.1. The Hepatic Artery and Portal Vein.. 9
I.2. The Sinusoids.. 10
I.3. The Acinus.. 13
I.4. Metabolic Zonation.. 15
I.5. Lymphatics.. 17
I.6. Nervous Innervation.. 17

Section II. Regulation of Hepatic Blood Flow.. 20

II.1. The Hepatic Artery.. 20
II.2. Intrinsic Regulation.. 22
II.2.A. The Hepatic Arterial Buffer Response.. 22
II.2A.i. Suggested Mechanisms for the Buffer Response.. 24
II.2A.ii. Significance of the Buffer Response.. 33
II.2B. Autoreglation of the Hepatic Artery.. 34
II.3. Extrinsic Control of the Hepatic Artery.. 35
II.3A. Nervous Control of the Hepatic Artery.. 35
II.3B. Humoral Control of the Hepatic Artery.. 38
II.4. Modulation of the Hepatic Artery.. 40
II.4A. Glucagon.. 41
II.4A.i. Glucagon Receptor and Second Messenger System.. 43
II.4A.ii. Glucagon-Like Immunoreactivity (GLI).. 45
II.4A.iii. Regulation of Glucagon Release.. 46
II.4B. Other Potential Modulators.. 48

Section III. Regulation of Portal and Intrahepatic Blood Pressure.. 50

III.1. Postsinusoidal Resistance.. 50

III.2. Presinusoidal Resistance.... 53

III.3. Autoregulation of Portal and Intrahepatic Pressure: Distensible Resistance Sites.... 54

Section IV. Blood Volume.... 63

IV.1. Definitions.... 63
IV.2. Changes in Hepatic Blood Volume.... 65
IV.3. Modulation of Blood Volume Responses.... 67

Section V. Complications of Hepatic Hemodynamics: Portal Hypertension.... 68

V.1. General Considerations.... 68
V.2. Development of Portal Hypertension.... 70
V.2A. Development of Increased Resistance.... 72
V.2B. Development of Increased Portal Inflow.... 74
V.2B.i. Circulating Humoral Agents.... 74
V.2B.ii. Hyposensitivity to Endogenous Constrictors.... 75
V.2B.iii. Development of Portal Systemic Shunting and Varices.... 78
V.3. Treatment of Portal Hypertension.... 78
V.3A. Surgical Therapy.... 79
V.3B. Pharmacological Therapy.... 80
V.3C. Chronic Bile Duct Ligation as a Model of Portal Hypertension in the Cat.... 84

Objectives of Thesis.... 91

Section VI. Methodological Developments for the Pharmacological Assessment of Vasoactive Agents in the Hepatic Artery.... 94

Introduction.... 94
Methods and Materials.... 96
Protocols.... 99
Results.... 104
Discussion.... 109
Summary.... 113

Section VII. Pharmacology and Functional Considerations of Glucagon in the Hepatic and Mesenteric Vascular Beds of the Cat.... 115

VII.1. Glucagon Pharmacodynamics and Modulation of Nerve-and Norepinephrine-Induced Constrictor Responses in the Hepatic Artery of the Cat.... 115

Introduction.... 115
Methods and Materials.... 116
Protocols.... 120
Results.... 123
Discussion.... 130
Summary.... 135

VII.2. Glucagon Pharmacodynamics and Modulation of Nerve-and Norepinephrine-Induced Constrictor Responses in the Superior Mesenteric Artery of the Cat.... 136

Introduction.... 136
Methods and Materials.... 137
Protocols.... 138
Results.... 140
Discussion.... 147
Summary.... 150

VII.3. The Phenomenon of Vascular Escape: Quantification and Expression of Results.... 152

Introduction.... 152
Methods and Materials.... 154
Protocols.... 155
Results.... 157
Discussion.... 168
Summary.... 173

VII.4. Modulatory Effect of Glucagon on Vascular Escape in the Hepatic and Mesenteric Vascular Beds of the Cat.... 174

Introduction.... 174
Methods and Materials.... 174
Protocols.... 175
Results.... 177
Discussion.... 182
Summary.... 186

Section VIII. Assessment of Chronic Bile Duct Ligation as a Model of Portal Hypertension in the Cat........ 188

Introduction........ 188
Methods and Materials........ 189
Protocols........ 193
Results........ 196
Discussion........ 226
Summary........ 240

General Summary........ 241

References........ 244

PREFACE

The liver possesses a wealth of vascular research projects owing to its dual perfusion by the hepatic artery and portal vein and its capacity to act as a blood reservoir. A vast number of investigations have been carried out in these areas, but, despite this, the hepatic vascular bed is still a poorly understood circulatory system. Furthermore, in some instances, outdated theories in respect to the regulation and roles of this vascular bed are still maintained. In recent years, however, exciting new advances have taken place at the technical and conceptual levels regarding the hepatic and splanchnic circulations. Reassessment and reevaluation of previously held ideas about the liver, in light of the new developments, has become imperative.

In the midst of an hepatic "renaissance" (of sorts), the objectives of my research program were to develop and assess methodologies and to apply these procedures to study and re-evaluate the pharmacology and physiology of the hepatic and splanchnic vascular beds. To this end, it was necessary to study the hepatic and splanchnic circulations in several complimentary directions, reaping valuable data and experience. Thus, the work presented in this thesis will discuss topics including the development of methodologies for the accurate investigation and interpretation of vascular responses and intrinsic regulation of the hepatic artery. Specifically, the unique relationship between hepatic arterial flow and portal venous flow is a major concern not only in respect to hepatic function, but also in respect to our ability to conduct meaningful pharmacological and physiological research in this area. Pharmacodynamic analysis of vasoactive agents on the hepatic and mesenteric arterial beds will be discussed as well as the physiological or modulatory role these

compounds may play in the regulation of these vascular beds.

The venous side of the hepatic vascular bed is of great significance, especially in respect to the maintenance of intrahepatic pressure which, in turn, has repercussions on the microcirculation and basic hepatic functions. Data and discussion on the preliminary development of a model of feline portal hypertension will address issues pertaining to the venous system of the splanchnic circulation.

What follows is a review of the complexities of the hepatic circulation and the multifaceted role of the liver in overall cardiovascular and metabolic homeostasis.

INTRODUCTION

The liver is the single largest gland in the human body and weighs approximately 1500 grams in the adult human. It is located just below the diaphragm and attaches to this muscle by the falciform ligament. In the adult, the liver extends from the right fifth intercostal space along the mid-clavicular line and down to the costal margin, thus being encased and protected by the rib cage. The liver is covered by a thin layer of connective tissue known as Glisson's capsule. This capsule is composed of type I and III collagen fibres, fibroblasts, and small blood vessels.

Hepatic tissue is a complex labyrinth of interconnected sheets or walls of hepatocytes referred to as hepatic plates. Using light microscopy or scanning electron microscopy, the morphology of the liver appears as a maze of strips with no particular order. The problem with such an interpretation is that this network of hepatic plates is presented primarily in only two dimensions. The true architecture of the liver must be conceptualized in a three dimensional manner with sheets of hepatic plates interconnecting with others at all angles and in all directions. Such a system of interconnecting walls is defined as a muralium (Elias and Sherrick, 1969). Between these plates is a system of interconnecting cavities known as the lacunae (figure 1). Within the lacunae reside the hepatic sinusoids.

The blood supply to the liver is through the hepatic artery and portal vein. This dual blood supply renders the hepatic vascular bed unique, not only in anatomical and functional terms but also in methodologies required to study this circulation. The hepatic artery, which is derived from the common hepatic artery, supplies the liver with well oxygenated blood. Portal venous blood is the sum total of venous drainage from the spleen, stomach, pancreas,

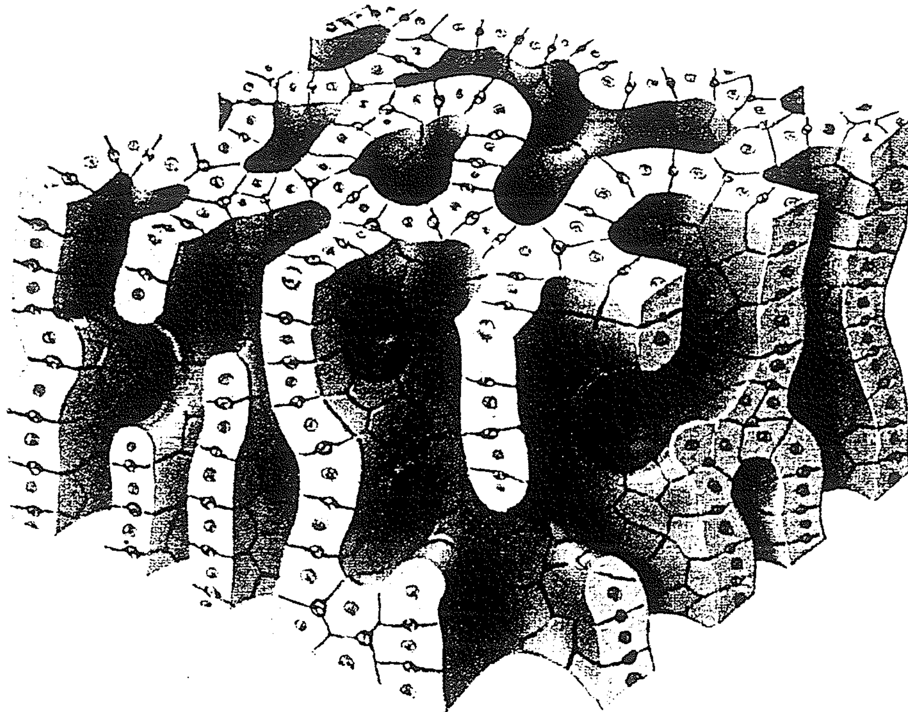


Figure 1: Stereogram of liver cell plate structure (without the hepatic blood vessels, lymph vessels or duct systems) depicting the lacunae within the muraliam of hepatic plates. Note the random orientation of the hepatic plates (Taken from the CIBA Collection of Medical Illustrations, Vol. 3, Digestive System Part III, plate 7).

mesentery, and intestine. Portal blood, therefore, is less well oxygenated than hepatic arterial blood and contains a gamut of absorbed nutrients and compounds from the intestinal tract and gastrointestinal hormones.

The liver is the main site for the handling of large amounts of absorbed nutrients, such as amino acids, carbohydrates, lipids, and vitamins, but is also central for detoxification, biotransformation and elimination of ingested pollutants, xenobiotics, and endogenous metabolites. These detoxification processes occur primarily in the endoplasmic reticulum of the hepatocytes via the mixed function oxidase system of enzymes (including the P450 cytochromes). The process of biotransformation (phases 1 and 2) makes nonplolar compounds more polar thereby facilitating excretion of these substances into the bile or urine (Gumucio and Chianale, 1988).

Pivotal to the metabolic and biotransformation functions of the liver are the processes of hepatic uptake and fluid filtration. Most of the approaches to studying hepatic uptake have been conducted in isolated perfused livers and models of hepatic uptake are primarily mathematical in nature. Neither technique has proven to be satisfactory for several reasons. In general, the isolated liver is only perfused by the portal vein and tends to produce inconsistent results. This is likely due to abnormal hemodynamics and the fact that regulatory systems, such as the hepatic arterial buffer response (see Intrinsic Control of the Hepatic Artery), are abolished in a preparation perfused solely by the portal vein. The mathematical models, in efforts to reduce the complexity of dynamic flow distribution and heterogeneity in enzyme distribution and sinusoidal structure, have incorporated inappropriate or over-simplified assumptions. The current models are the Goresky model, the equilibrium

model and the parallel tube model.

The Goresky model is based on the multiple indicator dilution technique (Goresky, 1981). Briefly, the time emergence in hepatic venous blood of substances injected into the portal vein is compared with that of substances with a known distribution in blood and extracellular and intracellular water. Uptake characteristics of compounds such as saturation kinetics and intracellular sequestration mechanisms can be determined with this technique (Goresky, 1981). The major disadvantage with the indicator dilution technique is that to obtain the dilution curves, 50 ml of hepatic venous blood must be rapidly withdrawn. In small animals, this can be considered to be a severe, acute hemorrhage and may trigger unwanted reflex hemodynamic and blood volume responses.

The equilibrium model incorporates the concept of intrinsic clearance, which is the maximum capacity of the liver to remove a specific compound from hepatic blood in the absence of any flow limitations. This model relates hepatic clearance and hepatic blood flow whereby the influence of blood flow on hepatic drug elimination depends on the intrinsic ability of the liver to remove the drug (Branch et al., 1973; Rowland et al., 1973; Wilkinson, 1975). With a low intrinsic clearance, blood flow has little effect on elimination half-life or hepatic clearance. Drug elimination and clearance will depend on flow when the liver has a high intrinsic clearance for that compound. In this situation, blood flow can be estimated from the calculated clearance of that drug.

One of the assumptions made in this model is that the substrate in the liver water is in equilibrium with emergent hepatic venous blood. This implies that the liver cells are in equilibrium with the substrate concentration in the hepatic venous blood. This last

assumption is unphysiological. Validation of this model has been difficult and has not been able to produce consistent results (reviewed by Greenway and Lautt, 1989).

The third model is the parallel tube model in which the liver is envisioned as a single tube or sinusoid with enzymes distributed along the sides. Substrate concentration declines along the length of the tube (Pang and Rowland, 1977). In this model, constituents in the hepatic blood are sequentially eliminated as blood passes through the sinusoids and develop concentration gradients along these vessels (Bass et al., 1976). These gradients, in turn, help to develop elimination rates for individual compounds. Like the equilibrium model, the parallel model predicts that if substrate uptake is low due to low metabolic processes, uptake will not be dependent upon blood flow. Uptake will be flow-dependent when substrate concentrations are decreased and/or when enzyme activities are high. This model seems to be inherently more correct than the equilibrium model based, in part, on the estimation of substrate concentration, which is assumed to be the logarithmic mean of the inflow and outflow concentrations of the agent in question. The assumption that the liver can be represented as a single uniform sinusoid, however, is not valid. This model has been revised to incorporate heterogeneity in the sinusoids resulting from differences in blood flow and in hepatocyte numbers within different sinusoids. This model is known as the distributed perfusion model (Bass et al., 1978).

Like hepatic uptake, hepatic fluid filtration is fundamental for substrate supply and metabolic and biotransformation processes in the liver. The liver is well suited for filtration of plasma and its contents owing to the sinusoidal fenestrae. Hepatic fluid filtration is

controlled according to Starling forces and is described by the equation:

$$F = CFC\ [(Pc - Pi) - r(OPc - OPi)]$$

F is the net fluid movement across the capillary wall; CFC is the capillary filtration coefficient and is determined by the product of vessel permeability and the surface area; Pc and Pi are the hydrostatic pressures in the capillary (c) and interstitial fluid (i) respectively; Opc and Opi are the colloid osmotic pressures in the capillary (Opc) and the interstitial (Opi) fluid respectively. The value r is the protein osmotic coefficient. The major physiological factor controlling net fluid exchange in the liver is sinusoidal hydrostatic pressure (Pc) (Lautt and Greenway, 1987) and as mentioned, fluid exchange and filtration occurs easily in the sinusoids due to large endothelial fenestrations. The fenestrae allow free movement of fluid in and out of the sinusoid, but restricts erythrocytes to the sinusoidal lumen (Granger et al., 1979; Wisse et al., 1985). Albumin filtration is partially restricted but at raised hepatic venous pressures the sieving effect of the fenestrae is reduced and liver exudate has been found to have a protein content approximating 90% of that of plasma (Greenway and Lautt, 1970).

The role of the liver is not restricted only to detoxification and bio-transformation of blood-borne constituents. In addition to excretory functions, the liver is also a major site of protein synthesis such as albumin and clotting factors. The liver is central to carbohydrate metabolism, switching between glycolysis and glycogen synthesis (and storage) and glycogenolysis and gluconeogenesis. Metabolism of lipoproteins, cholesterol, and triglycerides are also pivotal functions carried out by the hepatic parenchyma. Related to this, the liver also produces bile and bile salts which facilitates fat absorption from the gut

and waste excretion. Nitrogen metabolism, specifically ammonia removal and urea and glutamine synthesis, are also well defined processes occurring in the liver (Hausinger, 1989). The liver is a storage site for the B vitamin complexes and vitamin A compounds (retinol and related compounds) and vitamin K (Goodman, 1988), as well as the site of vitamin D hydroxylation and production of vitamin D binding proteins (Holick, 1988).

Apart from the metabolic functions, the liver plays a central role in cardiovascular and circulatory homeostasis. Owing to the high percentage of cardiac output received by the liver (25%), and the fact that the hepatic vascular bed is a highly compliant vascular system, the liver acts as a major blood volume reservoir. Approximately 25-30% of the liver volume is blood and the liver is capable of expelling 50% of this blood volume upon sympathetic stimulation without affecting normal liver functions (Greenway and Lautt, 1989).

Resistance to blood flow is almost entirely located at postsinusoidal sites in the normal state; sinusoidal and portal blood pressures are insignificantly different. During sympathetic stimulation, however, presinusoidal resistance develops such that portal pressure becomes greater than sinusoidal pressure. The exact location of the postsinusoidal resistance sites is not known, but is believed to be located in the small sublobular hepatic veins (approximately 1.5 mm diameter) although this is not universally agreed upon. The resistance sites are believed to be sphincter-like and have also been shown to be distensible. The existence of these distensible hepatic venous resistance sites partly protects the liver from changes in central venous pressure and helps to maintain a constant portal venous pressure during changes in portal blood flow.

It is clear from this very brief overview of the liver and its processes, that this organ

is central to many regulatory and control systems. The emphasis of the remainder of this introduction will, however, concentrate on the hepatic vasculature.

SECTION I

ANATOMY OF THE HEPATIC CIRCULATION

I.1. The Hepatic Artery and Portal Vein

The liver is supplied with blood from the hepatic artery (HA) and portal vein. The HA proper is derived from the common hepatic artery which is in turn derived from the celiac artery. The common hepatic artery also branches into the gastroduodenal artery at the site of the T-junction with the HA and common hepatic artery. Occasionally the small gastroepiploic artery exits from the common hepatic artery distal to the T-junction of the HA.

The HA enters the liver adjacent to the portal vein at the liver hilum. The artery divides into hepatic arterioles which empty into the sinusoids to bathe the liver parenchyma with freshly oxygenated blood. The branches of the HA also form a rete or network of vessels known as the peribiliary plexus which surrounds the bile ducts. This plexus has been identified in several species, including the rat and hamster, but is not present in the human (Yamamoto et al., 1985). In man, the hepatic arterioles empty directly into the sinusoids.

The portal vein is derived from the union of the splenic and mesenteric veins which drain the splanchnic organs. Approximate contributions to portal blood flow from these organs are 10% splenic, 20% gastric, 10% pancreatic, and 60% intestinal, but naturally will vary depending on the physiological state of the animal (Greenway and Lautt, 1989). The portal vein supplies the majority of blood to the liver, approximately 70% of total liver flow. The HA supplies the remaining 30% of blood flow. Immediately upon entering the liver, the portal vein divides into two primary trunks, the left and right. From each of these two

portal trunks arise innumerable branches supplying nutrient-rich blood to the sinusoids and hepatic parenchyma via the smallest vessels of the portal system, terminal portal venules. Along with lymph vessels, bile ducts, and the hepatic nerves, the branches of the portal vein and HA are encased in layer of hepatocytes collectively known as the limiting plate. The bundle of vessels, bound by the limiting plate is known as the portal canal. The vessels within the portal canal are referred to as a portal triad. Within the confines of the portal canal, the portal and arterial vessels branch and rebranch. The portal vessels eventually penetrate the limiting plate via inlet vessels and by this means distribute portal blood into the sinusoids (figure 2).

I.2. The Sinusoids

Sinusoids are specialized capillaries which possess a discontinuous membrane. The lumen of the sinusoids consists of endothelial cells with flattened processes perforated by fenestrae ranging in size from 0.1 to 0.2 µm. The fenestrae arranged in groups called sieve plates (Wisse et al., 1985).

As mentioned, the sinusoids lie within the cavities (lacunae) produced by the maze of interconnecting hepatic plates. A peri-sinusoidal gap, the space of Disse, exists between the exterior of the sinusoidal wall and the interior surface of the lacunae (see figure 2). Within this space, microvilli of the hepatocytes, making up the hepatic plates, provide an amplified surface area for uptake processes. Solutes within the sinusoidal blood collide with the endothelial wall of the sinusoids and pass through the fenestrations into the space of Disse where they make contact with the hepatocyte microvilli. Conversely, metabolites and

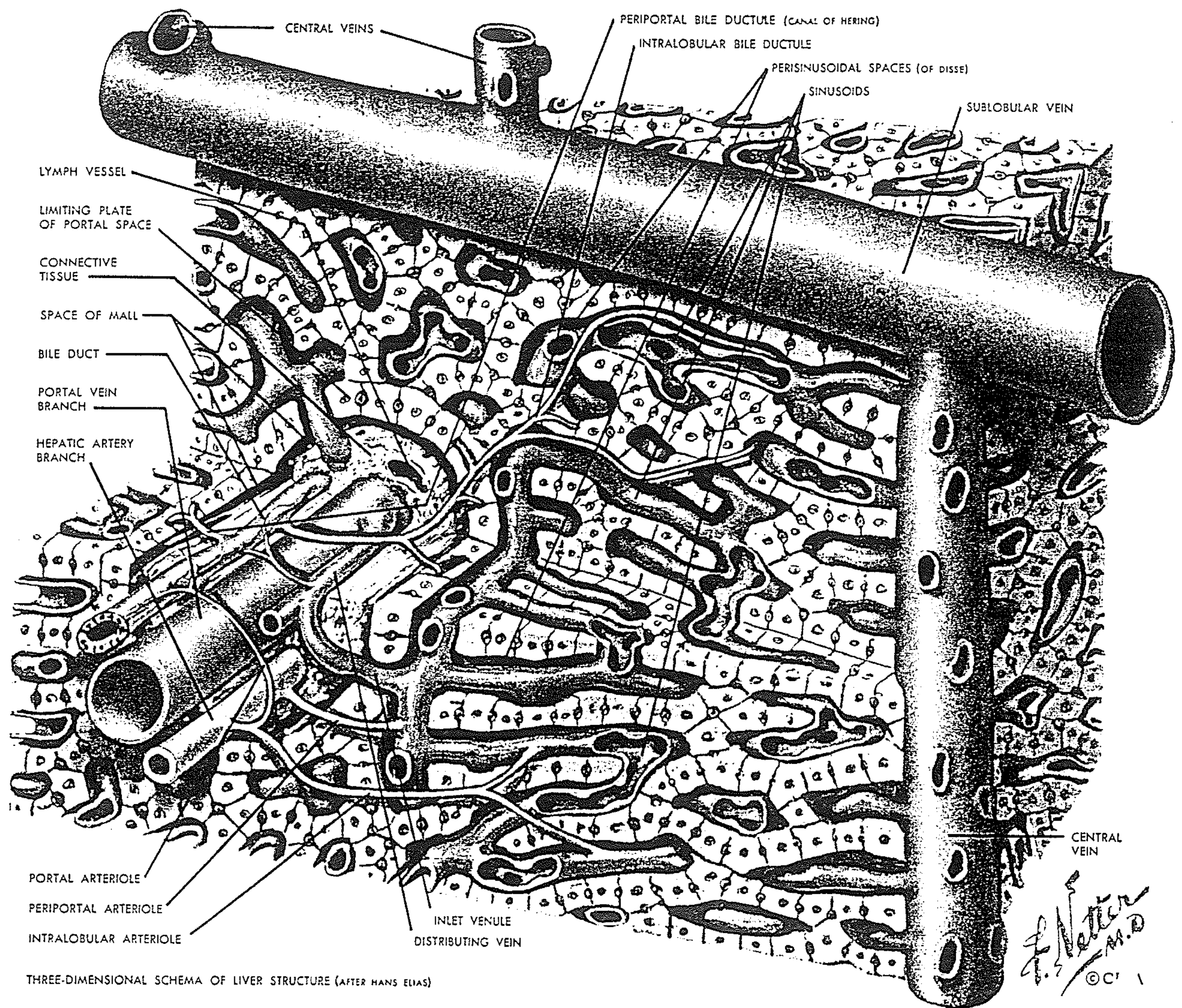


Figure 2: Three dimensional depiction of the liver structure and vascular arrangement in the normal liver (Taken from the CIBA Collection of Medical Illustrations Vol. 3, Digestive System Part III, plate 7).

products can exit the hepatocytes, traverse the space of Disse, and enter the blood through the fenestrae.

In man, the system of sinusoids course through the liver plates toward the central hepatic vein. Central veins enter almost perpendicularly into sublobular veins which converge to form collecting veins. These vessels unite to form the large hepatic vein.

The diameter and frequency of fenestrae differ in periportal and pericentral sinusoids. Diameters of fenestrae decrease slightly but increase in frequency as one progresses from the periportal to the pericentral region (Wisse et al., 1985) resulting in a slightly more porous pericentral region. Fenestrae size is altered under several conditions. Elevated hepatic venous pressure (Nopanitaya et al., 1976), CCl_4 (Fraser et al., 1982), endotoxin, and hypoxia (Frenzel et al., 1977) enlarge these gaps. Alcohol consumption has also been shown to enlarge fenestrae but also decrease the number of these structures (Mak et al., 1984). Serotonin and norepinephrine have been reported to decrease fenestrae size (Wisse et al., 1985). The possibility that fenestrae size may be regulated by actin and myosin filaments in endothelial cells has been suggested (Wisse et al, 1985).

The role of the fenestrae is to increase the permeability of the sinusoids and to enhance movement of fluid and particles (filtration) between the sinusoid and the space of Disse. Wisse et al. (1985) has measured sinusoidal size in rats and found that red and white blood cells are larger than the sinusoids especially in periportal regions. The blood cells squeeze through the sinusoid, greatly increasing sinusoidal cell interaction and may also contribute to the transport of particles and fluids in and out of the space of Disse. Two theories involving this process have evolved, forced sieving and endothelial massage.

According to the forced sieving theory, red blood cells force the plasma and its contents through the fenestrae into the space of Disse as this cell progresses through the sinusoid. Endothelial massage theorizes that white blood cells passing through the sinusoid compress the endothelial cell lining and the space of Disse lying directly below the endothelial cell. Fluid within the space of Disse is pushed downstream and flushes out of the space of Disse through the fenestrae. After the white blood cell passes over an endothelial cell, the space of Disse resumes its original shape causing fluid in the sinusoid to be drawn into the perisinusoidal space (Wisse et al., 1985).

I.3. The Acinus

For many years the hexagonal or classic liver lobule was considered to be the basic vascular and anatomical unit of the liver. In 1954, Rappaport et al., presented the concept of the liver acinus which today is considered to be the microvascular unit of liver function. The liver acinus is a small parenchymal mass, situated around a central axis consisting of a terminal portal venule, hepatic arteriole, and bile ductule (portal triad). The portal venule and hepatic arteriole give off branches and enter the sinusoids in a perpendicular angle through the portal triad. The sinusoids radiate from the portal triad toward the central vein (figure 3).

The acinar sinusoids are divided into three zones. Zone 1 is located in the periportal zone surrounding the triad, and zone 3 is furthest from the triad and closest to the central vein. Zone 2 sinusoids are located between zones 1 and 3. Zone 1 sinusoids and hepatocytes receive oxygen-rich and solute-laden blood from the portal venule and hepatic

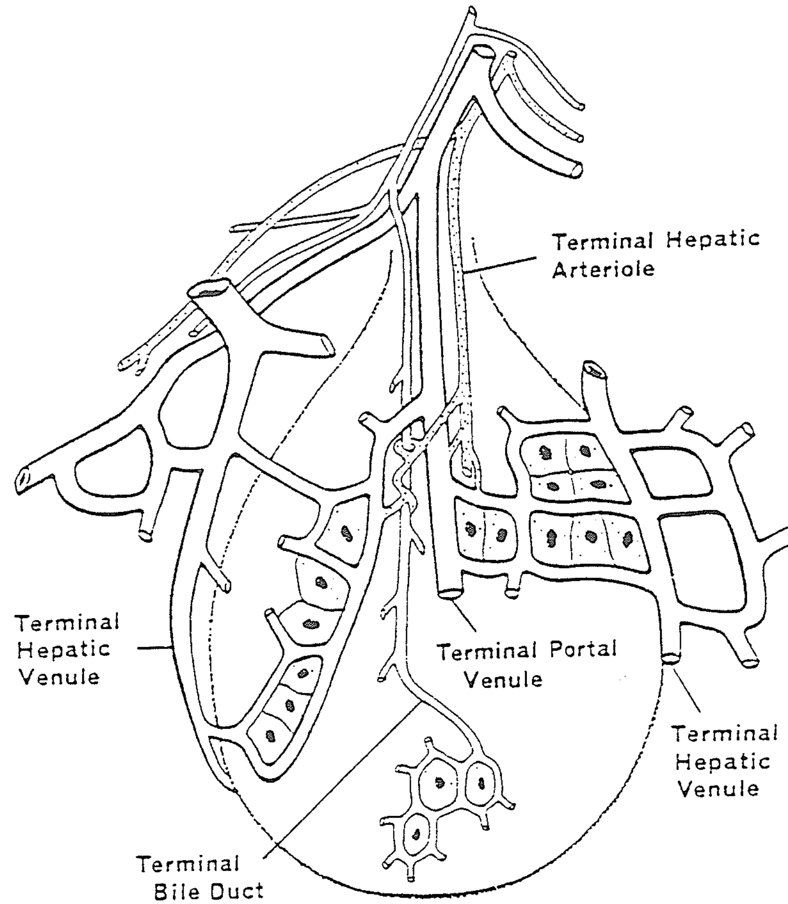


Figure 3: The acinus according to the description of Rappaport. The acinar parenchymal mass is situated around a central axis consisting of the terminal portal venule, an hepatic arteriole and a bile ductule. Blood enters the sinusoids from the portal and hepatic vessels and flows sequentially through zones 1, 2 and 3 of the acinus. Sinusoidal blood drains into the central veins located at the periphery of zone 3 hepatocytes (Taken from Lautt and Greenway, 1987).

arteriole. Zone 3 sinusoids receive the least oxygenated blood.

The sinusoids in zone 1 have been found to have a smaller volume fraction and larger surface-to-volume ratio than those in zone 3 (Miller, 1981). In agreement with reports utilizing corrosion casts, sinusoids of zone 1 are highly anastomotic and tortuous while those in zone 3 are straight and radially arranged (Hase and Brim, 1966). These two observations suggest that there is an increased probability of solutes coming into contact with endothelial cells in zone 1 sinusoids. This would increase the sieving action of solutes through the sinusoidal fenestrations and increase contact with the hepatocytes.

I.4. Metabolic zonation

One essential and interesting feature of the acinus concept is that blood flow through the acinus is unidirectional. All hepatocytes in this unit are perfused with blood originating from the same axioportal venule and hepatic arteriole. Consequently, as the blood moves through the sinusoid, substances are sequentially extracted or added to sinusoidal blood by the hepatocytes in a periportal to perivenous direction. The blood is progressively modified, implying that hepatocytes located at various positions between acinar inlet and outlet regions will be bathed by blood of varying composition. This results in the acinus becoming a unit of varying microenvironments, different in terms of substrate and enzyme availability, location and function. From these observations grew the concept of "metabolic zonation" within the liver acinus, a term coined by Katz and Jungermann (1976), describing the observation that different metabolic and catabolic processes appeared to take place in different regions along the sinusoid. Originally proposed to explain the compartmentation

of glucose metabolism in the periportal and perivenous zones of the acinus (Katz and Jungermann, 1976; Katz et al., 1977; Schmidt et al., 1978), it is now expanded to include many more metabolic processes. For example, oxygen-requiring processes such as fatty acid and amino acid breakdown predominate in the periportal region where oxygen levels are highest and mitochondria are more populous. Other examples of metabolic functions existing primarily in the periportal zone are respiratory chain processes and ATP production, glucose release, glycogen synthesis from pyruvate and bile and urea formation. The perivenous zone (zone 3) is less well oxygenated and has been found to possess increased concentrations of the enzymes involved in processes such as glycolysis and glycogen synthesis from glucose. Related to nitrogen and ammonia metabolism, glutamine formation occurs in pericentral regions as well as xenobiotic detoxification. Lipogenesis is also a process occurring in the perivenous region (Jungermann, 1988).

Metabolic zonation within the liver acinus is not a static compartmentalized phenomenon; hepatocytes are capable of carrying out all of the liver functions. The development and regulation of hepatocyte heterogeneity leading to metabolic zonation may be due to differences in substrate delivery to hepatocytes as well as selective gene expression of enzymes (Gumucio, 1989). Alternatively, Arber, Ariel and Zajieck et al. (1988), report that liver cells originate in periportal regions and progress toward pericentral regions as they mature. The possibility exists that as hepatocytes move from periportal to pericentral regions they progressively differentiate into more specialized cells (Gumucio, 1989; Grisham, 1962).

I.5. Lymphatics

The liver has a rich supply of lymphatic vessels which are located on the capsular surface of the liver and within the substance of the liver. Capsular lymphatics drain into the lymph nodes of the lumbar and celiac system or into the thoracic system. In man, lymphatic vessels have been found to possess valves and constrictions at 0.2 mm intervals. Intrahepatic lymphatics wind themselves around the portal vein or form a fine network around the bile duct. According to Magari (1990), lymph flow can lead from capsular vessels to intrahepatic vessels or in the opposite direction with the latter route predominating in man.

Fine lymphatic capillaries originate in the interlobular connective tissue and are in communication with the peri-sinusoidal space of Disse. The peri-sinusoidal spaces are also continuous with tissue spaces known as the space of Mall (Motta et al., 1978). Filtered and tissue fluid in these spaces are drained by the lymphatics.

Under conditions of elevated filtration, the lymphatics prevent edema formation in the liver by draining the tissue spaces. However, at filtration rates that exceed the lymphatic ability to collect the filtrate, this fluid can exit via the liver surface.

I.6. Nervous Innervation

The hepatic vasculature and liver tissue are innervated by two major nerve plexuses, the anterior and posterior nerve plexuses. The anterior plexus runs along with, and forms a dense sheath of nerves around, the HA. This plexus has been reported to contain sympathetic and parasympathetic fibres (Lautt and Wong, 1978a, 1978b). The posterior plexus surrounds the portal vein. The anterior and posterior plexuses join at the junction of

the HA and gastroduodenal artery and enter the liver. Nerve fibres also run within the hepatic ligaments. The relative contribution of the anterior and posterior plexuses in reflex sympathetic vasoconstriction is variable. Data suggest that the anterior plexus accounts for 59% of the response with the remainder accounted for by the posterior plexus and those fibres contained in the hepatic ligaments (Lautt, 1981a).

Stimulation of the hepatic nerves results in constriction of the HA, raising of portal venous pressure and contraction of the liver volume. Details of specific nerve responses in the hepatic vessels will be discussed in later sections (see Nervous Control of the Hepatic Artery) and have been extensively reviewed (Lautt, 1980a, 1983a; Greenway and Lautt, 1989). The hepatic nerves are not restricted to innervating only the hepatic vasculature. Parenchymal innervation is present in varying degrees depending on the species. At the one extreme, it appears that rat and mouse liver have the least extensive adrenergic innervation of hepatocytes (Reilly et al., 1978). Guinea pigs, conversely, have been reported to have an abundant innervation of the portal triads and parenchyma (Metz and Forssman, 1980). Humans have been reported to possess highly innervated hepatocytes (Nobin et al., 1977) as do monkeys, rabbits, pigeons and guinea pigs (Tsuneki and Ichihara, 1981). It is also an interesting observation that in species that do not have heavily innervated parenchyma, the number of gap junctions between cells are increased, suggesting a role for cell-to-cell communication (Forssmann and Ito, 1977).

Hepatic nerves innervating the parenchyma are also involved in the control of metabolic functions in the liver. This area has been reviewed recently (Jungermann et al., 1987). The data reported in this review however, have been taken primarily from studies

in the rat using the isolated liver technique. Briefly, sympathetic stimulation of the liver increases glucose output, shifts lactate uptake to output, decreases urea and glutamine formation and reduces ammonia uptake. Ketone body production and oxygen uptake were also reduced. These responses could be antagonized by alpha adrenergic blocking agents. Insulin was able to reduce the effect of sympathetic nerve-induced glucose and lactate responses. Glucagon-induced glucose elevations were enhanced by nerve stimulation.

Selective parasympathetic nerve stimulation was accomplished by pharmacological sympathectomy (Jungermann et al., 1987). In these animals, parasympathetic stimulation alone had little effect on glucose and lactate metabolism. Parasympathetic stimulation did, however, enhance insulin-induced glucose uptake/utilization and reduced glucagon-induced glucose output. Stimulation of the parasympathetic nerves by other investigators, however, has been demonstrated to affect glucose metabolism independently of insulin. Shimazu (1971) and Shimazu and Fujimoto (1971) reported a rapid and intense uptake of glucose and an increase in glycogen synthetase activity upon vagal stimulation in pancreatectomized rabbits. In the chemically sympathectomized cat, Lautt and Wong (1978b) reported that parasympathetic stimulation produced a rapid decrease in hepatic glucose output. Although the role of the sympathetic nervous system in hepatic metabolism has received greater attention than the role of the parasympathetic nervous system, the evidence does suggest that both branches of the autonomic nervous system are involved in hepatic glucose metabolism.

SECTION II

REGULATION OF HEPATIC BLOOD FLOW

II.1. The Hepatic Artery

The hepatic vascular bed is a low pressure vascular system implying that the basal vascular resistance is very low. The liver receives approximately 100-130 ml/min/100 g of blood. Portal flow is supplied and regulated by the outflows of the splanchnic organs and can vary widely under certain conditions. Although the portal vein supplies the majority of hepatic blood flow, the liver cannot regulate portal flow. The only control of flow within the liver is through the HA. Unlike most arteries that respond to the metabolic requirements of the tissue it supplies, hepatic arterial blood flow is regulated to maintain total hepatic blood flow at a constant level in response to a fluctuating portal blood flow, regardless of the metabolic demands. In short, the control of the hepatic blood flow via the HA is designed to subserve or maintain the metabolic homeostasis of the animal rather than the liver itself (Lautt, 1983b). This conclusion was based on several studies which altered oxygen supply or metabolic activity of liver. In one of these studies, oxygen delivery through arterial blood was reduced by normovolumetric hemodilution of venous blood (by addition of a 1:1 mixture of Ringer solution and dextran 75) in an extracorporeal blood reservoir (using a hepatic venous long-circuit methodology described by Lautt, 1976). In response to the reduced oxygen delivery, splanchnic arteries dilated yet mean hepatic arterial conductance remained constant (99 $\pm$ 5% of control) (Lautt, 1977a).

In another set of studies (Lautt, 1980b), hepatic metabolism was either increased using 2,4-dinitrophenol (DNP) or decreased using SKF-525A. DNP was shown to increase

liver metabolism as evidenced by a 24% increase in oxygen uptake and a significant decrease in portal venous and hepatic venous PO_2 and oxygen content. Arterial flow was slightly decreased from its control level and portal blood flow increased suggesting that the splanchnic vascular bed dilated in response to the increased metabolic rate. Overall hepatic blood flow was reported to be 104 $\pm$ 4% of this control level. To compensate for the lack of increase in oxygen delivery to the liver during the elevated metabolism, the oxygen demand was quenched by an increase in oxygen extraction. Had the HA been regulated by the metabolic demands of the liver, it would have been expected to dilate. This did not occur and suggests, in fact, that the hepatic arterial flow is not dictated by the local metabolic demands of the liver. SKF-525A was administered to cats to reduce the metabolic rate and again, total hepatic blood flow remained at the control level (Lautt, 1980b).

In a separate study, the relationship between bile flow and hepatic arterial flow was studied (Lautt and Daniels, 1983). The aim of this study was to dissociate the rate of bile flow from hepatic arterial flow since bile salt-induced bile flow has been concomitantly associated with hepatic arterial dilation and metabolic activity. Accordingly, this point has been suggested to account for metabolic control of the HA. In this study Lautt and Daniels were able to show that intraportal taurocholate did not produce simultaneous or equivalent changes in bile flow and hepatic arterial flow. This suggested that bile flow and metabolic rate were not linked to hepatic arterial activity.

It has since been determined that the HA is regulated by two processes. These processes are intrinsic and extrinsic regulation.

II.2. Intrinsic Regulation

Two mechanisms of intrinsic control of the HA have been defined, the hepatic arterial buffer response (HABR) and autoregulation.

II.2A. The Hepatic Arterial Buffer Response

In 1911, Burton-Opitz reported that a reciprocal relationship existed between the flows of the HA and portal vein. Although he observed that a reduced portal flow would result in an elevated hepatic arterial flow, this relationship is, in fact, not of a reciprocal nature; changes in hepatic arterial flow do not produce inverse changes in portal venous flow or resistance. The activity of the HA, therefore, is to counteract any changes in portal flow. Stated in another way, a change in portal blood flow will result in an inverse change in hepatic arterial flow (and conductance) to maintain total hepatic blood flow at a constant level. This relationship has since been renamed as the hepatic arterial buffer response (HABR) (Lautt, 1981b) in an effort to focus on the singular nature of the response (that is, the hepatic arterial response) and the role it plays from a functional point of view (vascular buffer).

An example of the HABR is illustrated in figure 4 which is a tracing taken from one cat. The preparation is such that superior mesenteric arterial (SMA) blood flow is equal to and represents portal blood flow. Point A represents the control state. Point B represents an occlusion of portal blood flow. When portal blood flow drops, there is a simultaneous increase in hepatic arterial blood flow (the buffer response) and blood pressure. At point C, despite hepatic arterial blood pressure being mechanically reduced to control levels, hepatic

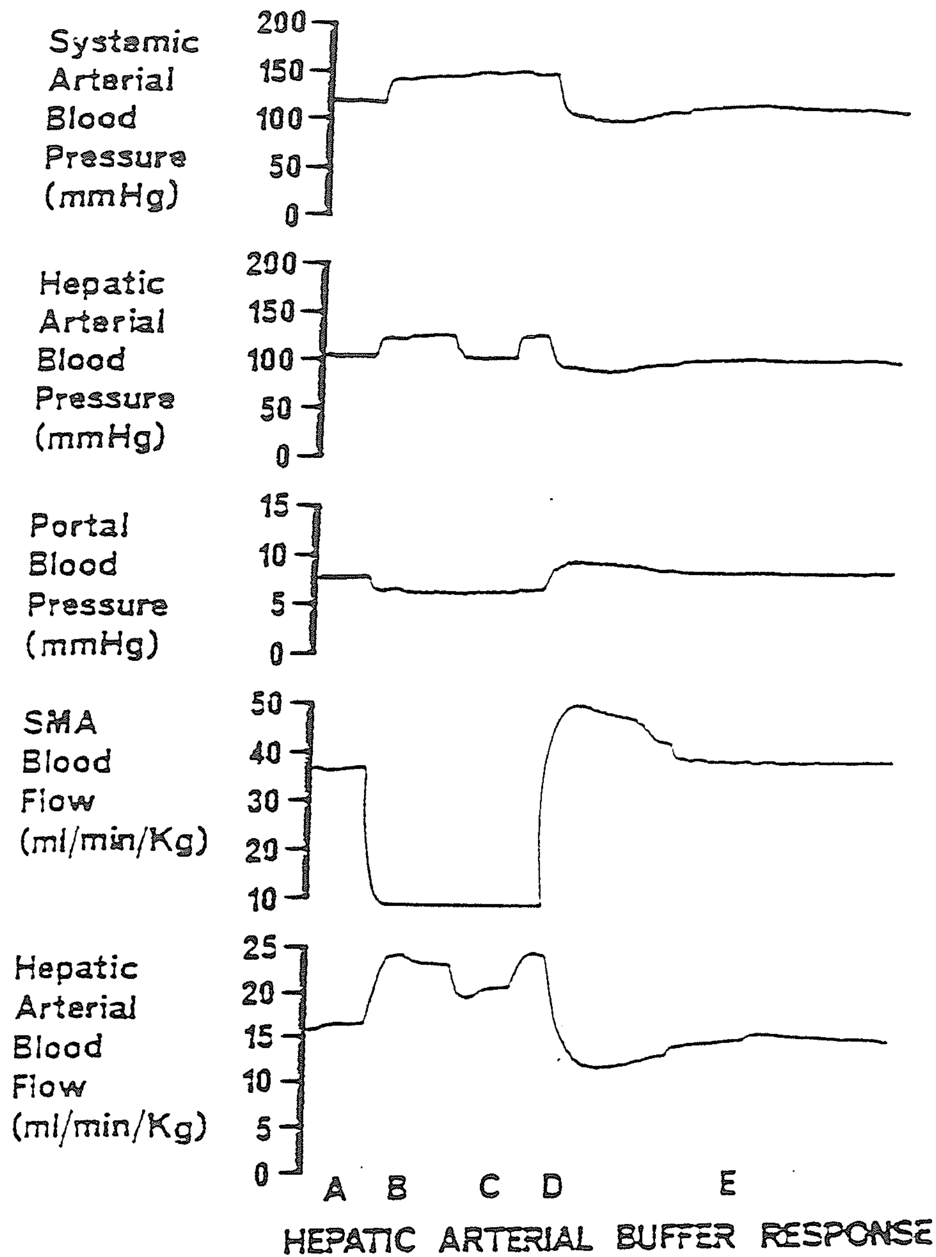


Figure 4: An example of the hepatic arterial buffer response taken from a tracing from one cat. Blood flow is measured by electromagnetic flow probes. In this surgical preparation, the only source of portal blood is by the superior mesenteric artery (SMA). Thus, portal blood flow is equal to SMA blood flow. See the text for a description of the experimental protocol.

arterial flow is still elevated and illustrates the buffer response without the influence of raised hepatic arterial pressure. Point D represents the release of the SMA occlusion and point E represents the return of the parameters to control levels.

The role of the HA in this portal vein-hepatic arterial relationship is apparent, it appears to compensate for the fluctuation in portal venous blood flow. The exact degree of compensation or efficiency of the HABR, calculated as the change in hepatic arterial flow divided by the change in portal venous flow, has averaged 25% but can be quite variable. It must be cautioned, however, that some aspects of the methodology utilized to study this phenomenon may predispose the calculated buffer efficiency to underestimate the true capacity of this response. For example, the animals are anesthetized and surgically stressed, which can cause mesenteric constriction and decreased portal blood flow thereby partially activating the buffer response. The animals have also been splenectomized, which reduces portal blood flow and may further activate the buffer response. The HA would then be in a more dilated state which could limit the extent of further dilation and the degree of buffer response by this vessel.

II.2A.i. Suggested Mechanisms for the Buffer Response

Several hypotheses have been put forward to account for the mechanism of the buffer response, however only one, the washout hypothesis, appears to be consistent with the data. The alternative hypotheses will be briefly discussed. A more thorough discussion of these hypotheses can be found elsewhere (Lautt, 1981b).

Myogenic Mechanism

Myogenic autoregulation has been indicated to play a role in controlling the HA (Hanson and Johnson, 1966). Elevations in hepatic venous pressure in dogs were shown to cause a constriction in the HA. On the other hand, in cats, raising hepatic venous pressure by 4.7 mmHg for 5 minutes did not induce any myogenic response in the HA, suggesting that the HA is not under this form of control (Lautt, 1978). It is possible that these conflicting results may be consistent with the proposed regulatory mechanism of the HABR. Details of this will be discussed more fully in that section (see Washout Hypothesis). Furthermore, despite the report by Hanson and Johnson (1966), the observations that portal venous and intrahepatic pressures are autoregulated (not portal blood flow) would suggest that these pressures are an unlikely means of controlling the HA (Lautt et al., 1987b, Greenway and Lautt, 1988).

Neural Mechanism

Complete hepatic denervation has not been shown to affect the buffer capacity (Sancetta, 1953; Mathie et al., 1980; Lautt, 1981a) and suggests a purely vascular or blood borne phenomenon regulating the HA.

Physical Mechanism

The physical mechanism of introducing a slower moving stream (portal flow) into the path of a faster moving stream (HA) has been suggested to account for the relationship between portal flow and hepatic arterial flow (Ternberg and Butcher, 1965). This theory,

however, is not very strong since the buffer response can disappear in the preparation despite unaltered pressure and flow parameters.

Quality of Portal Blood

There is the possibility that a blood-borne compound in the portal vein can act on the hepatic arterial resistance sites. We know that a transhepatic route exists whereby agents added to or infused into the portal vein can affect the HA (Lautt et al., 1984). While this is true, Lautt has clearly shown that the quality of portal blood per se does not control the buffer response. Altering oxygen content or PO_2 did not affect the HA to any significant extent (hemodilution series). Rather, hepatic arterial conductance correlates with changes in portal venous blood flow, not oxygen levels. Perfusing the portal vein with blood re-routed from the vena cava did not alter hepatic arterial conductance or calculated buffer capacity (Lautt, 1981b).

Production of Metabolites

As detailed in the description of the liver acinus there is no counter-current blood flow in the liver; blood flow through the acinus is unidirectional. Consequently, any metabolic byproduct released from the liver parenchyma into sinusoidal blood is washed downstream. There is no chance for shunting of blood back to the hepatic arterial resistance vessels.

Washout Hypothesis

The mechanism of the HABR can be accounted for by the adenosine washout hypothesis (Lautt et al., 1985; Note: As an undergraduate student, I was involved with these studies although these data will not be presented as a component of this thesis). The washout hypothesis states that adenosine, which is a well known vasodilatory agent and has potent effects on the HA, is produced in the space of Mall at a constant rate, diffuses through the fluid of the intrahepatic tissue spaces and comes into contact with the hepatic arteriole and portal venule (figure 5). Adenosine concentration is regulated by washout into the portal vein and HA. If portal blood flow is elevated, more adenosine is washed away with the portal blood. Adenosine levels in the space of Mall decrease and reduce the concentration of adenosine in contact with the HA. This causes the HA to constrict and decrease its flow in the face of elevated portal blood flow. Conversely, if portal blood flow is reduced, less adenosine is washed away and adenosine levels increase in the space of Mall. More adenosine is available and leads to a dilation of the HA with a subsequent increase in hepatic arterial flow and conductance (Lautt et al., 1985). Several lines of evidence support these claims and criteria have been met to indicate that adenosine is a putative dilator substance controlling the HABR:

1. Adenosine dilates the HA; pharmacological analysis of the dose-response curve for adenosine in the HA indicates that adenosine could produce a 245% dilation in the HA from basal levels (Lautt and Legare, 1985). The intra-arterial ED_{50} of adenosine in the HA was estimated to be 0.19 mg/kg/min (Lautt and Legare, 1985).

2. Portal blood must have access to hepatic arterial resistance vessels. The logic behind this

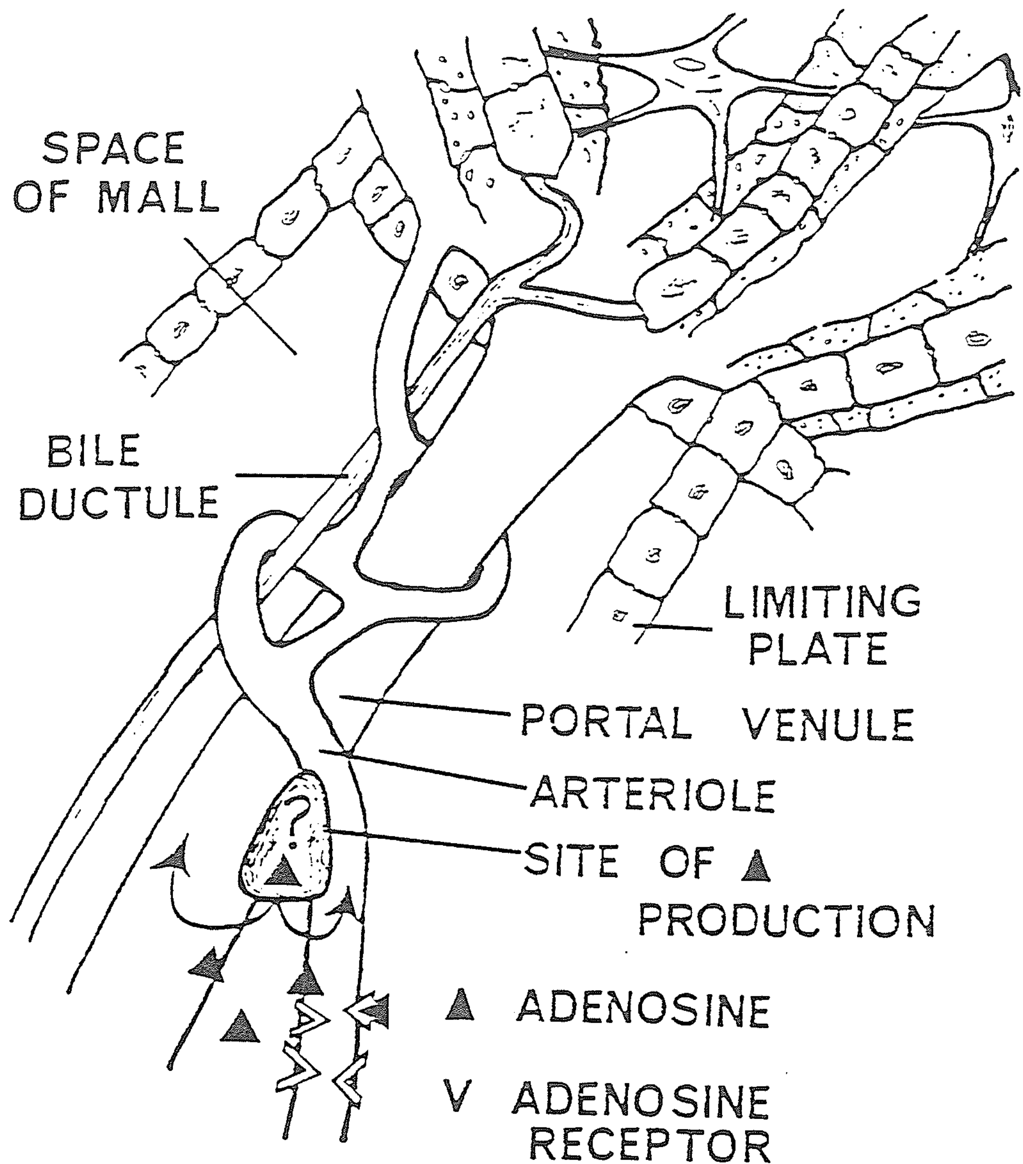


Figure 5: Description of the microvascular arrangement of the hepatic acinus in relation to the washout hypothesis mechanism mediating the buffer response. Adenosine is hypothesized to be produced at a constant rate in the Space of Mall and diffuses into the portal venules and hepatic arterioles. See the text for a detailed description of the washout hypothesis (Taken from Lautt et al., 1985).

criteria is that if portal venous blood regulates adenosine levels in the region of the hepatic arterial resistance vessels, portal venous blood flow must also have access to these sites. Intraportal venous infusions of vasoactive agents, including adenosine, have clearly shown effects on the HA (Lautt and Daniels, 1983; Lautt, et al., 1984; Richardson and Withrington, 1981).

3. Agents that potentiate the effects of exogenous adenosine should also potentiate the buffer response. Dipyridamole is an agent that blocks the uptake of adenosine into cells. Lautt et al. (1985) were able to show that dipyridamole potentiated exogenous adenosine and also potentiated the buffer response, that is, an endogenous adenosine-mediated response.

4. Antagonists of exogenous adenosine should reduce or inhibit the buffer response. The first studies testing this criterion utilized the adenosine blocking agents, aminophylline and theophylline (Lautt, 1983). These agents, however, also have unwanted systemic effects. Consequently, the dose required to block exogenous adenosine and the buffer response also produced systemic effects making their use unacceptable. Another compound, 3-isobutyl-1-methylxanthine (IBMX) was utilized as an adenosine antagonist but also had several undesirable systemic effects and a very brief half-life. Despite these problems, IBMX did produce a nearly complete block of the dilatory response to intra-arterial adenosine infusions and decreased the buffer capacity from 19% to 5% (Lautt et al., 1985).

8-Phenyltheophylline (8-PT), a more selective adenosine receptor antagonist, was found to be a very effective competitive antagonist to exogenous adenosine (Lautt and Legare, 1985). It was also very effective in inhibiting the buffer response which suggests very strongly that the buffer response is adenosine-mediated.

There are two important comments to be made in respect to the washout theory and figure 5. First, the statement of location for adenosine production and the assumption that this substance is produced at a constant rate have not yet been proven, but are consistent with the action of the buffer response. Second, the location of the adenosine receptors have been arbitrarily placed on the HA (figure 5); the exact sites of hepatic arterial adenosine receptors (luminal vs extraluminal, or both) has also not been delineated.

Recently, Mathie and Alexander (1990) have questioned the extent to which adenosine is involved in the buffer response. Using IBMX and 8-PT as adenosine receptor antagonists and dipyridamole to prevent adenosine uptake, these investigators concluded that adenosine was an important, but not sole mediator of this phenomenon. These investigators acknowledged using smaller doses of 8-PT compared to Lautt and Legare (1985) and this may explain why 8-PT was only 49% effective in inhibiting the buffer response compared to a complete inhibition reported by Lautt and Legare. There may also be the added complication of a species difference involved in these results since Mathie and Alexander utilized a canine model and Lautt and Legare used a feline model. Alternatively and speculatively, there may also be seperate pools of adenosine and adenosine receptors which may not be easily accessed by the different antagonists or in the doses used; Lautt and Legare (1985) reported that larger doses of 8-PT were required to block endogenous adenosine (mediating the buffer response) compared to the doses required to block the responses of exogenously infused adenosine.

Another recent study in the rat (Kawasaki et al., 1990) has also suggested that portal territory blood flow and hepatic arterial flow are regulated in a manner to allow independent

changes in the flow of each vessel. This conclusion was based on the observation of 7 distinct flow patterns of the portal venous blood and HA in response to a series of infusions of vasoactive agents and a single meal consisting of carbohydrates, protein and fat. While the authors did not necessarily refute the washout hypothesis for the control of the HA, they strongly suggested that such a mechanism could not account for the different flow patterns observed between the portal venous and hepatic arterial flow in response to the administered agents. This study, however, suffered from a major technical flaw; all drugs were administered through the left ventricle which, in terms of drug distribution is, equivalent to an intravenous infusion of the agent. This problem will be fully discussed in section VII of this thesis.

The different flow patterns for the portal vein and HA proposed by Kawasaki et al. (1990), can be accounted for by the adenosine washout theory for the HABR and by the action of the HABR in response to changes in portal blood flow and the direct action of the vasoactive agent acting on the HA itself. Furthermore, that the portal territory blood flow and hepatic arterial flow are independently controlled is not consistent with the concept and consequences of maintaining a constant total hepatic blood flow as will be discussed in the next section.

As mentioned earlier, when hepatic venous pressure was elevated, Hanson and Johnson (1966) reported data that was consistent with a myogenic mechanism of control in the HA, whereas Lautt (1978) found no such evidence. It is possible that these two apparently conflicting reports may still be consistent with the adenosine washout hypothesis for the control of the HA and that myogenic reflexes do not regulate this vessel. In both

studies, the elevated hepatic venous pressure caused portal venous blood flow to decrease. Under such conditions, an increase in hepatic fluid filtration out of the peri-sinusoidal space (including the space of Mall) occurs, resulting in a bulk transfer of fluid out of the liver. This fluid drains out of the liver by means of the hepatic lymphatic vessels, which originate in intrahepatic spaces that are continuous with the space of Mall. It is the fluid in this region that is proposed to contain the adenosine through which the HABR is mediated. Thus, with an increased rate of fluid filtration in this region, adenosine is washed away into the lymphatics and total adenosine levels in the space of Mall decline. This could result in a constriction of the HA, and explain the observations made by Hanson and Johnson (1966). Despite a decline in the total amount of adenosine in the space of Mall, the decrease in portal venous blood flow, observed in both studies, would tend to elevate adenosine levels (in the space of Mall) and trigger the HABR, opposing the constriction of the HA, the extent to which being related to the change in portal flow. Thus, there may be two processes simultaneously acting on the HA, one constricting the HA (a filtration-induced decline of adenosine levels in the space of Mall) and one dilating the HA (a rise in adenosine levels induced by less washout in portal venous blood, the HABR). In the study by Lautt (1978), portal flow was decreased by 42% and may have activated a powerful enough buffer response to fully counteract the proposed filtration-induced constriction of the HA. In the experiments by Hanson and Johnson (1966), portal flow decreased by only 26% and, therefore, the constriction of the HA may represent an overriding of the dilatory effect of the HABR.

The evidence for a washout mechanism of control for the HABR has been well

supported. Whether or not adenosine is the single mediator of the buffer response has been questioned, however, to date no other endogenous compounds have been definitively shown to be involved in the regulation of the HABR.

II.2A.ii. Significance of the Buffer Response

As the name implies, the HABR acts as a blood flow buffer to prevent excessive changes in total hepatic flow due to the potential wide fluctuations in portal flow. This is important for the maintenance of hepatic clearance of blood-borne compounds when clearance is blood flow dependent. Of specific concern are the regulatory peptides or hormones whose levels must be highly regulated and rapidly adjustable. This is accomplished by rapid turnover or metabolism by the liver in concert with rapid adjustment of hormonal output by the glandular source. If rapid and consistent turnover is to be accomplished, consistency of hepatic blood flow is necessary. Lautt and Greenway (1987) state that if hepatic blood flow was not prevented from rapid transient changes secondary to similar changes in portal flow, endocrine homeostasis would be imperiled. Thus, the HA acts to buffer flow changes occurring in the portal vein and minimizes the effect that altered portal flow would have on hepatic clearances.

The buffer may also play a role in overall cardiovascular homeostasis by maintaining hepatic blood volume (Lautt, 1985; Greenway, 1983; Mathie and Blumgart, 1983). Because the liver is a major blood reservoir in the mammalian system, active and passive changes in liver volume can mobilize a large amount of blood volume into the systemic circulation and have effects on venous return and cardiac output. Passive changes in hepatic volume,

occurring due to decreased hepatic flow, secondary to changes in portal flow, can be compensated for by the action of the HABR. Thus, a role of the HABR may be to minimize these passive volume shifts which would, in turn, minimize the effects on the rest of the cardiovascular system (Lautt, 1985).

Finally, the buffer response plays a significant role in oxygen delivery to the liver. Previous reports (Mathie and Blumgart, 1983) suggest that the degree of buffer observed in their preparation held oxygen levels steady, despite the decrease in portal flow. Furthermore, under conditions of severe stress, such as hemorrhage, the buffer may only partially compensate the change in portal flow, but supplies the liver with a highly oxygenated source of blood. Thus, although the buffer response has an important role in oxygen delivery to the liver, it is controlled by changes in portal blood flow, not oxygen requirements (Lautt, 1983b).

The HABR thus represents a unique control system in which overall metabolic homeostasis of the organism is served above and beyond the local oxygen demand required by the liver itself. The adenosine washout theory as the regulatory mechanism of control of the hepatic artery has been strongly supported. This is not to imply that other factors, such as vasoactive agents in the arterial or portal blood, or nerve and reflex responses cannot influence the buffer response.

II.2B. Autoregulation of the Hepatic Artery

Autoregulation of the HA is the second intrinsic regulatory process of the HA, and should not be confused with the buffer response. Autoregulation is a relationship between

arterial perfusion pressure and blood flow, the buffer response is not.

Hepatic arterial autoregulation is weak and is under the control of local adenosine levels (Ezzat and Lautt, 1987). The mechanism governing hepatic arterial autoregulation is very similar to that of the HABR. Like the buffer mechanism, adenosine is produced at a constant rate in the Space of Mall, diffuses to the HA and is washed away into the hepatic arterial blood. When perfusion pressure increases, arterial blood flow also increases and washes away more adenosine leading to a reduction in the adenosine levels in the Space of Mall. This, in turn, decreases the concentration of adenosine coming in contact with the HA and causes the vessel to constrict. Thus, adenosine levels are also determined by washout of this compound, except that in the case of hepatic arterial autoregulation, it is arterial blood that washes away adenosine, not portal blood (Ezzat and Lautt, 1987).

II.3. Extrinsic Control of the Hepatic Artery

Extrinsic control of the HA is under the influence of neural inputs, possibly a number of blood-borne compounds, and the compounding impact from the buffer response. Details of the influence of the buffer response on the extrinsic control of the HA will be discussed later. This discussion will focus on the neural and humoral influences.

II.3A. Nervous Control of the Hepatic Artery

The HA is abundantly innervated by the hepatic anterior and posterior plexuses. The hepatic nerves are a combination of parasympathetic and sympathetic fibres. Direct electrical stimulation of the anterior nerve plexus elicits frequency-dependent constriction of the HA

with a maximal stimulation frequency of 8-12 Hz in dogs and cats (Greenway et al., 1967). In cats, using frequency-response curves for nerve stimulation of the hepatic nerves, it was found that 2 Hz frequency stimulation was a good estimate of the Hz_{50} (the frequency that produces 50% of the maximal constriction) for constriction of the HA (Lockhart et al., 1988). The vasoconstriction elicited upon nerve stimulation has been determined to be mediated by alpha adrenergic receptors (Greenway et al., 1967). It has also been shown that a dilation of the HA can be produced upon nerve stimulation when alpha receptors are blocked with phenoxybenzamine. The dilation can be inhibited by the addition of beta blockers (Greenway and Lawson, 1969).

Maintained stimulation of the hepatic nerves or norepinephrine infusion results in an initial peak vasoconstriction of the HA in cats which occurs within 1-1.5 minutes after commencement of stimulation. With continued stimulation, the blood flow response of the HA (in an intact preparation) returns toward the baseline despite the maintained stimulus. This response eventually plateaus after approximately 3 minutes of stimulation and is known as vascular escape. The phenomenon of vascular escape is unusual in as much as it has been reported to occur in several species, but is not consistently present in all vascular beds. For example, vascular escape has been reported to occur in the intestinal and hepatic vascular beds of the cat. In dogs, vascular escape is observed in the intestinal vascular bed, but no such response has been reported in the HA (Greenway, 1984a).

It is reasonable to surmize that the role of vascular escape appears to be the prevention of excessive and prolonged vasoconstriction which could lead to tissue ischemia and hypoxia. The mechanism(s) of this response is not yet clear, but some ideas have been

put forth (Greenway, 1984a). Recently, Remak et al. (1990) proposed that neurogenically-induced escape may be due to afferent nerve fibres releasing vasodilatory peptides during stimulation. It has also been purported that vascular escape may occur in response to an inhibition of α_2-adrenoceptors by a local elevation in H^+ ions taking place during the constriction (Chen and Shepherd, 1991). In an investigation wherein adenosine deaminase was found to partially inhibit vascular escape, adenosine was implicated as having a role in this phenomenon (Crissinger et al., 1988). Other reports do not support this claim (Lautt et al., 1988b). The degree of vacular escape has been postulated to be directly related to the intensity of the initial constrictor response (Folkow et al., 1964a). This observation, and studies that claim to have modified the degree of vascular escape, must be viewed with some caution especially if the escape index has been calculated using vascular resistance. It is possible that calculations of vascular escape and vascular tone may be subject to mathematical artifacts if resistance is used, rather than conductance (the inverse of resistance) (Lautt, 1989). This is based on the observation that resistance is nonlinearly related to blood flow and conductance is linearly related to flow. Thus, when calculating vascular responses where blood flow changes, the use of resistance may induce an arithmetic error due to its nonlinear relationship with flow. These problems can be circumvented if conductance is used. This topic and that of vascular escape is a subject of study and further discussion in this thesis.

Although the hepatic nerves are involved in the metabolic and active hemodynamic functions of the liver, it appears that hepatic arterial resistance vessels are not under chronic control of these nerves in cats (Lautt, 1977b; Lautt and Carroll, 1984) dogs (Cohn and

Kountz, 1963; Mundschau et al., 1966) and rats (Ozier et al., 1989). Assessment of complete chemical denervation by phenol under acute or chronic conditions has been obtained and supports the lack of tonic control of the hepatic nerves (Lautt and Carroll, 1984). In the rat study (Ozier et al., 1989), surgical denervation, including sectioning of the hepatic branch of the vagus and cutting of the liver ligaments, was not found to disrupt basal hepatic and systemic hemodynamics. In contrast to these reports, Henderson et al., reported that hepatic blood flow (1989) and cardiac output (1992) are chronically elevated after liver transplantation in man (studied 3 and 8 months and 1 and 2 years post-transplantation). Because transplanted livers are completely denervated, Henderson suggested that this increase in blood flow may be due to a lack of normal vasomotor tone in the liver vasculature or to a hepatic-mediated neural feedback control system that regulates the splanchnic circulation. Alternatively the increased hepatic blood flow and cardiac output may possibly be due to a persisting hyperdynamic circulation caused by advanced pre-transplantation liver disease.

II.3B. Humoral Control of the Hepatic Artery

The potential for humoral control of the HA is great; portal blood is rich in agents released or secreted into portal blood and portal blood has access to hepatic arterial resistance vessels. It is possible that any vasoactive agent or metabolite in portal blood can, in part, affect hepatic arterial blood flow. Although this may appear to confound any concrete conclusions as to the specific humoral regulators of hepatic flow, it is possible to exclude some factors. Vasoactive gut hormones and many autacoids have been frequently

tested for their actions on the hepatic arterial resistance vessels. Many of these compounds do not appear to have vascular actions at their physiological concentrations. This includes VIP, insulin, serotonin, bradykinin, and histamine (Richardson and Withrington, 1981). Agents that may contribute to the control of the HA include the vasodilators, gastrin, secretin, and possibly cholecystokinin (Richardson and Withrington, 1981). Epinephrine, angiotensin, and vasopressin may have some effect although any actions would be weak at best. Whether prostaglandins or other arachidonic acid metabolites have any effect on hepatic arterial regulation is not known. Bile salts are also a group of compounds with potential to modulate the HA. Reabsorbed and recirculated via the enterohepatic circulation, bile salts clearly gain access to the HA through the portal blood (Richardson and Withrington, 1981). Direct infusion of taurocholate into the HA or mesenteric artery has been shown to produce dose-dependent dilations of the HA (Lautt and Daniels, 1983). Similarly, portal venous infusions of taurocholate dilate the HA but to a lesser extent than direct intra-arterial infusion (Lautt and Daniels, 1983).

Adenosine and glucagon are also of interest in this regard due to their ability to dilate vascular smooth muscle and reports of their modulatory action of vasoconstriction (see Modulation of Hepatic Artery).

Part of the problem with determining the influence of humoral factors on the HA, in addition to the multitude of potential agents, has been the methodologies used to investigate this area. Many studies have been conducted without consideration of the possible influence of the HABR. As a result, investigators have reported the results of a vasoactive agent that initially alters portal blood flow (secondary to affecting splanchnic blood flow) thereby

producing an opposite or unexpected response in the HA. Many of the earlier studies reported results from responses to bolus injections of compounds. Interpretation of these results can be confusing or misleading because the agents do not reach steady state levels. Instead a large highly concentrated wave of this compound interacts with the hepatic arterial resistance vessels and produces a large short-lived response which is not necessarily representative of the true pharmacodynamics of that agent (Lautt et al., 1988a).

II.4. Modulation of Hepatic Arterial Responses

Regulatory systems governing vascular responses are integrated and coordinated so that proper perfusion and metabolic homeostasis of the perfused tissue is maintained. These systems can be modified, modulated or fine tuned by local chemical or physical factors to produce the final vascular response. In most vessels it is the sysmpathetic nervous system that regulates much of the vascular activity and it is these nerves that are often the target in vascular modulation. The nerves can be affected at the pre-junctional terminals, generally by altering neurotransmitter release, or at the post-junctional site. Acidosis, hyperosmolality, elevations of potassium concentration and norepinephrine itself (via pre-junctional α_2 adrenceptors) have all been shown to inhibit norepinephrine release from sympathetic nerve terminals. Circulating humoral agents such as adenine nucleotides and adenosine, acetylcholine, histamine, serotonin have been documented to reduce or inhibit noradrenergic transmission (Shepherd and Vanhoutte, 1985).

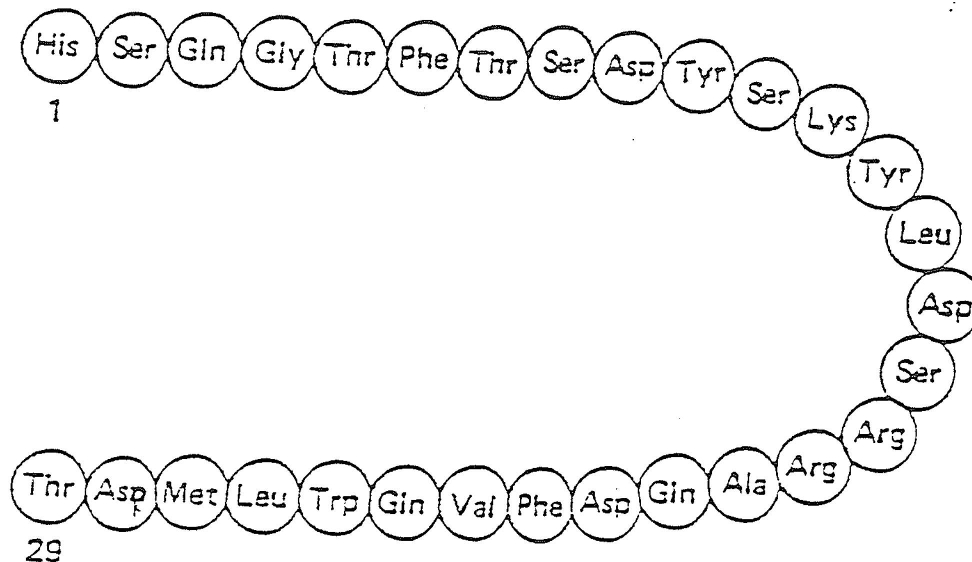
The nerve-induced constrictor response in the HA can be inhibited by adenosine (Lautt and Legare, 1986). This is in addition to the observations that adenosine also inhibits

vasoconstriction induced by norepinephrine, vasopressin, and angiotensin (Lautt and Legare, 1986). The effect of adenosine in the HA is consistent with a postsynaptic mode of action. Adenosine does not affect the resistance vessels of the portal venous or hepatic venous system (Lautt and Legare, 1986).

II.4A. Glucagon

Glucagon is a 29 amino acid polypeptide (molecular weight 3485) produced in the A-cells of the Islets of Langerhans of the pancreas (figure 6). Glucagon is produced by a series of proteolytic cleavages from an 18-19KDa polypeptide precursor, proglucagon and is also rapidly and efficiently degraded by proteolytic action in the liver, kidney and in the plasma with a half-life of approximately 3 to 6 minutes (Jaspan et al., 1981; Emmanouel et al., 1978; Unger and Orci, 1981). Glucagon is released directly into the portal blood by exocytosis. Thus, the liver parenchyma, portal venules and hepatic arterioles are subjected to the highest levels of endogenous glucagon; portal blood glucagon levels have been reported to be between 160 pg/ml to 5000 pg/ml (Blackard et al., 1974; Felig et al., 1974) and basal systemic plasma levels are estimated to be in the range of 100 pg/ml to 350 pg/ml (Smitherman et al., 1978).

Glucagon has been of interest for its role in liver-related metabolic processes, specifically glucose metabolism and its ability to produce ketones as an alternate energy substrate in periods of starvation and fasting (Unger and Orci, 1976), but also because of its cardiovascular effects. This peptide has long been known for its ß-adrenoceptor-like cardiotonic effects (Farah, 1983), but our interests have been related to its vasodilatory



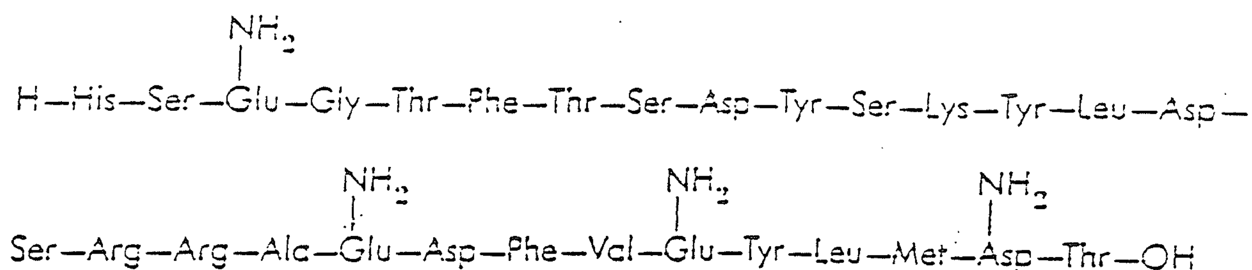


Figure 6: The amino acid sequence and structure of glucagon. Glucagon is a 29 amino acid, single chain polypeptide with a molecular weight of 3485 (Taken from Karam, 1987; Larner, 1985).

actions on arterial smooth muscle and potential for modulation of the hepatic vascular bed. Glucagon has been reported to dilate the HA in intact preparations upon intraarterial and intraportal infusions (Richardson and Withrington, 1976a, 1978a), the mesenteric vascular bed during intravenous, intraarterial and intraportal infusions (Kock et al., 1970b; Tibblin et al., 1970; Madden et al., 1971, Premen, 1987) and renal arteries in response to intravenous infusions (Kock et al., 1970b). When given intravenously, glucagon has a selectivity for dilating the splanchnic vascular bed (Kock et al., 1970b). It is also interesting to note that while intravenous glucagon dilates the mesenteric vasculature, it has also been reported to constrict the HA at the same time (Ross 1970, Krarup and Larsen, 1974) despite the well documented dilatory effect of glucagon on the HA during intraarterial administration. This apparent inconsistency is likely due to the action of the HABR, which would tend to constrict the HA in response to the glucagon-induced rise in mesenteric and portal blood flow. This concept will be tested and discussed in greater detail later in the thesis.

Glucagon has also been reported to inhibit nerve and norepinephrine-induced peak constrictions of the hepatic and mesenteric artery of dogs (Kock et al., 1971, Tibblin et al., 1971; Richardson and Withrington, 1976b, 1977, 1978a). Doses in these studies were, however, extremely large and in many instances were bolus injections and therefore did not reach steady state concentrations. These findings have not been reproduced in cats (Greenway, 1981a) and will be the subject of study and discussion in this thesis.

II.4A.i. Glucagon Receptor and Second Messenger System

The structure of the glucagon receptor has not yet been fully determined, but it has

been reported to be a 60 KDa glycoprotein located on the plasma membrane. Glucagon-receptor interactions result in an increase in the second messenger, cAMP (Sutherland et al., 1968) although recent studies have revealed that a second glucagon receptor may exist which is linked to the generation of diacylglycerol (DAG) and inositol trisphosphate (IP_3) (Murphy et al., 1987). The relaxant effect of glucagon on vascular smooth muscle has been linked to cAMP generation. Phosphodiesterase inhibitors were shown to potentiate this effect of glucagon in isolated vessel preparations (Gagnon et al., 1978; 1980).

Glucagon-induced responses have also been linked to calcium activity, but the results of investigations in this area are mixed. Friedmann and Park (1968) reported an increase in calcium efflux from the liver, suggesting a net output of calcium from the hepatocytes. In isolated hepatocytes, calcium uptake was reported to be increased (Andia-Waltenbaugh et al., 1978). Elevations in intracellular calcium have been reported (Sistare et al., 1985) and may possibly be due to a combination of extracellular calcium influx and release from intracellular calcium stores, presumably the endoplasmic reticulum (Combettes et al., 1986). The intracellular release of calcium may be induced by a glucagon-mediated rise in cAMP and activation of A-kinase which may be the mechanism for calcium release (Staddon and Hansford, 1989). IP_3 levels have also been reported to be elevated by glucagon and glucagon analogues in cAMP dependent and independent mechanisms which can unlock intracellular stores of calcium (Combettes et al., 1986; Wakelam et al., 1986). In vascular smooth muscle, increases in intracellular calcium would tend to promote activation of the contractile apparatus and produce an increase in tension or vasoconstriction. Gagnon et al. (1980), using the rabbit renal artery, has shown, however, that glucagon may actually

decrease intracellular calcium levels by extruding this ion from the smooth muscle cell, thereby contributing to relaxation of the vessel. This work has also led to the suggestion that the glucagon receptor may be linked to a calcium channel. Thus, the data would be suggestive of an essential difference between hepatocytes and vascular smooth muscle in respect to the action glucagon has on calcium activity. However, the increase in calcium uptake reported by Andia-Waltenbaugh et al. (1978), was linked to a calcium sequestration mechanism by the mitochondria which is clearly a means of decreasing cytosolic calcium levels. The report by Combettes et al. (1986), that there was a release of calcium from intracellular stores was also found to be related to an activation of a plasma membrane calcium pump and an efflux of calcium from the cell. Calcium efflux from the cell into a calcium free medium was taken as a measure of the rate of calcium release from the endoplasmic reticulum. It can also be interpreted as a means of ridding the cell of free cytosolic calcium. This information, considered in conjunction with the general observation by Friedmann and Park (increased efflux of calcium from the liver) and Gagnon's work, suggest glucagon may induce a reduction of cytosolic calcium in both hepatocytes and vascular smooth muscle. This area is still under investigation and a definitive answer is pending.

II.4A.ii. Glucagon-Like Immunoreativity (GLI)

Several glucagon-like compounds which immunoreact to glucagon anti-serum have been identified. Glicentin, a 100 amino acid polypeptide is found in the A-cells of the pancreas along with glucagon and in the L-cells of the intestine. The L-cells of the intestine

also secrete enteroglucagon, a glucagon-like immunoreacting compound, and along with glicentin, are considered to be a family of glucagon-like polypeptides. The L-cells of the intestine appear to lack the proteases required to produce glucagon from glicentin and enteroglucagon. Biological activity of these glucagon-like compounds has been reported to be less potent than glucagon (Holst, 1975; Sasaki et al., 1975). Glucagon has also been found to contain homologous amino acid sequences with vasoactive intestinal peptide (VIP), gastrointestinal inhibitory peptide (GIP) and secretin (Brown and Otte, 1978; Dockray, 1979) and suggest the possibility that these compounds developed from a common peptide ancestor. Glucagon also has an extremely well conserved structure with identical sequences shared between human, rat, bovine, porcine and rabbit glucagons. Moreover, mammalian and chicken glucagon have only a single residue difference (Dockray, 1979).

II.4A.iii. Regulation of Glucagon Release

As a general statement, it can be said that the plasma glucose level is the main regulator of glucagon levels; low levels of glucose stimulate the release of glucagon and high levels reduce glucagon output in a joint effort with insulin to maintain an adequate level of glucose in the plasma to sustain the activity of the animal. However, this is a rather simplistic veiw of how release of this peptide is controlled; regulation of glucagon levels is a complex and highly regulated event. In the Islets of Langerhans where the glucagon secreting A-cells are located, insulin secreting B-cells, somatostatin producing D-cells and pancreatic peptide secreting PP-cells also exist (Matthews and Clark, 1987). Insulin, glucagon and somatostatin are in a unique local paracrine relationship whereby each

compound has some degree of control over the production of the others. Glucagon has stimulatory effects on insulin and somatostatin release while somatostatin has inhibitory effects on glucagon and insulin secretion. Insulin, which is also under the control of plasma glucose levels, does not appear to have any effect on somatostatin release but has been shown to be inhibitory towards glucagon (Unger and Orci, 1981). Thus, even at the local level, glucagon release is balanced by a number of factors.

Extrinsic factors such as neuronal input, circulating factors and ingested food also play a role in glucagon release. Parasympathetic and sympathetic nerves innervate the pancreas (Matthews and Clark, 1987; Yamaguchi, 1992). Cholinergic (Kaneto et al., 1981; Bobbioni et al., 1983; Ahren and Lindquist, 1986) and sympathetic (Marliss et al., 1973; Holst et al., 1981; Bloom and Edwards, 1985; Ahren et al., 1987) activation have been shown to release glucagon, with norepinephrine (Ribes et al., 1984; Ahren et al., 1987) and acetylcholine (Kaneto and Kosaka, 1974) producing a similar response. There is evidence that the acute rise in glucagon levels is mediated by ß-adrenoceptors in dogs during hemorrhage (Lindsey et al., 1975) and upon ß-adrenoceptor agonist administration (Kaneto et al., 1975; Ahren and Lindquist, 1987). In contrast to the findings from Lindsey et al. (1975), however, is a report from Lautt et al. (1982) which found that glucagon levels initially decreased upon hemorrhage and only became elevated after 90 minutes at a maintained blood pressure of 50 mmHg. There is also accumulating evidence that α-adrenoceptors are also involved in glucagon secretion in mice, dogs, rabbits, sheep and goats (Skoglund et al., 1987; Samols and Wier, 1979; Muggaberg and Brockman, 1982; Knudtzon, 1984; Oda et al., 1986) despite reports to the contrary in dog, man and rats (Iversen, 1973;

Gerich et al., 1974; Narimiya et al., 1981; Schuit and Pipeleers, 1986). In a recent study by Skoglund et al. (1987), the authors used selective α_1 and α_2 adrenoceptor agonists, phenylephrine and clonidine to implicate both receptor types in activating the release of glucagon in the murine model. The elevations, however, were quite small; the highest dose of phenylephrine (50 nmol/kg) roughly doubling glucagon levels (approximately 150 pg/ml to 300 pg/ml) and clonidine raising levels from approximately 150 pg/ml to 200 pg/ml and raises questions as to the extent to which these receptors are involved.

Hormones released during stress, epinephrine, cortisol (Marco et al., 1973), growth hormone (Tai and Pek, 1976) and endorphins (Ipp et al., 1978) stimulate glucagon secretion. At meal time, anticipatory stimulation of glucagon release may occur, but much of the glucagon response depends on the amount of carbohydrates consumed. Large amounts of carbohydrates elicit a greater rise in insulin than glucagon to prevent postprandial hyperglycemia and favors hepatic accumulation of ingested glucose (Shulman et al., 1978). A protein meal will stimulate a larger rise in glucagon compared to insulin. With a higher glucagon secretion, hepatic glucose production increases and prevents a hypoglycemia arising from the protein-induced insulin secretion (Unger et al., 1969; Felig et al., 1976). Oral or intravenously administered amino acids, either alone or in combination with other amino acids are glucagon-stimulating (Unger and Orci, 1976).

II.4B. Other Potential Modulators

Whether other endogenous compounds are capable of modulating nerve-induced constriction of the HA is not known. Certainly there is the potential for such interaction.

Of particular interest are the arachidonic acid metabolites. Studies have shown that the gut is capable of contributing large amounts of prostaglandins and thromboxanes into the portal blood. Iwai et al. (1988; Iwai and Jungermann, 1987) have suggested that prostaglandins and thromboxane A may partially mediate or modulate nerve-induced metabolic and hemodynamic responses in the rat liver. However, these studies were carried out in in situ perfused rat livers with erythrocyte-free perfusate and perfused only through the portal vein. The results, therefore, can only be considered in respect to the portal resistance vessels and cannot be directly extrapolated to the HA.

SECTION III

REGULATION OF PORTAL AND INTRAHEPATIC BLOOD PRESSURE

Portal venous pressure is regulated by presinusoidal and postsinusoidal resistance sites in the liver. The location of the presinusoidal resistance is believed to be in the portal venules. The site of postsinusoidal resistance has not been definitively agreed upon.

III.1. Postsinusoidal Resistance

It was previously held that the major site of resistance to portal venous flow was in the portal venules and that the pressure drop from the portal vein to the vena cava occurred across presinusoidal sites. This belief was held despite the observations that wedged hepatic venous pressure and portal venous pressure were reported to be similar (Child, 1954). These conclusions were drawn on the assumption that wedged pressure did not represent intrahepatic pressure. Rather it represented an upstream pressure transmitted through a static column of blood produced by the occlusion of the outflow vessel by the measuring cannula (Groszmann and Atterbury, 1982). The pressure being recorded was the pressure at the first site of the hepatic collateral vessels, which, according to Wanless et al. (1981), were the portal venules, and according to Groszmann and Atterbury, were the sinusoids. In the normal liver, evidence suggests that collaterals exist throughout the liver including hepatic veins proximal to the hepatic venous resistance sites (Lautt et al., 1986). Lautt et al. (1986), have demonstrated postsinusoidal resistance sites in the cat by passing a sealed tip catheter with side-holes into the vena cava via the jugular vein. As the catheter entered the hepatic

veins, the pressure recorded from the catheter was equal to or similar to central venous pressure being simultaneously recorded. Further advancement of the cannula resulted in a sudden rise of the recorded pressure to portal venous pressure. The cannula was found to be residing in third order branches of the hepatic vein. It was concluded that the cannula had passed through a narrow region of the hepatic vein which represents the major resistance sites to portal venous flow. This is consistent with reports by Greenway et al. (Greenway and Oshiro, 1973; Greenway et al., 1985), who pioneered the use of the hepatic venous cannula described above. Deysach (1941) reported anatomical, physiological and pharmacological evidence suggesting that the sublobular hepatic veins are the major site of vascular resistance to hepatic blood flow in the cat. The hepatic venous resistance sites in the cat constrict in response to norepinephrine, angiotensin, and sympathetic nerve stimulation in dose and frequency-dependent manner (Lautt et al., 1987a).

The pressure recorded from the sealed tip cannula in the studies by Lautt has been defined as lobar venous pressure (LVP) and represents a true physiological pressure and a measure of intrahepatic pressure which, in the normal state, is very similar to, or equals, portal venous pressure. It is different from wedged hepatic venous pressure in several ways. The cannula is not wedged at the site just proximal to the resistance site. The cannula tip is sealed and records pressure from side-holes several mm back from the tip. Lautt assumes that the ability to measure pressure proximal to the resistance sites but distal to the sealed tip is dependent upon a collateral circulation proximal to the resistance sites.

In a comparative study, Legare and Lautt (1987) repeated the lobar venous pressure studies in the dog and reported similar results. The difference in the dog studies was that

the site of resistance was located downstream in the larger terminal portion of the hepatic vein. This is consistent with previous investigations in the dog (Walker, 1960; Lautt and Legare, 1987) and with reports that the dog possesses a large amount of smooth muscle in the caval ends of the hepatic veins (Arey and Simonds, 1920; Bauer et al., 1932). This would appear to set the dog apart, morphologically, from most species, including man, cats and rabbits (Arey, 1941; Deysach, 1941). However, in 1973, Greenway and Oshiro had excluded the large hepatic veins as sites of increased resistance in response to histamine and norepinephrine in dogs. Rather, they suggested that these agents likely acted at the level of the sublobular veins of the hepatic venous system. Walker (1960) and Lautt and Legare (1987) on the other hand, reported that histamine produced constrictions of the hepatic veins near the ostia of the vena cava. In the dog, the resistance sites respond to the same agents noted in the cat studies, but also constrict in response to histamine (Lautt and Legare, 1987).

Recently, Bohlen et al. (1991), raised arguments against the contention that postsinusoidal sites constitute the major site of resistance to hepatic flow. In dogs, rats and rabbits, using a micropuncture technique, these investigators recorded hepatic venous pressure to be intermediate between PVP and caval pressure and concluded that postsinusoidal resistance contributed only 30% of the total resistance across the liver (PVP-IVCP) at rest. In a following study by Maass-Moreno and Rothe in dogs (1992)(also co-authors on the study by Bohlen), it was reported that hepatic venous pressure and LVP (measured using catheters) were insignificantly different from each other, but significantly larger than CVP. However, they concluded that hepatic venous pressure was erroneously high due to technical artifacts, namely, a catheter-induced increase in resistance to flow

which produced the elevated intrahepatic pressure. Both of these studies have, however, several inconsistencies at the technical and conceptual levels which leaves their results in question. For example, the micropuncture technique used by Bohlen et al. lacks serious validation and appears in this instance to be a very difficult technique to use. Norepinephrine infusions also produced suspiciously poor PVP responses in the dog and rat which questions the apparatus involved and the viability of the preparation per se. In the report by Maass-Moreno and Rothe (1992), the argument for an artifactually raised hepatic venous pressure is simply not substantiated by their data.

Henriksen and Lassen (1988) suggested that as an alternative to the hepatic veins, the major site of resistance to hepatic blood flow may be provided primarily by the confluence of the sinusoids with central veins. A shortcoming of this hypothesis is that it is unclear whether the sinusoidal-central vein junction would be capable of active responses.

There is a good deal of evidence supporting the concept that postsinusoidal sites are the major sites of resistance to hepatic blood flow. The exact location of these sites and their histological constitution is debatable, but there may also be a species-specific aspect to this debate.

III.2. Presinusoidal Resistance

In the basal state of the normal liver, portal venous and lobar venous pressures are insignificantly different which suggests that the vascular resistance proximal to the hepatic venous sites is negligible. Thus, hepatic venous resistance constitutes the major site of vascular resistance to portal blood flow in cats and dogs (Lautt et al., 1986; Legare and

Lautt, 1987). Under the influence of norepinephrine and sympathetic nerve stimulation the hepatic venous resistance increases and raises LVP and PVP. This stimulation also produces dose and frequency-dependent increases in presinusoidal resistance which can cause PVP to become larger than LVP. It was found that the proportion of rise in PVP attributed to hepatic venous sphincter contraction decreased from 90% at 2 Hz stimulation of the hepatic nerves to 59% at 10 Hz frequency (Lautt and Legare, 1987). Interestingly, although a significant portal to lobar pressure gradient develops, a vascular escape in the presinusoidal resistance component occurs during a maintained stimulation and PVP returns toward and may equal LVP (Lautt and Legare, 1987; Lautt et al., 1987a). LVP remains consistently elevated. Once presinusoidal vascular escape has occurred, the rise in PVP can be entirely accounted for by the rise in LVP, that is, by elevated postsinusoidal resistance (Lautt et al., 1987a). Histamine has no effect on the presinusoidal resistance sites. Thus, although not always present, a presinusoidal component of resistance can affect PVP when hepatic vessels proximal to the venous sphincters are subject to sympathetic stimulation.

III.3. Autoregulation of Portal and Intrahepatic Pressure: Distensible Resistance Sites

Portal venous resistance sites do not control portal blood flow. The role of these portal and hepatic venous resistance sites, therefore, appears to be to autoregulate PVP and intrahepatic pressure (LVP). By maintaining PVP at relatively normal pressures during changes in hepatic blood flow, sinusoidal collapse is prevented and allows proper and continuous perfusion of these vessels. As a result, hepatic metabolism and uptake and

exchange processes do not become jeopardized.

That the liver autoregulates portal pressure in the face of changing hepatic blood flows is consistent with the concept that hepatic venous resistance sites are passively distensible. That is, the resistance at these sites varies with changes in blood flow and venous pressure and large changes in portal flow lead to very small changes in PVP (Siderys et al, 1964). It was not until recently however, that Lautt, Legare and Greenway (1987b), and Greenway and Lautt (1988) interpreted these sites as being passively distensible. Several important concepts were advanced at this time. These authors were able to relate changes in hepatic venous resistance to the distending pressure of the resistance sites. The distending pressure was estimated to be the average of the upstream and downstream pressures for the resistance site. Because there are 2 sites of resistance, the distending pressure is different for each site; presinusoidal distending pressure is calculated as (PVP + LVP)/2 and postsinusoidal distending pressure is (LVP + CVP)/2. The precise calculation of these pressures depends on accurate measurement of LVP. Furthermore, Greenway and Lautt suggested that the liver passively autoregulates PVP and LVP because of the passive distensibility of the resistance sites. Since that time the understanding of the relationship between resistance and the distending pressure has been refined. It is now proposed that resistance (R) is related to distending pressure (Pd) by a constant, referred to as the Index of Contractility (IC), where $IC = R \bullet Pd^3$ (units being $mmHg^4$/ml/min/kg body weight) and is a reflection of the contractile state of the vascular bed (Lautt et al., 1991a). Whereas resistance at the pre- and postsinusoidal resistance sites vary with changes in blood flow and venous pressure, IC remains constant and provides further evidence for the passively

distensible nature of the resistance sites.

The plot of resistance versus $1/Pd^3$ is linear and is depicted in figure 7. Table 1 details experimental results in which IC remains unaltered while changes in blood flow produce passive changes in resistance. Changes in IC occur in response to alterations in the active contractile state of the liver, such as during norepinephrine infusion (Lautt et al., 1991a) which is also illustrated in figure 7.

Within physiological limits, changes in IC do not significantly affect the ability of the liver to autoregulate intrahepatic and portal venous pressures (Lautt and Legare, 1992). Using experimentally (figure 8) and theoretically derived curves (figure 9) for the flow-pressure relationship, Lautt and Legare demonstrated that the slope of the flow-pressure curve was not affected when portal venous and hepatic venous IC were doubled. However, flow-pressure curves derived from the theoretical data with high IC values did suggest that the slope of the flow-pressure curve is greater as the IC increases (figure 9). The consequence of this steeper slope is that, for a given decrease in portal flow, the effectiveness of PVP autoregulation is diminished and PVP would decrease to a greater extent.

Data also suggest that, because of the passive distensibility of these sphincters, elevations in central venous pressure (CVP) can be partially transmitted back toward the hepatic vascular bed (Lautt et al., 1987b). In this case, the sphincters also passively distend, reduce the resistance at the sphincters and decrease the pressure gradient between LVP and CVP. The hepatic venous sphincters do not act as classical waterfall- Starling resistors (Greenway and Lautt, 1970; Lautt et al., 1986; Lautt et al., 1987b) which contrasts with

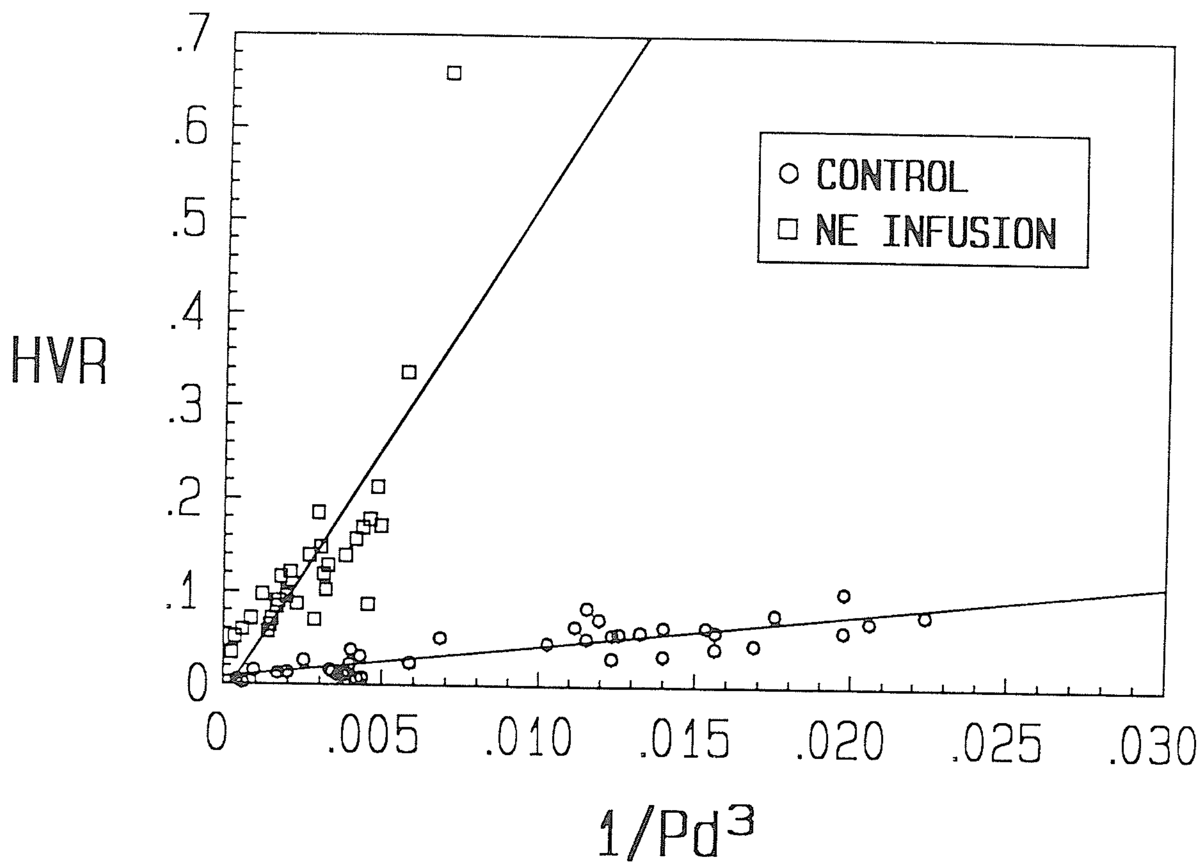


Figure 7: Plot of $1/Pd^3$ vs. hepatic venous resistance (HVR). This plot represents data from one cat demonstrating the relationship between HVR and Pd according to the equation $IC=HVR \cdot Pd^3$. Control data were obtained by changing blood flow over the range of 10-50 ml/min/kg in several steps at a normal IVCP of 3 mmHg and at a raised IVCP of 6 mmHg. Flow was then held steady and IVCP was raised in steps between 3 and 15 mmHg. These same procedures were repeated during a constant infusion of norepinephrine (1.25 μg/kg/min). It can be seen that IC (the slope of the line) is increased during a norepinephrine infusion, indicating that IC increases in response to active contraction of the hepatic vascular bed (Taken from Lautt et al., 1991a).

TABLE 1

Mean values of distending pressure, hepatic blood flow, percentage hepatic arterial flow and index of contractility values across the liver and total resistance across the liver, at low, mid and high hepatic blood flows

Flow	Pd_L	HBF	HAF%	IC_{PV}	IC_{HV}	IC_L	R_L
High	7.7	43.7	24.9	48.8	30.3	79.1	0.121
Mid	7.1	34.2	44.8	49.6	29.1	78.7	0.139
Low	6.6	22.9	75.4	52.9	25.8	78.7	0.198
SEM	0.1	1.5	3.3	5.8	2.0	7.7	0.019

The vascular long-circuit was used to alter portal blood flow to validate the IC model. Portal flow was roughly doubled and reduced to 25% to cause the distending blood pressure across the liver to decrease (Pd_L=(PVP + IVCP)/2, mmHg). Abbreviations: total hepatic blood flow (HBF, ml/min/kg body weight), the proportion of HBF accounted for by hepatic arterial blood flow (HAF%), R_L=added resistances of PV and HV (mmHg/ml/min/kg body weight), IC_{PV}=index of contractility for the portal vein, IC_{HV}=index of contractility for the hepatic veins, IC_L=IC_{PV} + IC_{HV}. All values except for IC_{PV}, IC_{HV}, and IC_L were significantly altered by changes in blood flow (blocked ANOVA, n=8, $p<0.05$, taken from Lautt and Legare, 1992)

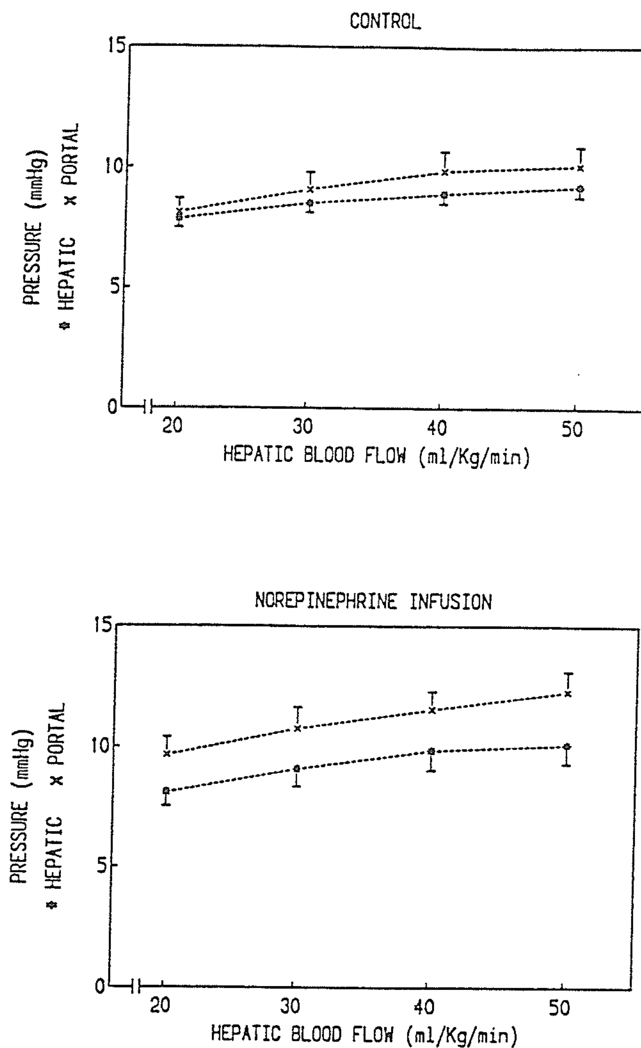


Figure 8: Effect of changes in hepatic blood flow, produced using a vascular circuit, on in vivo intrahepatic pressure (lobar venous pressure, * hepatic), proximal to hepatic veins and portal venous pressure (x portal) with the hepatic artery intact and IC within physiological ranges (IC = 30.7 $\pm$ 7.0 mmHg/ml$\cdot$kg$^{-1}\cdot$min^{-1}). The control curves (top panel) were conducted before and after the curve during norepinephrine infusion was obtained (1.25 µg/kg/min, IC = 78.1 $\pm$ 27.1 mmHg/ml$\cdot$kg$^{-1}\cdot$min^{-1}). The slope of the curve is not significantly altered by norepinephrine (n=9 cats; taken from Lautt and Legare, 1992).

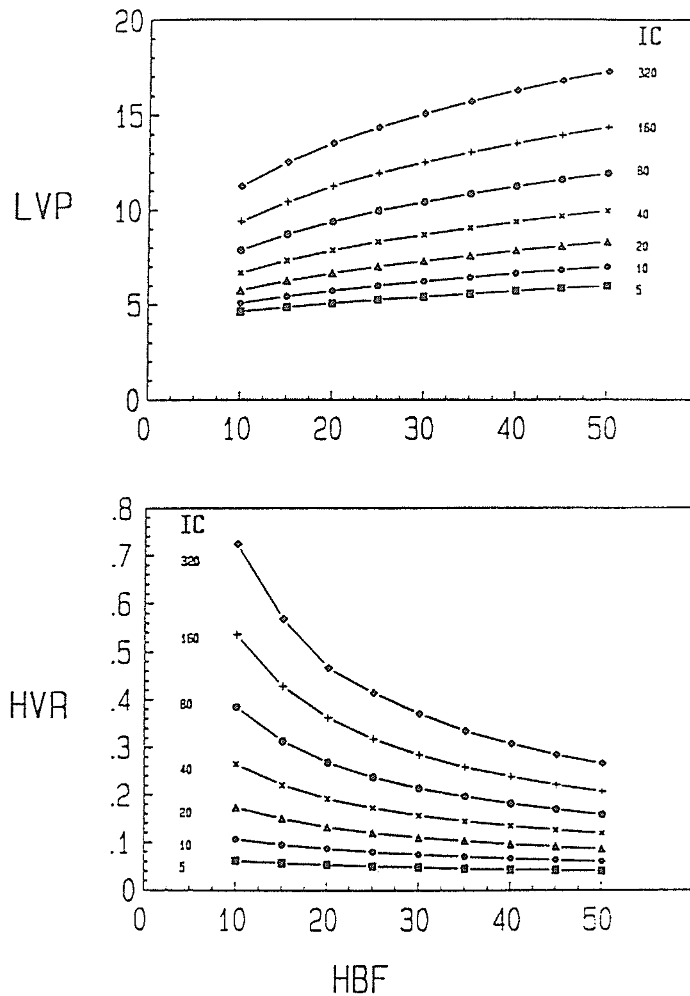


Figure 9: Theoretical data for the predicted lobar venous pressure (LVP mmHg) upstream from the hepatic venous resistance sites and hepatic venous resistance (HVR mmHg/ml/min/kg) as total hepatic blood flow (HBF ml/min/kg) is altered. The family of curves is obtained for a range of IC values generated from computer-assisted estimates. Downstream pressure was assumed to be constant at 4 mmHg. Absolute HVR change per unit change in HBF is greater at high IC values but the percentage change is greater for the low IC values. The HVR increase as HBF decreased from 50 to 10 was 49% at IC = 10, but only 31% at IC = 320 (Taken from Lautt and Legare, 1992).

earlier findings by Mitzner (1974) and Green (1975). If the sphincters operated through this mechanism, the venous pressure would be required to overcome an external or upstream pressure before any pressure would be transmitted upstream. In the liver, even very small elevations in central pressure can be partially transmitted upstream to the sinusoids, although at low central venous pressures the percent transmission of pressure upstream is very small (Lautt et al., 1987b). The larger the acute rise in central venous pressure, the greater the percent transmission of pressure, presumably due to a greater dilation of the hepatic venous sphincters. The percent transmission of central pressure to the sinusoids does depend on the initial tone and therefore, resistance, existing in the hepatic venous sphincters. The greater the tone and resistance, the less transmission of central pressure to LVP occurs presumably because the sphincters will be more constricted (figure 10) (Lautt et al., 1987b). Such an event may help to reduce the potential rise in central pressure and protect the heart from an excessive elevation in preload (Greenway and Lautt, 1988). Conversely, the sphincters may also afford some protection for portal and intrahepatic pressures from changes in CVP.

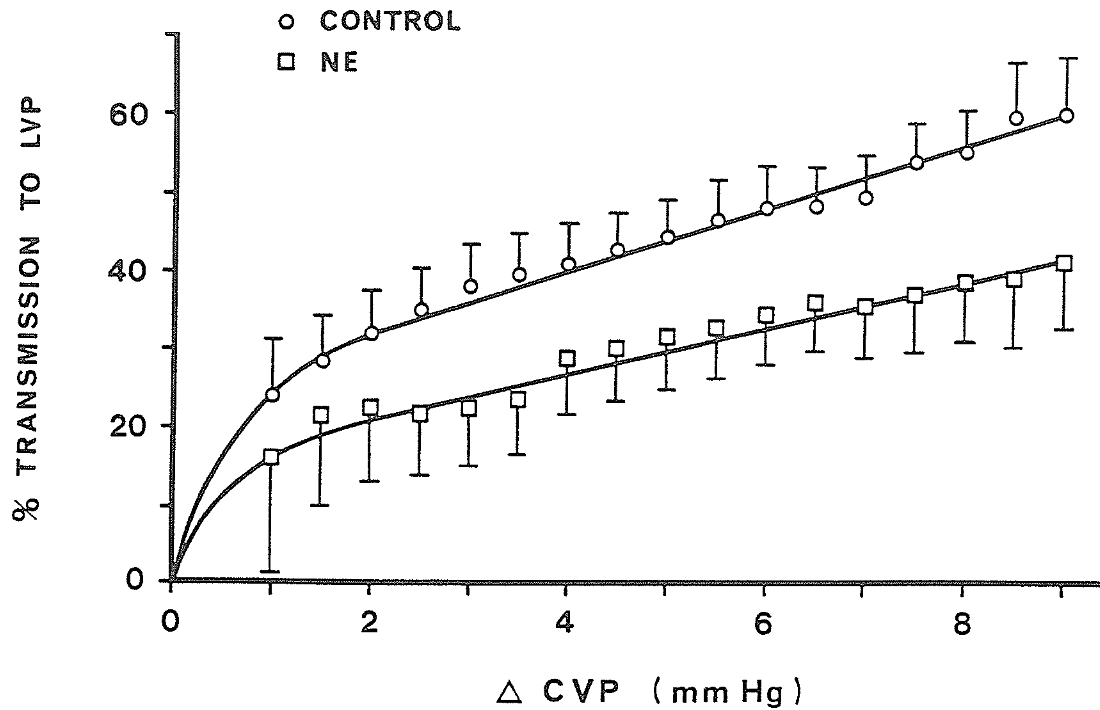


Figure 10: Data calculated to show the percentage of a rise in central venous pressure (CVP) transmitted past the hepatic sphincter to the upstream lobar venous pressure (LVP) site. Curves were obtained in the control state and during a norepinephrine infusion (1.25 μg/kg/min) into the portal vein to increase the resistance at the hepatic venous sphincters. Percent transmission of CVP to LVP in the control state, increased as the distending pressure ([LVP + CVP]/2) increased, until at a change in CVP of 9 mmHg, 59% transmission occurred. During norepinephrine infusions, only a 41% transmission of an elevation of CVP by 9 mmHg occurred. The slopes of the control and norepinephrine regressions were significantly different ($p<0.001$; taken from Lautt et al., 1987b).

SECTION IV

BLOOD VOLUME

The liver acts as a major blood volume reservoir because of its capacity to store and mobilize blood into the systemic circulation. Characteristics of the liver that support such a role include its compliant nature and that the major site of resistance to hepatic blood flow is postsinusoidal.

IV.1. Definitions

The primary determinants of blood volume are the distending transmural pressure, compliance of the vessels (and elasticity of the surrounding tissues), and unstressed volume (Greenway and Lautt, 1989). Compliance is defined as the extent to which the volume of a vessel changes in response to alterations in transmural pressure, that is, change in volume (ml) divided by the change in pressure (mmHg). In the liver, the appropriate pressure to use is LVP. In the normal liver, the intrahepatic pressure-volume relationship is linear suggesting that compliance is constant over the range of pressures and volumes which have been plotted (Greenway et al., 1985). Hepatic compliance, calculated using intrahepatic pressure, is 2.5-3.0 ml x $mmHg^{-1}$/100 g liver weight (Greenway et al., 1985). According to a graph of pressure versus volume, if this relationship is extrapolated to zero pressure, a positive volume intercept is obtained which is the theoretical unstressed volume (Greenway and Lautt, 1989) (figure 11).

Unstressed volume is the volume of blood required to passively fill the vascular spaces of the liver (or organ), i.e. the blood volume in the liver at zero distending pressure.

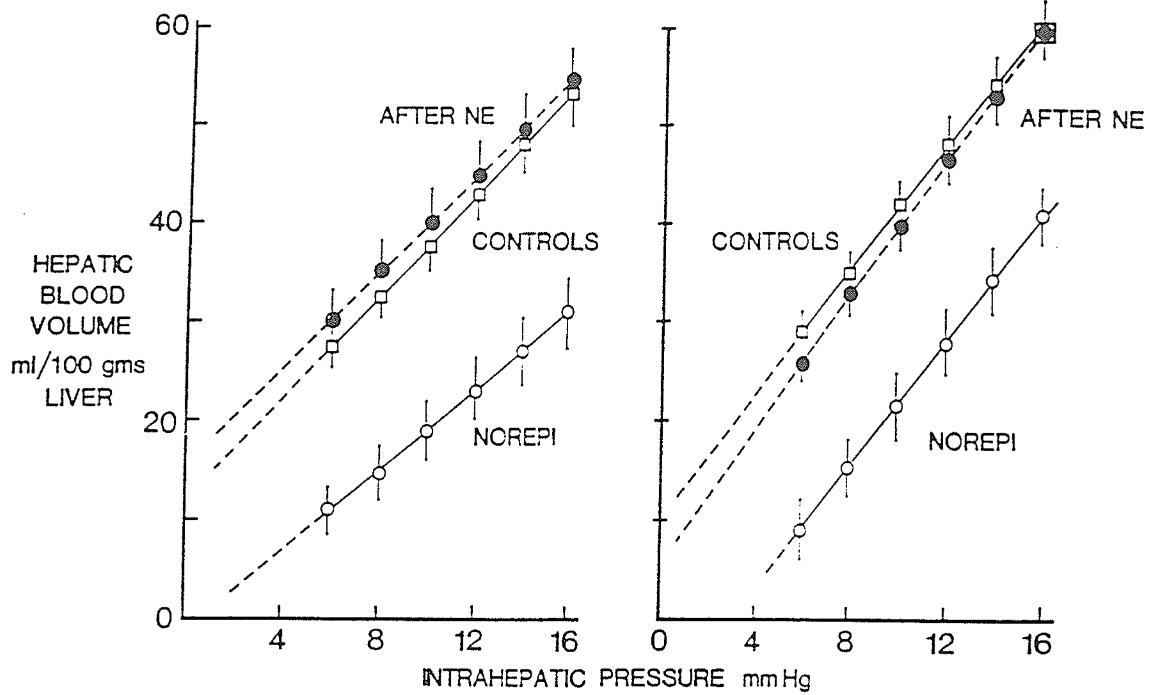


Figure 11: Relationships between hepatic blood volume and intrahepatic pressure determined by varying portal flow (left panel) or by varying hepatic outflow pressure (right panel), before, during, and after infusion of norepinephrine (NE). Values represent the mean ± SEM, n = 9 (Taken from Greenway et al, 1985).

This volume is about 40% of the total liver volume. This amounts to about 12-15 ml/100 g liver tissue in the denervated liver with total liver capacitance being approximately 37 ml/100 g of liver tissue at a portal venous pressure of 8 mmHg (Greenway et al., 1985). Stressed volume is the volume of blood (in an organ) which causes distension of the blood vessels thereby producing intravascular pressure. Stressed volume is the product of compliance and transmural pressure (ml/mmHg x mmHg). The functional difference between unstressed and stressed volume is that stressed volume plays a role in hemodynamic events; it is the blood volume that generates blood pressure and blood flow. Unstressed volume, therefore, is that volume of blood required to passively fill the vascular spaces.

IV.2. Changes in Hepatic Blood Volume

Changes in hepatic blood volume can occur by passive or active mechanisms. Passive changes in liver blood volume occur secondary to alterations in hepatic blood flow or hepatic venous pressure without concomitant active changes in the hepatic capacitance vessels. In this situation intrahepatic pressure is passively changed and therefore the change in blood volume occurs by a change in stressed volume. The hepatic vascular bed tends to minimize the alterations in flow and pressure. Blood flow is generally kept constant despite changes in portal flow via the action of the hepatic arterial buffer response. Intrahepatic pressure is autoregulated by the distensible hepatic venous sphincters.

Active expulsion of blood from the liver (in response to a sympathetic stimulation) occurs through a contraction of the capacitance vessels. The liver responds in this manner under physiological stress such as hemorrhage or elevated sympathetic tone. Many studies

(reviewed by Greenway and Stark, 1971 and Greenway and Lautt, 1989) have consistently demonstrated that active hepatic volume responses can mobilize up to approximately 50% of the hepatic blood volume and transfer it into the systemic circulation. In dogs and cats, Greenway and Oshiro (1972) found that 36% and 47% of total hepatic blood volume was expelled upon mid-frequency (4-6 Hz) nerve stimulation.

Active decreases in liver volume can occur either by decreasing compliance (the slope of the pressure volume relationship) or by decreasing unstressed volume (and thereby increasing stressed volume). Referring to the pressure-volume relationship in figure 11, Greenway et al. (1985), have shown that in the cat, norepinephrine does not change hepatic compliance when intrahepatic pressure was used in the calculations. This suggests that capacitance responses in the liver occur through changes in unstressed volume. This makes teleological sense and has important physiological consequences. If compliance changed rather than unstressed volume, the amount of blood actively mobilized would decrease as intrahepatic pressure decreased. In situations where mobilization of blood is crucial, such as during hemorrhage, the amount of blood mobilized during sympathetic stimulation would become progressively smaller as the hemodynamic condition of the animal declined. But, because compliance remains the same, unstressed volume is mobilized to the same extent regardless of the intrahepatic pressure (Rothe, 1983). The unstressed volume, therefore, represents a blood volume reserve which can replace the stressed volume of the animal under conditions of blood loss. Unstressed volume will also be used to increase stressed volume to maintain cardiac preload and cardiac output under conditions of exercise and to compensate for stressed volume which has pooled in dependent parts of the body (Greenway

and Lautt, 1986).

IV.3. Modulation of Blood Volume Responses

Other than mediators of the sympathetic nervous system, angiotensin and histamine may be the only other endogenous compounds that may alter hepatic blood volume. According to Greenway and Lautt (1972) exogenous angiotensin (estimated to produce high endogenous levels) actively reduced hepatic blood volume by 20%. Exogenous vasopressin in doses within the range produced by the posterior pituitary had only very weak effects on hepatic blood volume and was concluded to have little physiological significance.

Histamine in the dog, but not in the cat, has been shown to produce an excessive volume increase in liver capacitance (Greenway and Oshiro, 1973). This is presumed to be due to constriction of the postsinusoidal resistance sites resulting in a passive increase in the liver volume rather than having any direct effect on capacitance vessels (Lautt and Legare, 1987).

Recently, Lautt et al. (1991b) found that infusions of glucagon modulated nerve-induced volume responses in the liver. Although the maximal volume response was not affected, glucagon caused the Hz_{50} (frequency required to produce 50% of the maximal volume response) to increase from 3.5 ± 1.1 to 5.6 ± 0.07 Hz. Doses of glucagon used in this study were extremely large and suggest that a physiological role for glucagon in this regard is unlikely. Large doses of adenosine had no effect on the Hz_{50} but significantly decreased the maximal response for nerve-induced blood volume changes from 11.1 ± 2.3 mls to 8.1 ± 2.0 mls.

SECTION V

COMPLICATIONS OF HEPATIC HEMODYNAMICS: PORTAL HYPERTENSION

V.1. General Considerations

A hallmark of the hepatic vascular bed is that it is a low pressure-high blood volume circulatory system. Disturbances of the fine hepatic architecture due to disease states as well as non-parenchymal-induced alterations can result in circulatory disruptions. Liver cirrhosis is one example of such a pathology. Cirrhosis is defined and characterized anatomically as a diffuse process with fibrosis and nodule formation which usually follows hepato-cellular necrosis. The growth of regenerative nodules and the deposition of collagen fibres (fibrogenesis) and formation of a basement membrane in the space of Disse (capillarization of the sinusoids) increases the resistance to hepatic blood flow and disrupts the normal circulatory patterns leading, in many cases, to portal hypertension (Phillips et al., 1987; Sherlock, 1989).

Cirrhosis is the most common cause of portal hypertension (Sherlock, 1989) and accounts for 90% of all the cases in developed countries (Bosch et al., 1989). Portal hypertension is generally classified into 3 broad categories, pre-hepatic, intra-hepatic and post-hepatic depending on the site of the lesion.

The cardiovascular complications of portal hypertension involve the portal vascular bed and the systemic circulation. When portal hypertension is fully manifested it has been found to involve chronically elevated PVP and a hyperemic splanchnic circulation. This hyperemia, a result of decreased arterial resistance in the splanchnic vascular bed, leads to

elevated portal venous inflow. The combination of the increased resistance and flow to the liver can cause the development of a portal-collateral or portal-systemic circulation, whereby portal blood is shunted around the high resistance hepatic vascular bed into the systemic circulation resulting in the formation of blood engorged veins otherwise known as varices. These varices can be life-threatening if they hemorrhage. In addition, peripheral vascular resistance and arterial pressure are also decreased in portal hypertension and are associated with an increase in cardiac output. Finally, ascites formation may be present in some instances.

The cardiovascular complications of portal hypertension noted above have been recognized for many years in man, but the precipitating mechanism(s) has eluded researchers. Because of the inherent complexities in using humans to study portal hypertension, investigators have had to rely on animal models of cirrhosis and portal hypertension to study the development of this condition. The majority of the hemodynamic studies have utilized the partial portal vein-stenosis model (pre-hepatic portal hypertension) in the rat. Carbon tetrachloride administration and bile duct ligation models of cirrhosis in the rat and other species have also been used although to a lesser extent. The bile duct ligation technique has also been used to study the biochemical changes occurring in obstructive jaundice in several species including the cat. However, hemodynamic studies using this technique in the cat have not been conducted. More about this model will be discussed at the end of section V.

V.2. Development of Portal Hypertension

Portal pressure has generally been considered to be determined by the interrelationship between portal blood flow and portal vascular resistance.

$$\text{pressure} = \text{portal blood flow} \times \text{portal venous resistance}$$

In this equation, pressure is the pressure gradient between PVP and central venous pressure. However, in light of the recent advances in the understanding of hepatic hemodynamics, the equation should use total hepatic blood flow, rather than portal flow. Total hepatic flow is used because of the finding that the major site of vascular resistance is postsinusoidal and therefore it is total hepatic blood flow passing across the resistance site. Technically, portal venous resistance is, therefore, the sum of pre- and postsinusoidal vascular resistance, although it has been shown that presinusoidal resistance in the basal state is very small.

Portal pressure can increase either by increasing blood flow or resistance, or both. That portal hypertension is developed and maintained solely by an increase in resistance is known as the "backward flow" theory (Moreno, 1967; Whipple, 1945). Conversely, the "forward flow" theory proposes that portal hypertension is maintained due to elevated portal venous inflow in the face of increased portal venous resistance (Vorobioff et al., 1983; Witte et al., 1974). As with most biological systems, which are rarely governed or regulated by a single mechanism, it has recently been shown that the development and maintenance of portal hypertension is likely due to a combination of increased portal venous resistance and

elevated portal venous blood flow. Using theoretical and experimental results in the partial portal venous stenosed rat, Benoit et al. (1985) suggested that the forward flow theory could account for 40% of the increase in portal venous pressure and the backward flow theory accounts for 60% of the rise in portal venous pressure. In a study by Sikuler et al. (1985), the temporal development of portal hypertension in the partial portal vein stenosis model of the rat was studied over a 14 day period. They concluded that in this model, the initial mechanism for portal hypertension is the increase in portal venous resistance (as a result of the ligation) but the subsequent elevation in portal venous inflow plays a more important role in maintaining the hypertension. However, high portal venous inflow per se (forward flow theory) is not sufficient to produce an increase in portal venous pressure in a normal low resistance hepatic vascular bed (Sikuler and Groszmann, 1986a).

There are two important additional points that should also be considered: 1. In the portal vein ligation model of portal hypertension, any active change in portal venous resistance occurs in the portal collateral vascular bed since the resistance site on the portal vein is fixed. 2. Kroeger and Groszmann (1985) found that the resistance of the portal collateral circulation is inappropriately high for the elevated portal venous flow. That is, at a given portal venous flow the calculated portal venous resistance was greater in the portal hypertensive rats than in the sham animals. Thus, the elevation of portal venous pressure depends not only on the elevated portal venous inflow but also on the high resistance to portal venous blood flow by the portal-collaterals.

V.2A. Development of Increased Resistance

In the experimental model of portal vein-stenosis-induced portal hypertension, the development and site of increased resistance is clearly prehepatic, without any liver pathology. In intrahepatic forms of portal hypertension, whether experimentally or non-experimentally-induced, the exact site(s) or cause(s) of increased resistance to hepatic blood flow have been less easily defined. The cause of increased resistance in cirrhotic portal hypertension has been ascribed to the formation of regenerative nodules and fibrosis, which distorts the hepatic venular system causing vascular compression (pre- and postsinusoidal) (Popper and Zak, 1958; Hales et al., 1959; Popper, 1977). There are reports, however, that portal hypertension occurs in chronic alcoholics without evidence of cirrhosis, necrosis, or regenerative nodules (Reynolds et al, 1969; Leevy et al., 1970). Similarly, architectural disarray or the presence of inflammation, fat, alcoholic hyalin, terminal hepatic vein sclerosis (Orrego et al., 1981) or fibrosis (Krogsgaard et al., 1984) has not correlated with wedged or intrahepatic pressure in portal hypertensive patients.

In 1979, Orrego et al., reported that a good correlation existed between clinical manifestations of portal hypertension and the amount of collagenization in the space of Disse in alcoholic patients. According to these investigators, the amount of collagen in the space of Disse was the best structural parameter that predicted the severity of the clinical condition in liver biopsies that showed fatty liver damage or cirrhosis. In a following study, Orrego et al. (1981), further confirmed this finding but also reported that the amount of collagen in the space of Disse and hepatocyte surface area were both positively correlated to intrahepatic pressure. Further work by these authors (Blendis et al., 1982) revealed that changes in

hepatocyte surface over time appeared to be more closely related to changes in intrahepatic pressure than collagen in the space of Disse. In this same study, however, a subpopulation of patients, representing approximately 13% of the entire study group, did not show any correlation between hepatocyte surface area and intrahepatic pressure (r=0.24). More recently, Krogsgaard et al. (1984), refuted the findings of Orrego and Blendis by reporting that no apparent correlation existed between hepatocyte surface area and intrahepatic pressure. Their results appear to be in agreement with the early findings of Popper, that the elevated portal pressure in portal hypertension is due to architectural destruction. While this may be true, it is worth noting that the subjects in the Krogsgaard study and those in the studies by Orrego and Blendis were not selected according to the same criteria, which may account for the differences in results.

It is worth noting that in all of these studies, collagen and fibrosis analysis was only semi-quantitative. That is, only relative or arbitrary values have been reported for the fibrosis (eg. scales from 1 to 5). To my knowledge, there are no studies of this nature that have precisely quantitated the degree of fibrosis occurring in the histological sections. The possibility also exists that the particular histological section analyzed may not necessarily be representative of the changes taking place in the liver during the disease state. Despite the reports from Orrego and Blendis, it is difficult to envision that the extensive fibrosis and nodule formation observed in cirrhosis (of various etiologies) is not responsible, at least in part, for the raised intrahepatic and portal venous pressures recorded in portal hypertensive patients. That hepatocyte swelling, leading to an increased surface area is solely responsible for raised portal pressures has certainly not been proven. The reported correlations between

hepatocyte surface area and intrahepatic pressure do not prove a causal relationship. The increased surface area may be a result of the increased pressure. Likewise, fibrogenesis in the space of Disse and vessels in the liver (and possibly bile ducts during biliary obstruction) may also be a result, rather than a cause of the raised intrahepatic pressure. Thus, it is still a matter of some debate as to the exact mechanism of increased resistance to hepatic blood flow in portal hypertension.

V.2B. Development of Increased Portal Venous Inflow

Mechanisms for the splanchnic hyperemia and increase in portal venous inflow have also not been fully defined, but two predominant theories have been put forward.

V.2B.i. Circulating Humoral Agents

One theory proposed to account for the systemic arterial hypotension and splanchnic hyperemia is that there is an elevation in the number and concentration of endogenous vasodilatory factors in the circulation. Benoit et al. (1984, 1986), using the portal vein-stenosed rat, suggested a role for circulating humoral agents as a mechanism for dilating the splenic vascular bed (Benoit et al., 1984). These investigators strongly suggested that elevated levels of circulating glucagon in portal hypertension and cirrhosis may account for up to 40% of the decrease in intestinal vascular resistance associated with portal hypertension. Using a specific glucagon anti-serum Benoit et al. (1986), reported a 30% drop in the intestinal hyperemia in portal hypertensive rats. Glucagon, which has been found to be hyper-secreted in cirrhosis (Sherwin et al., 1978), is well known to have vasodilatory

actions and have a specificity for dilating the splanchnic vascular bed (Kock et al., 1970b; Krarup and Larsen, 1974). Elevation of glucagon and other blood-borne agents normally cleared by the liver may also occur by escaping hepatic metabolism due to portal systemic shunting.

As attractive as the glucagon theory sounds, it has not been fully embraced. Others have argued that the lack of correlation between glucagon levels and the hyperdynamic level does not support the glucagon theory (Sikuler et al., 1985; Sikuler and Groszmann, 1986b). These and other authors suggest that an alternative to glucagon may be prostaglandin I_2 (PGI_2) (Sikuler et al., 1985; Sitzman et al., 1989). Endothelial-derived relaxing factor, considered to be nitric oxide, has been hypothesized to mediate some of the splanchnic hyperemia involved in cirrhosis (Vallance and Moncada, 1991). In support of nitric oxide being involved in the hyperdynamic circulation, Claria and collegues (1992) have implicated this compound in the arterial hypotension occurring in cirrhotic rats.

In an alternative theory, Lee et al. (1992b) reported that the degree of portal venous shunting, not necessarily circulating humoral factors or mesenteric venous hypertension, was the critical factor in the development of a hyperkinetic circulation in portal hypertension. From this study, it appears that the increased cardiac output and splanchnic organ blood flows are the result of markedly reduced portal tributary resistance rather than elevated mesenteric or portal hypertension.

V.2B.ii. Hyposensitivity to Endogenous Constrictors

A second and possibly co-existing mechanism behind the splanchnic hyperemia may

be a decreased sensitivity to endogenous vasoconstrictors. Several authors have shown a decreased sensitivity in man and animals to norepinephrine (Finberg et al., 1981; Kitano et al., 1982; Kiel et al., 1985), angiotensin II (Laragh et al., 1963; Finberg et al., 1981; Murray and Paller, 1985), and vasopressin (Mesh et al., 1991) in the peripheral and splanchnic vasculature. There are, however, many conflicting reports about the effect of portal hypertension on norepinephrine sensitivity and they are summarized in a review on this topic by Bomzon (1990). Part of the problem arises from the fact that many different models of portal hypertension and variations within these models have been studied, which, in itself, can produce different results. Furthermore, in many studies, it is my opinion that inappropriate vessel types have been used to study vascular reactivity. For example, the aorta has been used on several occasions in this type of investigation. The aorta is an example of a conducting vessel, not a resistance vessel, and is a poor choice of a vessel to study vascular reactivity to vasoactive agents.

Despite the reduced sensitivity to norepinephrine and angiotensin in portal hypertension and cirrhosis, circulating levels of norepinephrine and activity of the renin-angiotensin system have been shown to be elevated in these conditions (Gaudin et al., 1989, 1991; Henriksen et al., 1991, Moreau et al., 1987). Hepatosplanchnic spillover of norepinephrine has also been observed in cirrhotic patients (Henricksen, et al., 1987). The increase in norepinephrine has been determined to be due to an increased norepinephrine output from nerve endings rather than decreased metabolism (Henriksen et al., 1984, 1989; Nicholls et al, 1985, Willet et al., 1985). This is supported by the findings of Finberg et al.(1981, 1982) who found only minor changes in mono-amine oxidase activity and, recently

by Floras et al. (1991) who reported that muscle sympathetic burst frequency was elevated in man with decompensated cirrhosis.

In 1985, Murray and Paller suggested that the decreased vascular reactivity to angiotensin II was a specific but, as yet, undefined post-receptor defect, not a generalized defect of the vasculature. Down regulation of cardiac ß-adrenergic receptors has been reported by Lee et al. (1990) in the portal hypertensive rat and the existence of false neurotransmitters in liver disease has also been suggested (Fischer and Baldessarini, 1971) but requires further investigation (Henriksen et al., 1991). In contrast to angiotensin, Mesh et al. (1991) found that the attenuation of vasopressin responsiveness could be due to a general inhibition of vasoconstriction rather than a specific inhibition of vasopressin.

The decreased sensitivity to norepinephrine, angiotensin II, and vasopressin is contrasted by a hypersensitivity to serotonin in cirrhosis and portal hypertension. Cummings and colleagues (1986) found that isolated mesenteric veins from portal hypertensive rats exhibited a three-fold enhancement of the force of contraction to low doses of serotonin compared to in vivo sham-operated controls. Other investigators using the serotonin receptor antagonists, ketanserin and ritanserin, support Cummings' report (Hadengue et al., 1987; Mastai et al., 1989). The supersensitivity of serotonin may be involved, to some extent, in the elevated portal venous resistance found in portal hypertension, but conversely, may also act on the arterial vasculature to compensate for a decreased systemic arterial pressure found in this condition.

V.2B.iii. Development of Portal Systemic Shunting and Varices

As portal hypertension becomes more severe, the collateral circulation can form blood engorged varices in pre-existing anastomotic connections between the portal venous system and the caval circulation. Much of this shunted blood passes into the azygos circulation and can be measured in this system as an indication of the degree of shunting (Bosch et al., 1989).

The formation of esophageal varices requires that the hepatic venous pressure gradient (HVPG, portal venous pressure - central venous pressure), be elevated above 10 mmHg. A gradient above 12 mmHg, however, can have severe consequences for the patient; massive upper gastrointestinal bleeding from ruptured esophageal and gastric varices (portal hypertensive gastropathy) is the main complication of portal hypertension and represents one of the leading causes of death in patients with cirrhosis other than liver failure (Bosch et al., 1989). The factor that appears to be critical to the rupture of the varices is not the swallowing of food or necessarily hydrostatic pressure within the varix. It is now the current belief that this factor is the wall tension of the vessel, according to the Law of Leplace: $T = (P_1 - P_2) \times r/W$, where T is the wall tension, P_1 is the intravariceal pressure, P_2 is the pressure in the esophagus, r is the radius of the varix and W is the wall thickness. Thus, the larger the varix and the greater the intravariceal pressure, the greater is the tension in the vessel wall which, if great enough, can lead to the rupture of the varix.

V.3. Treatment of Portal Hypertension:

The primary objective of therapy for portal hypertension, whether pharmacological

or surgical, is to decompress the portal venous system and thereby diminish the chances of hemorrhage from the varices. Variceal bleeding is considered to be a medical emergency with a variable but high mortality rate in the range of 20% to 80% per bleeding episode.

V.3A. Surgical Therapy

The objective of surgical therapy is to reduce portal venous pressure, maintain hepatic blood flow, including portal venous blood flow, and minimize the incidence of hepatic encephalopathy. To this end, several procedures, the porta-caval, the meso-caval, and spleno-renal shunts, have been developed. The complicating consequence of hepatic encephalopathy has been high with the porta-caval shunt procedure and therefore, the frequency of this technique has been reduced (Sherlock, 1989). The meso-caval shunt is similar to the porta-caval technique except that the superior mesenteric vein is connected via a graft to the vena cava. The concept behind the spleno-renal shunt proposed by Warren over twenty years ago (Warren et al., 1967) was to separate the portal venous circulation into two components, a decompressed gastro-splenic component and a hypertensive superior mesenteric component. Postoperative encephalopathy has been shown to be significantly less frequent with this technique compared to the non-selective shunts (Rikkers, 1988) but does not provide any advantages over other shunts in respect to survival rates (Rikkers, 1990).

Endoscopic variceal sclerosis has recently become the most popular treatment for acute and chronic control of variceal hemorrhage. This technique, first successfully used over 50 years ago by Crafoord, is generally used as a treatment after varices have already hemorrhaged (Sherlock, 1989). Sclerotherapy, in itself, is not a curative procedure. It has

been shown to be effective in decreasing the frequency of rebleeding, but as a prophylactic therapy it has not proven to be successful in decreasing the risk of the first bleed in patients (Sherlock, 1989). Furthermore, new varices can develop that may also require sclerotherapy. Approximately one-third of all patients undergoing sclerotherapy will require shunt therapy and/or hepatic transplantation in the case of end-stage liver disease (Rikkers, 1990).

V.3B. Pharmacological Treatment

Theoretically, portal pressure can be lowered either by decreasing portal vascular resistance or portal venous blood flow according to the equation: P = resistance x blood flow, which was introduced earlier in this section. To this end, vasoconstrictor agents and vasodilatory agents or combinations thereof have been used to battle portal hypertension and bleeding varices.

Vasopressin has been used for over 30 years (Galambos, 1982) in the management of bleeding varices despite very limited success with this constrictor. Somatostatin has also been used as a vasoconstrictor and like vasopressin, reduces PVP by constricting the splanchnic arterioles and decreases portal venous inflow (Bosch, 1985; Rector, 1986; Reichen, 1990). In the treatment of acute variceal bleeding, vasopressin has only been effective in approximately 45% of the cases reported in 2 recent studies (Gimson et al., 1986; Tsai et al., 1986) and survival rates have not been improved. Somatostatin has been shown to be roughly equal to or more effective than vasopressin in this regard (Kravetz et al., 1984; Jenkins et al., 1985). Somatostatin is also known to be an inhibitor of glucagon release from the pancreas. Because of the putative role of glucagon in the hyperdynamic

circulation it would be an interesting additional mode of action by which somatostatin worked. Indeed somatostatin and its analog, SMS-201-995, decreased glucagon levels in cirrhotic patients as well as decreased the WHVP (Wahren and Eriksson, 1986).

A serious strike against vasopressin is that this agent has several untoward cardiovascular effects such as increasing afterload, decreasing cardiac perfusion and increasing end diastolic pressure in the dog (Zito et al., 1983) and man (Groszmann et al., 1982) and may also increase portal resistance. The side-effects monitored in the Jenkins and Kravetz studies indicated that somatostatin produced fewer cardiovascular complications than vasopressin.

The joint administration of nitrovasodilators (nitroglycerin) with vasopressin has been shown to reduce the unwanted systemic effects of vasopressin and to improve the effectiveness of controlling variceal bleeds compared to vasopressin alone (Groszmann et al., 1982; Gimson et al., 1986; Tsai et al., 1986; Moreau and Lebrec, 1990). The added benefit from this therapy however may only be minor and mortality was not improved.

Glypressin is a pro-drug of vasopressin which requires an enzymatic cleavage of the N-terminal glycine to obtain its activity. Like vasopressin, it is also a vasoconstrictor and acts to decrease WHVP by decreasing splanchnic inflow. Moreover, glypressin has also been shown to arrest variceal bleeding and lacks many of the untoward cardiac effects produced by vasopressin (Reichen, 1990).

Lebrec's 1980 report on propranolol has been followed by an enormous number of studies on this and other beta-blockers with mixed results. Propranolol, opposes $beta_1$-receptors in the heart to decrease cardiac output and decreases portal venous perfusion.

$Beta_2$ blockade in the splanchnic vascular bed allows unopposed alpha adrenergic-induced constriction of the mesenteric vessels and further reduces portal perfusion. Hepatic resistance is not affected (Reichen, 1990). Controlled studies on the effect of beta blockers preventing the first esophageal bleeding episodes (Pascal and Cales, 1987; Ideo et al., 1988) showed that beta blockers significantly decreased the bleeding episodes compared to placebo. The benefits gained by beta blocker administration versus placebo, however, are not particularly large and not all patients responded. Furthermore, contraindications to the use of the blockers reduces the number of patients available for such therapy.

At the opposite end of the adrenergic spectrum is the pharmacology of the α-adrenoceptors. The α_2 agonist, clonidine may be a promising agent in the treatment of portal hypertension. Studies by Willet et al (1986) and Moreau et al. (1987) found that clonidine decreased circulating levels of norepinephrine and decreased WHVP possibly by reducing intrahepatic resistance (Willet et al., 1986). In cirrhotic rats, Roulot et al. (1989) reported that clonidine decreased portal pressure by reducing portal tributary flow.

Serotonin receptor antagonists (S_2 receptors) ketanserin and ritanserin may play a role in counteracting the hypersensitivity to serotonin in the portal venous system reported to exist in portal hypertension. Ketanserin has been shown to reduce WHVP acutely but systemic effects (arterial hypotension) may limit its usefulness. Ritanserin, a newer serotonin receptor antagonist, has been shown to decrease WHVP and has the advantage of having fewer systemic effects than ketanserin.

Studies with verapamil have produced mixed results. Reichen and Le (1986) found that verapamil decreased portal venous pressure in isolated perfused livers by decreasing

intrahepatic vascular resistance (rather than by decreasing portal venous blood flow) and potentially improved hepatic function by increasing the extravascular space in cirrhotic rat livers. A long-term study in cirrhotic rats by Reichen et al. (1986) also supports these findings. In a clinical study from Taiwan, Kong et al. (1986) noted a small decrease in wedged hepatic venous pressure and the hepatic venous pressure gradient over a three month period. This is encouraging but it must viewed with guarded enthusiasm since wedged hepatic venous pressure is not necessarily a true measure of portal venous pressure if a presinusoidal resistance component exists. Additionally, several studies in cirrhotic patients have not shown beneficial effects of the calcium antagonists (Macmathuna et al., 1987; Navasa et al., 1988; Merkel et al., 1988).

Nitrovasodilators alone have been reported to decrease WHVP but also cause systemic arterial hypotension and possibly tissue hypoxia (Moreau et al., 1989; Moreau and Lebrec, 1990). These compounds may be working through cardiopulmonary and arterial baroreceptor reflexes to constrict the splanchnic vasculature in response to venous pooling (Hirsh et al., 1989), decreases in basal pulmonary wedged pressure (Rector et al., 1990) and arterial hypotension (Johnson et al., 1974; Abboud et al., 1979). There is evidence however, that arterial baroreceptor reflexes are impaired in cirrhotics (Koshy et al., 1989).

Other investigators utilizing isolated perfused rat livers have reported that nitrovasodilators decreased intrahepatic resistance. While this may be true, the viability of the preparation is questionable; five times the pathophysiological levels of norepinephrine produces only a 32% increase in portal venous resistance (Marteau et al, 1989).

Angiotensin-converting enzyme (ACE) inhibitors, antihistamines, and prostanoid

antagonists have been studied and the results summarized in an excellent review by Reichen (1990). Reichen concludes that the ACE inhibitors and antihistamines have limited usefulness in pharmacotherapy for portal hypertension. Prostanoid antagonists have been tested owing to the evidence that arachidonic acid metabolites may play a role in the hyperdynamic circulation. Prostacyclin (PGI_2) has been postulated to be involved as a vasodilator in the splanchnic hyperemia. However, by virtue of the roles in renal perfusion, Reichen suggests that prostaglandin synthesis inhibitors are not a realistic therapy in portal hypertension. There may, however, be a realistic role for specific inhibitors of the thromboxanes.

V.3C. Chronic Bile Duct Ligation as a Model of Portal Hypertension in the Cat

Chronic ligation of the bile duct (CBDL) produces a form of extrahepatic cholestasis which, in experimental animals, is able to produce hepatic cirrhosis with hemodynamic (blood flow and blood pressure), histological and biochemical changes comparable to that found in human cirrhosis and portal hypertension. Animal models of CBDL have also been developed to specifically study portal hypertension in the dog and rat (Bosch et al., 1983; Franco et al., 1979) although studies of this nature date back to the early 1930's (Cameron and Oakley, 1932). There have been several early studies utilizing this technique in the cat model primarily in respect to changes in enzyme activities resulting from biliary stasis (Flood et al., 1937; Carlsten et al., 1961; O'Brien and Mitchum, 1970; Kelly et al., 1975; Hoffmann et al., 1977; Everett et al., 1977a; Everett et al., 1977b; Center et al., 1983). To my knowledge, no studies of hemodynamic changes resulting from biliary obstruction have

been reported for this species and this is the reason for our interest in working with this model. The ensuing information is thus garnered from work conducted primarily on the rat, dog and rabbit.

The major differences between the CBDL model and the more popular portal vein-stenosis technique of producing portal hypertension is that CBDL is a form of intrahepatic portal hypertension (possibly pre or postsinusoidal) produced most likely from the development of biliary cirrhosis. Portal vein-stenosis is a purely pre-hepatic form of portal hypertension without liver damage. Portal vein-stenosis induces an immediate increase in portal pressure. Portal pressure elevation in CBDL is related to the duration of biliary obstruction and is therefore a progressive event. Individual reports diverge in respect to the actual portal venous pressure measurements recorded, mainly due to differences in duration of the study. Reports do indicate that pressures begin to elevate after about 2 to 3 weeks post-ligation (Bomzon and Blendis, 1990). Levels as high as 17 mmHg (Ohlsson et al., 1970a; Abergel et al., 1992) and as low as 6.3 mmHg (Mathie et al., 1988) have been recorded. Recordings of wedged hepatic venous pressure have not been widely conducted in CBDL but have been found to be elevated in the dog, along with significant increases in the hepatic venous pressure gradient (Bosch et al., 1983).

The initial histological observations in the liver after CBDL is a proliferation and dilation of the bile ducts with the early development of a fibrotic stroma surrounding the ductules. As the duration of obstruction increases, the number and size of bile ducts further increases along with the degree of fibrosis. It has been reported in rats that up to 50% of the total liver area can be occupied by bile ductule tissue (Sirica et al., 1991) and

hepatocytes may be transformed into ductular cells (Sherlock, 1989). The fibrosis has been found to form bridges between the portal and central regions of the lobules (Kountouras et al., 1984; Sherlock, 1989). Areas of necrosis have also been observed to increase with time (Trams and Symeonidis, 1957).

Biochemical indices of liver damage and biliary proliferation such as bilirubin levels, alkaline phosphatase activity, gamma glutamyltranspetidase (GGTP) activity and the activities of the serum transaminases, alanine transaminase (ALT, SGPT) and aspartate transaminase (AST, SGOT) are elevated in CBDL. Levels of albumin and cholesterol have also been incorporated into the batteries of tests to assess liver function (McIntyre, 1983). Generally a decrease in albumin and an increase in cholesterol would be suggestive of impaired hepatic function. Elevated serum bile acids is another typical finding in CBDL and, along with bilirubin, is believed to be one of the possible causative agents in producing some of the histological and functional disruptions occurring in CBDL (Yamamoto et al., 1978; Green et al., 1984). Patients and animals with obstructive jaundice have been shown to have an increased susceptibility to postoperative shock and renal failure that in many cases has been fatal (Zollinger and Williams, 1956; Williams et al., 1960; Yamamoto et al., 1978; Green et al., 1984). This phenomenon has usually been explained by the observed peripheral vasodilation and refractoriness to pressor actions of vasoconstrictors and the sympathetic nervous system which occurs in obstructive jaundice (Green et al., 1984; Bomzon, 1990). There has been some evidence to suggest that cardiac function may be impaired (Green et al., 1984; Binah et al., 1985) but there is little consistency in the literature to make a firm conclusion in this regard (Shasha et al., 1976; Better, et al., 1980; Hishida et al., 1980; Alon

et al., 1982).

The binding of bile acids and bilirubin to albumin normally reduces the toxicity of these agents. During liver disease when albumin synthesis may be compromised and levels of this protein are decreased, unconjugated bilirubin, by binding to phospholipids, can become imbedded in cellular and subcellular membranes such as that of the mitochondria. Bile acids can act as detergents and destroy the integrity and function of cells (Kanai et al., 1991). In 1956, Zetterstrom and Ernster reported that bilirubin was capable of uncoupling oxidative phosphorylation. Mitochondrial energy indices such as phosphorylation potential (mitochondrial ATP:ADP ratio) and energy charge were shown to be depressed in rats with CBDL (Yamamoto et al., 1978). Hemorrhage caused a drop in these indices in CBDL and control rats. Upon reinfusion of the hemorrhaged blood, these values returned to normal in the control rats but remained severely depressed in the CBDL rats (Yamamoto et al., 1978). Kanai et al. (1991) also reported that ATP production was inhibited in rat livers during obstructive jaundice. Whether these deleterious effects of bile acids and bilirubin are responsible for the spectrum of disruptions that occur in CBDL is not clear and I am not aware of any studies that directly link the bioenergetic data to the hemodynamic or cardiovascular refractoriness data.

The hemodynamic changes that occur with CBDL are far from being concretely determined. In respect to total hepatic blood flow, increases (Aronsen et al., 1969; Ohlsson et al., 1970b), decreases (Aronsen, 1968, Latzina et al., 1968; Aronsen et al., 1969; Hunt, 1979) and no changes (Sakoda and Atik, 1970; Hall et al., 1977) have all been reported. Recent work conducted using electromagnetic flow probes, however, has shown an overall

decrease in total hepatic blood flow in dogs (Bosch et al., 1983; Mathie et al., 1988). There are several reasons why such discrepancies may have occurred. The early reports have used a variety of blood flow measuring techniques including clearance of Xe^{133}, Au^{198}, and BSP, microsphere determinations and timed blood volume measurements. The clearance techniques can be suspect because of the possibility that an altered reticuloendothelial system or hepatocyte injury can disturb normal clearance process in the liver and misrepresent the true hepatic flow (Aronsen et al., 1969). Other reasons for the differences may lie in the fact that several different species, including rabbits, rats, dogs and humans, have been used to obtain these results and that most studies have had variable durations of cholestasis ranging from one hour to several weeks.

The specific changes occurring in hepatic arterial and portal venous blood flow are also variable. In some investigations, portal venous and hepatic arterial flow decrease to the same extent (Mathie et al., 1988) while others have reported that portal venous and hepatic arterial flow both increase in the first week post-ligation but return towards pre-ligation levels by the second week after ligation of the bile duct (Ohlsson et al., 1970b). Portal venous blood flow has also been found to be decreased with a "reciprocal" or partial compensatory increase in hepatic arterial flow (Ohlsson et al., 1970b; Bosch et al., 1983). Whether this increase in arterial flow is due to the hepatic arterial buffer response is not clear. The variety of responses in the hepatic vessels may be accounted for by the fact that liver structure can be severely altered in CBDL which can alter the delicate hepatic microvasculature. Observations that extensive duration-dependent changes occur in the vascular patterns of the hepatic arterial and portal venous microcirculation have been

reported (Del Rio Lozano and Andrews, 1965). Using the rabbit liver, these investigators found that, instead of receiving a mixture of arterial-portal venous blood, most sinusoids appeared to be supplied entirely by an arterial source after biliary obstruction. The development of new portal channels around occluded areas (possibly due to blood clots) forming a collateral circulation and possibly porto-hepatic venous anastomoses were also observed. Certainly such changes can influence the normal regulatory systems that govern hepatic blood flow, such as the hepatic arterial buffer response.

The development of porto-systemic shunt formation also occurs in CBDL but the progression of these anastomoses is slower compared to the portal vein-stenosis model. Using radioactive microspheres, Bosch et al. (1983) and Dunn et al. (1991), reported a 49% shunt index after 7 to 13 weeks post-ligation in the dog and a 46% shunt index after 21 days in the rat. Ohlsson et al. (1970a) on the other hand found only slight increases in porto-systemic shunting in dogs after 4 weeks of bile duct ligation.

The degree of hepatic pathology produced by CBDL is also related to the site of ligation along the biliary tree and whether the bile duct has been transected. In rabbits, ligation of the hepatic ducts produced an early and severe form of liver damage compared to the damage produced when the ligation was positioned on the common bile duct. Cystic duct ligation in the rabbit had no effect (Bauer, 1950). In rats, a species without a gall bladder, low position ligation of the bile duct (near the duodenum) had only mild hepatopathic effects. High position ligation of the bile duct produced severe liver damage (Koch-Weser et al., 1952). Furthermore, rats with bile duct excision displayed irreversible and severe liver damage over a 4 week period compared to rats that had the ligation around

the mid portion of the bile duct for the same time period. It was also found that in rats with only ductal ligation, a recanulization of the common bile duct occurred such that bile flow returned to normal in the new bile duct. Any early stage liver disruptions that occurred prior to the recanulization was reversed and the liver appeared normal (Trams and Symeonidis, 1957). It is also important to note that relief of the biliary obstruction within approximately 2 weeks after ligation of the bile duct produces a reversible form of liver damage in rats (Franco et al., 1979), dogs (Bomzon and Blendis, 1990) and humans (Aronsen, 1968). Cholestasis of longer durations tend to result in irreversible histological and hemodynamic alterations (Aronsen, 1968; Franco et al., 1979)

The model of portal hypertension produced by CBDL has been shown to be effective in producing a pathology similar to that found in man. It does not produce effects as immediately as the portal vein-stenosis model but it may be considered to have a more realistic etiology of portal hypertension, that is, through the development of hepatic injury and cirrhosis. The model may be more difficult to assess due to the somewhat labile nature of development of liver injury and the variability in duration of obstruction reported in the literature. Nevertheless, little if any information on the hemodynamic changes occurring in the portal hypertensive cat with this model has been documented and investigation in this area may lead to an acceptable research model.

OBJECTIVES OF THESIS

A doctoral research program is more than just investigations into the unknown. It is also a training ground to develop the proper skills and attitudes towards science. I realized early on that to obtain meaningful results in whole animal studies one must be surgically competent and be able to maintain the viability of a complex, living, breathing biological system, skills not to be taken lightly. I was also fortunate to be given the opportunity to train junior students and new personnel joining the research unit and to work in a spirit of collaboration with these individuals. These skills and philosophical approaches to science may, on occasion be taken for granted, but, I do consider them to be important and omnipresent goals in any research program and underlie the scientific objectives of this dissertation.

There were 3 major objectives in my doctoral research program. The first was to develop techniques to properly assess the pharmacology of the HA, with special reference to the influence of the hepatic arterial buffer response, the primary intrinsic control mechanism of the HA. The second major research objective was to study the pharmacology of the HA using the techniques developed pursuing the first objective. In particular, the pharmacolology of the pancreatic peptide, glucagon, was assessed in the HA. My interests in the pharmacology of glucagon were related to the possible role this peptide may play in the control of vascular function in the hepatic vascular bed. Specifically, I was interested in whether the sympathetic control of the HA could be modulated by glucagon. In the interest of expanding these findings, comparative studies in the mesenteric arterial vascular bed were also conducted in both research areas.

The third major objective of this thesis was to develop a model of intrahepatic portal hypertension in the cat and investigate the site(s) of increased resistance to portal blood flow. This project was the culmination of several of my research interests, such as: 1) the study of the hepatic microvascular disruptions occurring in portal hypertension, 2) the opportunity to gain experience with a new technique to measure intrahepatic blood pressure (lobar venous pressure), and 3) extension of my research training and experience from primarily basic research to include clinically relevant research projects.

Inherent in this final project was the development of procedures necessary to deal with animals undergoing a progressive time-dependent pathology. Unlike acute experimentation, innovative housing and maintenance techniques had to be devised, and the animals required constant monitoring of their clinical and mental health status over varying time periods. Thus, this project gave me first-hand experience in caring for the chronic experimental animal, and conducting surgery on cats with hepatic injury and possibly disrupted vascular systems. Moreover, having this experience greatly improved my ability to critically assess work of a similar nature.

The results of the research projects contained in this thesis will be presented in 3 sections. The first section (section VI) will discuss the methodologies developed for the investigation of the pharmacology of vasoactive agents in the hepatic vascular bed. Section VII, consisting of 4 research units, will detail studies which assessed the pharmacology and functional roles of glucagon in the hepatic and mesenteric vascular beds. Finally, in section VIII, results obtained in the development and assessment of a new model of portal hypertension, chronic bile-duct ligation in the cat, will be presented and discussed. In each

section (or unit), an introduction will be given, followed by complete details of the methods and materials utilized in the investigation, results and discussion of the results.

SECTION VI

METHODOLOGICAL DEVELOPMENTS FOR THE PHARMACOLOGICAL ASSESSMENT OF VASOACTIVE AGENTS IN THE HEPATIC ARTERY

INTRODUCTION

It has been shown that changes in portal blood flow lead to inverse changes in hepatic arterial flow, tending to maintain total hepatic blood flow at a constant level, by means of the hepatic arterial buffer response (HA buffer response). The HA buffer response, its mechanism of action (adenosine washout), and its implications have been reviewed in the general introduction.

Manipulation of portal flow, either mechanically or by vasoactive drugs, should change hepatic arterial flow and conductance in an opposite direction to that of portal flow. For example, if portal flow increases, the hepatic artery (HA) will constrict and decrease its blood flow, if portal flow is reduced, the hepatic artery will dilate and increase its blood flow.

Previous pharmacological assessment of vasoactive drugs in the hepatic artery has been plagued by confusion and erroneous conclusions. The hepatic artery has often been referred to as a very unusual vessel in terms of its responsiveness to such drugs. For example, intravenously infused dilator agents produce an elevation of portal blood flow secondary to dilation of the splanchnic arteries, yet the hepatic artery often appears to be insensitive to the dilator (Einzig et al., 1980; Krarup, 1975; Angerhn et al., 1980; Lindberg and Darle, 1976) even though it can be shown that direct infusion of the same agent into the

hepatic artery causes flow and conductance to increase (Lautt et al., 1988a; Richardson and Withrington, 1978a). However, most, if not all the pharmacological work on the hepatic artery, until recently, was conducted without knowledge or consideration of the HA buffer response and with inadequate techniques. It is possible that the unusual reactivity of the hepatic artery, in response to intravenously administered vasoactive drugs, is due to the combined influences of the buffer response, counteracting the changes in portal blood flow (secondary to the drug-induced changes in the splanchnic arterial vascular tone), and the direct action of the drug on the hepatic artery. Thus, if the influence of the buffer response is eliminated, the direct effect of the infused drug should be observed in the hepatic artery. Thus, the object of this investigation was to test the hypothesis that the HA buffer response counteracts the direct dilatory effects of intravenously administered drugs on the HA. To this end, it was necessary to select a specific vasoactive agent to use as a test compound on the HA. Glucagon was chosen because it is known to dilate the HA (Richardson and Withrington, 1976a, 1977) and is physiologically relevant in the portal and hepatic blood.

This investigation also deals with the larger issue of using proper techniques to accurately study the splanchnic vascular bed and the hepatic circulation in particular. As mentioned above, most of the previous pharmacological studies conducted on the HA have been conducted using inappropriate methods. By proving the stated hypothesis of this investigation, it will be possible to make concrete suggestions for improved techniques with which to study the physiology and pharmacology of the hepatic vascular bed.

METHODS AND MATERIALS

Cats were fasted overnight and anesthetized with pentobarbital sodium (32.5 mg/kg) by an intraperitoneal injection and a cannula was placed in the brachial vein for administration of anesthetic as required. Systemic arterial blood pressure and central venous pressure were measured by cannulas in the femoral artery and vein, respectively. A tracheal cannula was inserted to maintain a patent airway. After a laparotomy, electromagnetic flow probes were placed to measure hepatic arterial flow and superior mesenteric blood flow. Figure 12 illustrates the vascular preparation used. The hepatic arterial flow probe (Carolina Medical Electronics EP406) was placed on the celiac artery. All branches of the celiac artery were ligated, including the gastroduodenal artery, which was cannulated to allow for close intrahepatic arterial infusions of drugs. In this way, all flow in the celiac artery entered the hepatic artery. A small vein in the cecum was cannulated to measure PVP. This cannula advanced into the portal vein until its tip was 1.5 cm from the hilum of the liver. The spleen was removed and the splenic artery was cannulated to measure hepatic arterial blood pressure (HA pressure). A micrometer-controlled screw clamp was used to control pressure or flow within the celiac (hepatic) artery. The second flow probe (Carolina Medical Electronics EP407) was placed on the superior mesenteric artery (SMA), and systemic arterial blood pressure was monitored from a cannula in the femoral artery. The inferior mesenteric and gastric arteries were ligated. A second micrometer-controlled screw clamp was placed on the SMA proximal to the flow probe to determine zero base line and to reduce SMA blood flow. This methodology allows measurement of blood flows in the only arteries left perfusing the liver. All blood entering the portal vessel must derive from the

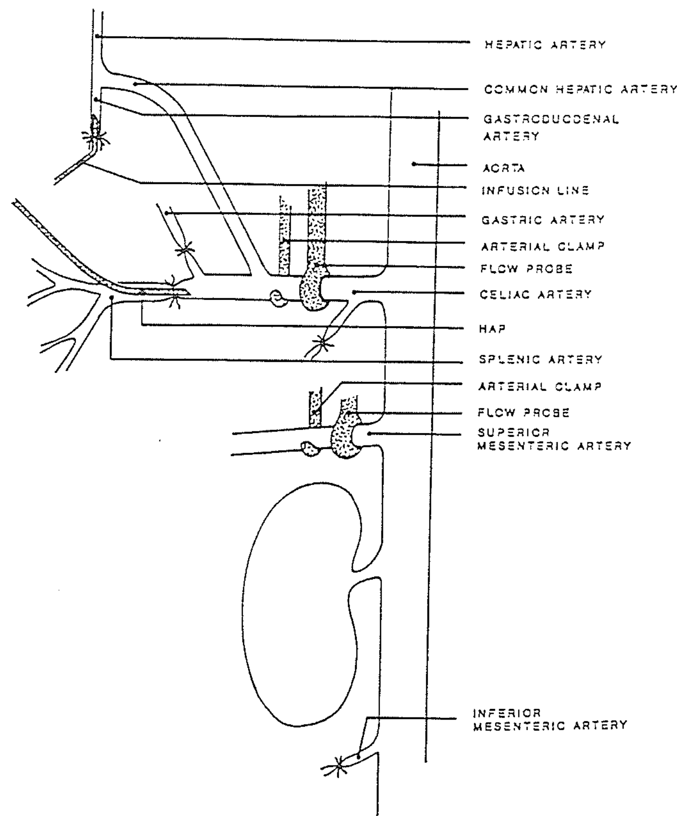


Figure 12: Preparation used to study the impact of the HA buffer response on HA conductance in response to i.v. infusions of glucagon leading to elevated portal blood flow. By removing the spleen and ligating the gastroduodenal, gastric and inferior mesenteric arteries, all portal blood is derived from the SMA. Portal flow can be regulated by an arterial clamp on the SMA in the same manner that HA pressure is regulated when SMA flow is reduced. HA flow is equal to celiac arterial flow. HA and SMA blood flows were measured by electromagnetic flow probes on the celiac artery and SMA, respectively. Central venous pressure was measured from a femoral vein and portal venous pressure was measured by a cannula in the portal vein (not shown).

SMA. Anastomotic connections of the SMA provide adequate blood flow to the regions normally supplied by the gastric and inferior mesenteric arteries as judged by a lack of gross evidence of ischemic regions and the presence of microspheres in all tissues when injected via the SMA (Lautt et al., 1985). Thus, in this preparation, SMA blood flow was synonymous with and equal to portal blood flow. This methodology has been extensively evaluated and verified (Lautt, 1981a; Lautt et al., 1985). The flow probes were calibrated in situ at the end of each experiment by the following procedure: After the experimental protocols were completed, approximately 50 mls of blood was removed from the cat and retained in a heparinized flask. The animals were then killed by an overdose of anesthetic. The SMA was cannulated towards the aorta from a location proximal to the flow probe, with a PE tubing with a diameter approximately the same size as the SMA. This cannula was used as a drainage cannula during the calibration process. The cannula previously inserted in the splenic artery to record HA pressure was also used as a drainage cannula during calibration of this vessel. The aorta was cannulated with PE tubing (PE 205) well below the origin of the SMA. This cannula was advanced towards the SMA until the cannula tip was approximately 5 mm below the SMA. The cannula was tied in place in this position. The aorta was then ligated just above the origin of the celiac artery and the common hepatic artery (the only branch of the celiac artery remaining intact) was ligated. With flowprobes in place on the celiac artery and the SMA, the blood retained in the flask was pumped into this vascular circuit via the aortic cannula by a non-pulsatile rotary pump (Cole Palmer Masterflex). As the blood passed through the SMA and celiac artery, a deflection of the recording pens occurred according to the rate of blood flow through the vessels. Timed

blood volume measurements were made separately for each vessel. At a given pump rate, multiple timed blood volume recordings were made after which the pump rate was changed and timed blood volume measurements repeated. The difference in flow rates (ml\min\kg) between these pump settings was divided by the difference in pen deflection (mm) to calculate the calibration factor for each vessel (ml/min/kg/mm). The height of blood flow responses (in mm) recorded during the experiment were then multiplied by the calibration factor to obtain the actual blood flow for that point.

Body temperature was monitored and held at 37.5°C by use of a rectal probe and a thermal control unit (Yellow Springs Instruments, model 72) operating heating rods in the surgical table. Statham pressure transducers were used for measuring blood pressure; blood pressures and flows were recorded on a Sensormedic type R-611 dynograph.

PROTOCOLS

Dose-Response Curves

Constant infusion, stepwise intra-arterial dose-response curves for glucagon in the hepatic artery and intravenous dose-response curves for glucagon in the SMA were obtained in every cat prior to the experimental protocol. The intra-arterial curves for the hepatic artery were conducted to demonstrate that the hepatic artery was capable of dilating in response to glucagon. The highest dose used in the intra-arterial hepatic dose-response curves was the largest dose not causing systemic recirculation, as indicated by dilation of the SMA. A single dose of glucagon, capable of producing a significant dilation of the SMA, was selected from the intravenous SMA dose-response curve.

The Hepatic Arterial Buffer Response

An inclusion criteria for animals in this investigation was that they had to be able to elicit an HA buffer response. To produce a buffer response, portal blood flow was decreased to zero by means of the vascular screw clamp on the SMA. In response to the drop in portal flow, the hepatic artery dilates and systemic arterial and hepatic arterial pressures rise. The elevation in hepatic arterial blood flow is due to two mechanisms, the action of the HA buffer response and the increase in arterial perfusion pressure. To eliminate the latter mechanism and demonstrate the lone effect of the buffer response, hepatic arterial pressure was decreased to the pre-buffer control level using the vascular screw clamp on the celiac artery. Any remaining elevation in hepatic arterial flow was then attributed to intrinsic vascular dilation, that is, the HA buffer response.

Effect of Glucagon on the Hepatic Arterial Buffer Response

Because glucagon was being used as a tool to study the HA buffer response, it was necessary to determine whether this hormone had any effect on the buffer response, per se. Thus, buffer responses were obtained before and during a constant intravenous infusion of glucagon (taken from the SMA/glucagon dose-response curve). The calculated buffer capacity (see calculations) was assessed for any changes due to the presence of glucagon.

Effect of the Hepatic Arterial Buffer Response on Hepatic Arterial Conductance Responses to Intravenously Infused Glucagon

Aside from calculating the buffer capacity, the presence of the HA buffer response

can be assessed by monitoring changes in hepatic arterial conductance (HA conductance) in response to alterations in portal blood flow. Alternatively, the impact of the buffer response on hepatic arterial conductance can be examined by eliminating the buffer response and monitoring the responses in the HA during changes in portal blood flow. Eliminating the buffer response can be accomplished in 2 ways:

Method 1

In 9 cats, an intravenous dose of glucagon capable of producing a significant dilation of the SMA was selected from the SMA/glucagon dose-response curve and infused. Responses in the SMA and hepatic artery were allowed to plateau. At this point, portal flow (SMA flow) was returned to its pre-infusion base line level and changes in hepatic arterial flow monitored. If hepatic arterial pressure (HAP) increased, it was also reduced to its control level. Changes in SMA conductance and HA conductance were calculated at the stable point of each experimental manoeuvre.

Method 2

In a separate group of cats (n=3), the buffer response was eliminated by pharmacological means. Because the buffer response has been shown to be primarily mediated by adenosine (Lautt et al., 1985), selective blockade of adenosine receptors should prevent the action of the buffer response and allow the HA to dilate in response to intravenous glucagon. This was accomplished by direct intrahepatic-arterial administration of 8-phenyltheophylline (8-PT), a selective competitive adenosine receptor antagonist. 8-PT has previously been shown to selectively block adenosine responses, in vivo, as well as

effectively inhibit the HA buffer response (Lautt and Legare, 1985).

In these cats, intra-hepatic arterial and intravenous dose-response curves for glucagon were obtained to demonstrate glucagon-induced dilation in the HA and SMA, respectively. A low dose of 8-PT was injected into the hepatic artery followed by a test infusion of adenosine, and a buffer response, to determine if adenosine responses were blocked. If the adenosine response and the buffer response were still present, the dose of 8-PT was doubled and the adenosine infusion and buffer response were repeated. This procedure was repeated until adenosine and buffer responses were absent. A test dose of isoproterenol was also administered before the first dose of 8-PT was injected and after adenosine receptor blockade had been achieved. This was to ensure that inhibition of adenosine responses was selective for adenosine and not for vasodilatory agents, in general. Intravenous glucagon was then infused and the vascular responses in the SMA and hepatic artery allowed to plateau. The remainder of the protocol follows that of method 1 described above.

Calculations

All vascular responses in this investigation, and in this thesis, are reported in terms of vascular conductance, rather than the more traditionally used, vascular resistance (Lautt, 1989). Conductance is preferable to resistance as an index of vascular tone, in vivo, where changes in vascular tone are primarily reflected as changes in blood flow rather than perfusion pressure. Resistance is nonlinearly (inversely) related to blood flow and is calculated as: perfusion pressure/blood flow. Consequently, extreme changes in flow, such as during an intense vasoconstriction, could result in a calculated resistance approaching or

equaling infinity, a value that cannot be manipulated mathematically. Conductance, on the other hand, is linearly related to blood flow and is calculated as: blood flow/ perfusion pressure. Changes in the calculated conductance, unlike resistance, parallel the changes in blood flow resulting from changes in vascular tone. Using the intense vasoconstriction example, even if blood flow was reduced to zero, calculated conductance becomes zero, and is a value that can be easily manipulated mathematically. Thus, because of the nonlinear relationship with blood flow, even simple mathematical functions, such as calculating means can be rendered inaccurate. The linear relationship between blood flow and conductance therefore allows mathematical manipulation of the data without incurring distortion of the data.

As noted above vascular conductance was calculated as: arterial blood flow/arterial blood pressure minus venous blood pressure. Femoral arterial blood pressure and portal venous pressure were used for SMA conductance calculations. HA blood pressure, measured via the splenic arterial cannula, was used for calculating HA conductance along with portal venous pressure since portal venous pressure is insignificantly different from hepatic sinusoidal pressure (Lautt et al., 1986).

HA buffer capacity is expressed as: change in HA blood flow divided by the change in portal blood flow x 100% (Lautt et al., 1985). HA buffer capacity is an index of the ability of the HA to compensate for changes in portal blood flow and as discussed above, SMA flow is equal to portal blood flow in this vascular preparation.

Glucagon (E. Lilly & Co.) was dissolved in the injectable solvent containing 1.6% glycerine and 0.2% phenol which was then diluted in warm Ringer's solution to be infused

in a volume of 0.103-0.203 ml/min. 8-PT (Research Biochemical Inc.) was dissolved (0.294 mg/kg/ml) in warmed NaCl (0.1M) solution at a pH of 11.6 adjusted with 1N NaOH. Adenosine (Sigma) was dissolved in warm Ringer's solution (1.94 mg/kg/min). Statistical comparisons were by paired analysis. The results are reported as the mean $\pm$ standard error of the mean.

RESULTS

Dose-Response Curves

Intrahepatic arterial infusions of glucagon produced dose-dependent dilations of the hepatic artery. The mean of the highest doses of glucagon not producing systemic recirculation was 0.31 $\pm$ 0.04 µg/kg/min (range 0.1-0.5 µg/kg/min) and produced an average increase in HA conductance of 55.1 $\pm$ 7.1%. This dose of glucagon and change in HA conductance should not be confused with the pharmacodynamic estimates of ED_{50} (dose of glucagon required to produce 50% of the maximal vascular response) and Rmax (maximal vascular response produced by glucagon), which will be discussed in the following research units.

Intravenous infusions of glucagon produced dose-dependent dilations of the SMA. The mean dose of glucagon selected to produce a significant elevation in SMA flow was 1.22 $\pm$ 0.17 (range 0.05 - 2.5 µg/kg/min) .

Effect of Glucagon on the Hepatic Arterial Buffer Response

The HA buffer response was measured prior to glucagon infusion by reducing SMA

flow from control levels to zero flow. The control buffer capacity was 19.1 ± 6.3%. Buffer capacity in the presence of glucagon was 21.7 ± 6.3% and was not significantly different from the control buffer capacity.

Impact of the Hepatic Arterial Buffer Response on Hepatic Arterial Conductance

Method 1

Intravenous infusions of glucagon caused SMA flow to rise from 16.2 ± 1.7 to 26.6 ± 3.0 ml/kg/min and significantly increased SMA conductance from 0.162 ± 0.013 to 0.287 ± 0.014 ml/kg/min/mmHg. At the same time that SMA flow was allowed to rise in response to glucagon, hepatic arterial flow did not increase significantly (16.6 ± 1.4 ml/kg/min to 19.0 ± 1.5 ml/kg/min). Only a minor, insignificant, rise in HA conductance occurred (0.186 ± 0.024 to 0.241 ± 0.042 ml/kg/min/mmHg). When SMA flow was reduced to control levels with the vascular clamp (15.2 ± 1.5 ml/kg/min) to remove the influence of the buffer response, HA flow rose significantly to 22.4 ± 1.1 ml/kg/min. HA conductance also increased significantly to 0.282 ± 0.044 ml/kg/min/mmHg.

Method 2

In this group of cats, direct hepatic arterial injections of 8-PT were used to pharmacologically remove the influence of the HA buffer response by blocking adenosine receptors. Buffer capacity in the control state was 21.8 ± 2.5% and was reduced to 8.5 ± 3.6% in the presence of 16 mg/kg of 8-PT. Exogenous adenosine responses in the hepatic artery were inhibited, whereas the percent change in HA conductance induced by

isoproterenol was not significantly different from the control values (21.4 ± 9.6% vs 18.5 ± 12.0%), confirming that 8-PT selectively blocked endogenous (HA buffer response) and exogenous adenosine responses.

The impact of the buffer response on HA conductance was assessed during intravenous infusions of glucagon (1.0 µg/kg/min in all cats), before (figure 13, left panel) and after inhibition of the buffer response by 8-PT (figure 13, right panel). In figure 13, point A in both panels refers to the pre-infusion control values. At point B in the control responses, glucagon dilated the SMA (89.4 ± 17.4% increase in SMA conductance), but, HA conductance did not rise significantly (6.7 ± 20.8%). At point C, the elevated SMA blood flow (portal flow) was returned to its pre-infusion level to mechanically remove the impact of the HABR. HA pressure was also returned to its control level to eliminate the effect of raised HA pressure on HA blood flow (point D). Thus, at point D in the control state, the HA was no longer subjected to the constrictor influence of the HABR and this vessel dilated in response to glucagon. In the presence of 8-PT, glucagon dilated the SMA to the same extent prior to 8-PT blockade (89.7 ± 28.7% increase in SMA conductance). However, after 8-PT administration and adenosine receptor blockade, HA conductance increased by 86.0 ± 29.8% (n=3, p=0.08). While the responses are not significantly different statistically, owing to the small number of subjects in this group, it is apparent that after the constrictor effect of the HABR was blocked by 8-PT, the hepatic artery dilated substantially more to the intravenous glucagon. Following 8-PT blockade of the adenosine receptors, when the buffer response was already greatly inhibited, mechanical elimination of the HA buffer response (point C) and controlling of HA pressure (point D) was without significant additional effect.

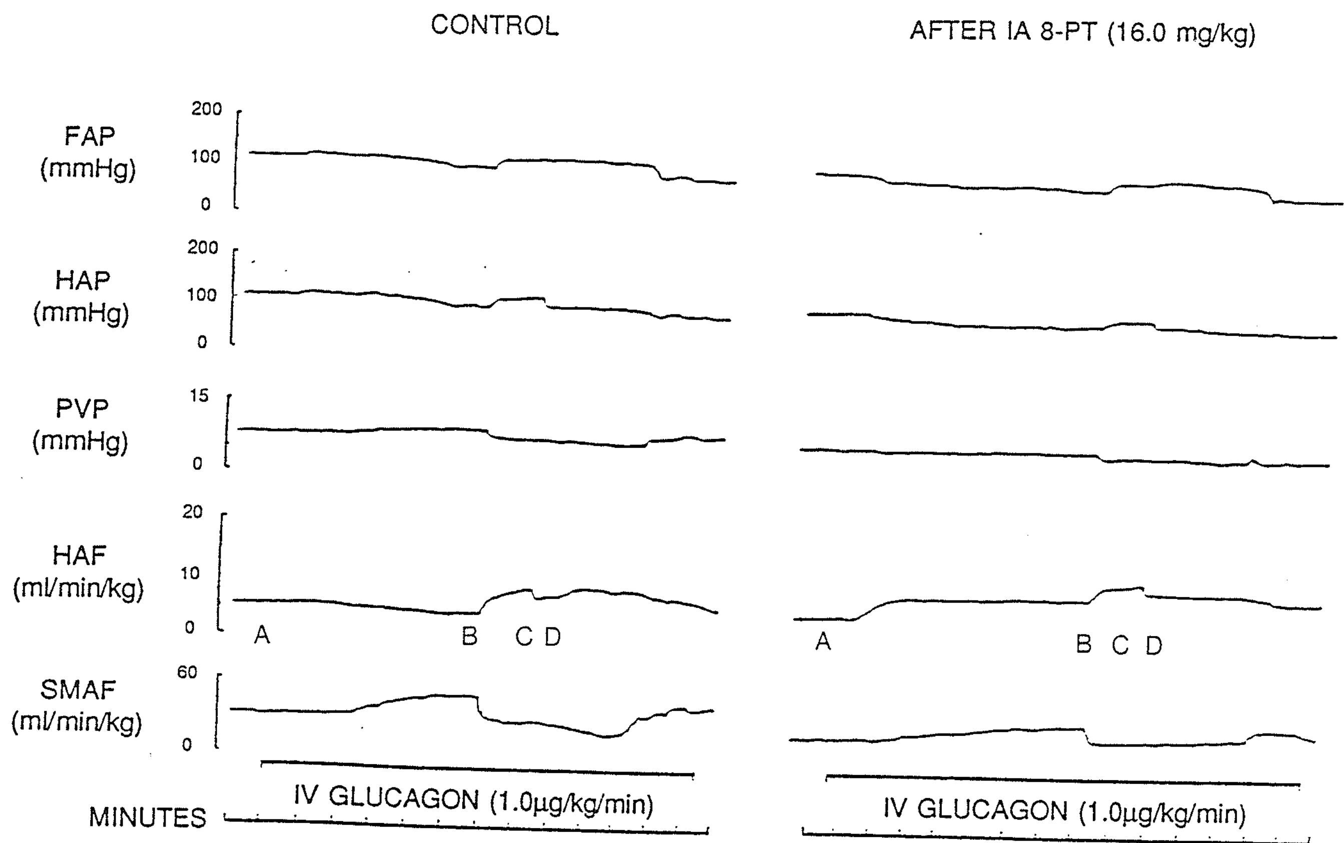


Figure 13: Effect of eliminating the impact of the hepatic arterial buffer response, mechanically and pharmacologically, on hepatic arterial blood flow (HAF) when portal blood flow (SMAF) is raised by an intravenous infusion of glucagon. Point A in both panels is the pre-infusion base line. When the buffer is mechanically eliminated by controlling SMAF (control panel, point B), the constrictor effect of the buffer response is removed and the HA dilates in response to glucagon. When the HA buffer response is eliminated by blocking adenosine receptors with 8-PT (right panel) the HA dilates from the start of the glucagon infusion. Refer to the text for details.

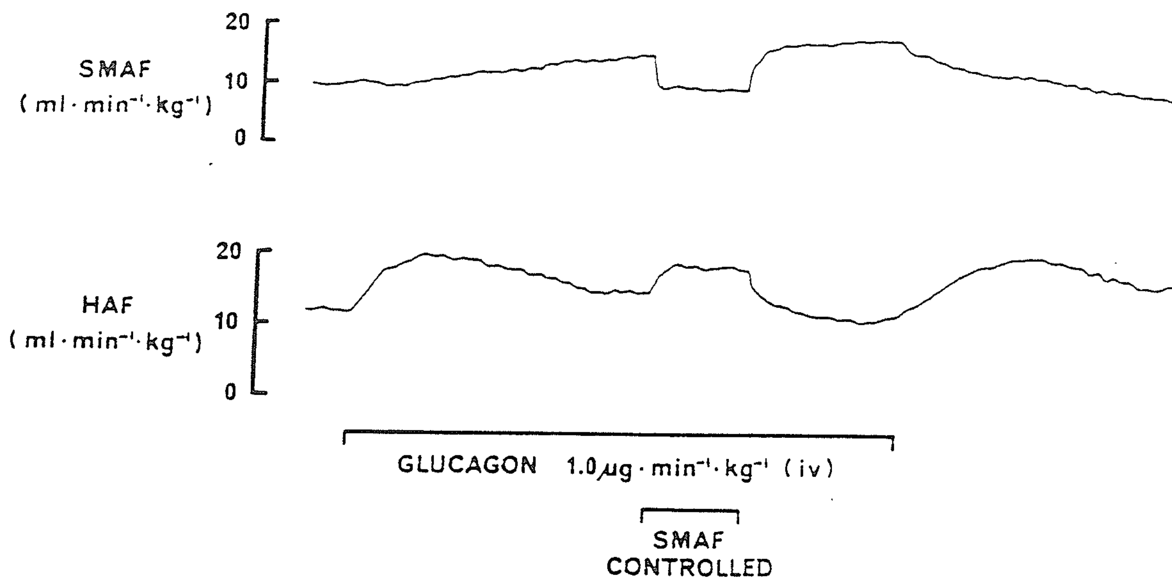


Figure 14: An example of the interaction of the hepatic arterial buffer response and intravenous glucagon (1.0 µg/kg/min) infusion on hepatic arterial blood flow (HAF). The more rapid onset of dilation in the HA than in the superior mesenteric artery (SMA) was consistently (but not always) seen. As the SMA dilated and portal blood flow increased, HAF decreased despite the maintained infusion of glucagon. When the SMA flow was returned to control levels, the HA dilated indicating that the secondary decline in HAF had been due to the buffer response, activated by the elevation in portal blood flow. The buffer capacity in this example was large (65%) compared to the mean response of nine animals using the same protocol (19.1 $\pm$ 6.3) but the qualitative relationships were similar in all animals.

Figure 14 is a tracing taken from one cat showing the SMA and HA blood flow response to an intravenous infusion of 1.0 μg/kg/min of glucagon. As opposed to the response in figure 13, the HA underwent an initial dilation in response to glucagon. This underscores the multitude of observations reported in the previous literature in regard to the effect of vasoactive agents on the HA. Figure 14 also demonstrates the dual action of the buffer response, that is, constriction and dilation. For example, as the slowly developing elevation in SMA flow (portal blood flow) occurs, the HA constricts and blood flow decreases from its elevated state. When SMA blood flow is reduced to its pre-infusion base line, the HA dilates.

DISCUSSION

The object of this investigation was to test the hypothesis that the hepatic arterial buffer response (HA buffer response) counteracts the direct dilatory effects of intravenously administered drugs on the hepatic artery. This hypothesis was based on the observation that changes in portal blood flow have been shown to activate the buffer response, resulting in changes of HA flow in a direction opposite that occurring in portal flow. It was not clear, however, what the interaction between portal and HA flow is during intravenous infusions of vasoactive compounds. As noted in the introduction of this section, there are several examples of where the HA response to intra-arterial drugs is opposite to that seen with intravenous administration of the same agent. It seemed probable that general dilator agents could result in elevated portal blood flow which would activate the HA buffer response and lead to a constriction of the HA rather than the anticipated dilation. Similarly, a constrictor

agent could reduce portal blood flow and lead to dilation of the HA.

The present data show that the HA was induced to dilate to direct arterial infusions of glucagon. Furthermore, glucagon did not affect the ability of the HA buffer response to function since the calculated buffer capacity was unaltered in the presence of this compound. However, with intravenous infusions, the dilator effect on the superior mesenteric artery (SMA) led to an elevated portal blood flow and no statistically significant net vasoactive effect on the HA. In this condition the HA received arterial blood with the same drug concentration as that delivered to the other arteries including the SMA, which underwent dilation. The direct dilator effect of glucagon on the HA appeared, however, to be counteracted by the constrictor effect of the buffer response activated by the elevated portal flow. This interpretation is verified by the observation that if SMA flow was returned to control levels by use of a vascular clamp, the HA dilated in the presence of the intravenous infusion. Moreover, no net vasodilator effect on the HA was seen with intravenous glucagon infusions unless the rise in SMA flow was prevented.

In the presence of 8-PT, the HA buffer response was inhibited. As a result, the rise in portal flow in response to intravenous glucagon cannot activate the buffer response. The direct actions of glucagon, therefore, produced a rise in HA blood flow and conductance from the onset of the infusion (figure 13). In addition, mechanical elimination of the buffer response by reducing SMA flow to the pre-infusion level did not produce any further dilation of the HA in response to the glucagon infusion. The results are clear, that, by removing the influence of the HA buffer response, either mechanically or pharmacologically, the HA is fully capable of dilating in response to intravenously infused glucagon. These results support

the hypothesis that the HA buffer response counteracts the direct dilator effects of glucagon on the HA.

Several implications, in respect to the study of the pharmacology and physiology of the hepatic arterial vascular bed, arise from these results. To assess the direct response of vasoactive agents in the HA, one must assure that portal flow has not changed, thereby activating the buffer response. There are many examples in the literature where the confounding effects of the buffer response may have lead investigators to erroneous conclusions regarding the reactivity of the HA. Adenosine infused intravenously caused dilation of all arteries measured except for the HA, which constricted (Lagerkranser et al., 1984) despite reports of a dilating effect of adenosine on the HA (Lautt et al., 1985). Prostacyclin infusion led to 18-65% increases in splanchnic organ flows and portal flow, despite no dilation of the HA (Einzig et al., 1980). Histamine, given by intravenous infusion, elevated portal blood flow and reduced HA flow (Krarup, 1975) whereas arterial administration leads to hepatic arterial dilation (Richardson and Withrington, 1978b). Intravenous dopamine in cats (Kullmann et al., 1983), dogs (Hirsch et al., 1975; Peschl, 1978) and man (Peschl, 1978; Angehrn et al., 1980; Grimaud et al., 1981) results in elevated portal and decreased hepatic arterial blood flow. Vasopressin, a potent constrictor, has been reported to lead to hepatic arterial dilation (Heimburger et al., 1960; Krarup, 1975) or no response (Richardson and Withrington, 1978c) concomitant with portal flow reduction, yet, vasoconstriction occurs with arterial infusions (Shanbour and Hinshaw, 1969; Richardson and Withrington, 1978c). Glucagon infused intravenously is reported to show wide species variation for effects on the HA with the cat showing elevation of portal flow, pigs mainly

showing increased portal flow and dogs showing elevation in both flows (Gelman et al., 1987; Krarup and Larsen, 1974; Lindberg and Darle, 1976; Richardson and Withrington, 1981). An example of this variability, taken from the current investigation, is illustrated in figure 14. In this case, HA blood flow initially increases due to the dilatory effect of glucagon. As the slowly developing elevation in SMA (portal) blood flow occurs, the buffer response is triggered and HA flow begins to decline. Returning SMA flow to its pre-infusion level eliminates the HA buffer response and HA flow returns to an elevated level. Upon removing the restraint on the SMA, flow in this vessel rises and reaches a plateau. The stimulus for activating the HA buffer response (the rise in portal flow) is again present and the HA constricts back to its pre-infusion level. Cessation of the glucagon infusion causes an immediate decline in SMA flow and a rapid increase in HA flow. The increase in HA flow is likely due to a combination of the deactivation of the constrictor effect of the buffer response on the HA (as SMA flow decreases) and the dilatory effect of residual glucagon still present in the circulation.

This figure illustrates several points: 1) The interaction of the buffer response and pharmacological agents is a complex event which can result in a variety of responses in the HA. In this figure, the HA initially dilates in response to glucagon. In figure 13, despite infusing the same dose of glucagon, the HA did not initially dilate, yet in both cases, the buffer response was present. 2) This figure also demonstrates the dual action of the buffer response, that is, constriction and dilation. For example, as the slowly developing elevation in SMA flow (portal blood flow) occurs, HA flow decreases from its elevated state. When SMA blood flow is reduced to its pre-infusion base line, the HA dilates. 3) Without prior

knowledge of the buffer response, or if portal blood flow was not controlled (or monitored), one might conclude that glucagon had a biphasic effect on the HA while having purely dilatory effects on the mesenteric vasculature. Thus, it should be reiterated, that, when assessing the responses of the HA to vasoactive agents, portal flow must not change, or must be monitored, to properly interpret hepatic arterial responses. This is of obvious relevance when drugs are given intravenously, but also holds true for intrahepatic arterial or portal venous administration of a drug at doses that produce recirculation of the drug in the systemic circulation.

While it is not proven that the HA buffer response is the cause of the differential effects of intravenous versus intra-arterial infusions on the HA in the cited examples, the present data indicate that this interpretation does account for such observations in the case of glucagon in the cat. The existence of the buffer response is known in a variety of species, including man, (reviewed by Lautt, 1985) and it appears highly likely that the splanchnic vascular response to intravenous infusions of any vasoactive compound will be confounded significantly by the effect of altered portal flow on the hepatic artery.

SUMMARY

This investigation demonstrated that the adenosine-mediated HA buffer response can interfere with the direct dilator effects of glucagon on the HA when glucagon is administered systemically, due to elevation of portal blood flow. Although it is not direct proof, these findings do suggest a possible explanation for the confusion in the previous pharmacological literature regarding the reactivity of the HA. These results also address the larger issue of

the techniques needed to properly study the pharmacology of the HA. It can be concluded with certainty that when assessing the pharmacology of any vasoactive agent on the HA, whether with systemic or direct arterial administration of the agent, portal blood flow must not change or at the very least, portal blood must be monitored in order to make accurate conclusions about the responsiveness of the HA.

SECTION VII

PHARMACOLOGY AND FUNCTIONAL CONSIDERATIONS OF GLUCAGON IN THE HEPATIC AND MESENTERIC VASCULAR BEDS OF THE CAT

In the preceding section, glucagon was used as an investigative tool to study the interaction between portal venous and hepatic arterial blood flows in the phenomenon of the hepatic arterial buffer response. In section VII, the hepatic and mesenteric vascular beds are used as research tools to assess the pharmacology and functional role that glucagon may have in these circulatory systems.

Section VII.1. Glucagon Pharmacodynamics and Modulation of Nerve and Norepinephrine-Induced Constrictor Responses in the Hepatic Artery of The Cat

INTRODUCTION

The pancreatic peptide, glucagon, has long been of interest for its role in liver-related metabolic functions, particularly glucose metabolism. In respect to the liver, glucagon is also of interest from a cardiovascular point of view. It has been reported that this peptide was able to dilate the hepatic artery (HA) in a dose-dependent manner when infused directly into the HA of the dog (Richardson and Withrington, 1976a). Equivocal vascular responses were reported for glucagon when it was infused into the portal vein (Richardson and Withrington, 1978a). This is suggestive of glucagon recirculating into the systemic circulation and activating the HA buffer response, as described in the previous section. In the 1970's, it was

also reported that intra-arterial and intraportal venous infusions of glucagon were able to inhibit constrictor responses in the HA, induced by direct stimulation of the hepatic nerves and by intra-arterial infusions of angiotensin, vasopressin and norepinephrine (Richardson and Withrington, 1976b, 1977, 1978b). These findings, however, have not been extended to other species and little work has followed the initial reports. At a symposium on hepatic circulation, Greenway (1981a) reported that he had been unable to confirm this effect using similar doses of glucagon utilized in the previous investigations. Furthermore, with advances that have been made in the understanding of hepatic hemodynamics and improvements in the techniques to study the hepatic vasculature, the reported inhibitory effects of glucagon may be seriously questioned. Thus the aim of this study was two-fold. One objective was to determine if this peptide was capable of modulating neural-mediated and norepinephrine-induced vasoconstriction of the HA. To this end, pharmacological assessment of intra-arterial and intraportal infusions of glucagon in the HA was carried out and constitutes the other primary objective of this investigation. The methods used to conduct this aspect of the investigation stem directly from the suggestions made in the previous study presented in this thesis (Section VI). The effect of glucagon on the phenomenon of vascular escape (reviewed by Greenway, 1984a) was also investigated, but will be presented in a separate section (section VII.4.).

METHODS AND MATERIALS

Cats of either sex were fasted overnight and anesthetized with pentobarbital-sodium (32.5 mg/kg) via an intraperitoneal injection. Anesthesia was maintained throughout the

experiment by supplemental doses (6.5 mg) of the anesthetic through a brachial vein cannula. Body temperature was maintained at 37.5°C by means of a rectal probe and a thermal control unit which regulated heating rods in the surgical table.

Systemic arterial blood pressure was monitored from a catheter in the right femoral artery. Central venous pressure was monitored from a cannula inserted via a femoral vein into the inferior vena cava. A tracheal cannula was inserted to maintain a patent airway. Abdominal surgery was initiated by a midline incision from the xyphoid process to the umbilicus. The common hepatic artery was exposed and the anterior plexus of the hepatic nerves was gently separated from this vessel. The nerve bundle was ligated, transected, and the peripheral end was placed in a circular bipolar stimulating electrode. The gastroduodenal artery was cannulated to allow direct intra-arterial infusions of drugs into the HA and to measure HA blood pressure. All vascular branches of the common HA except for the HA proper were ligated. This ensured that all blood flow through the common HA was equal to HA flow. An electromagnetic flow probe (Empco, Carolina Medical Electronics, size 4) was placed on the common HA to measure HA blood flow. Distal to the flow probe a vascular balloon occluder was also placed on the common HA. A string was looped around the HA at its junction with the common HA (T-junction) and, along with the balloon occluder, was used to determine the zero flow baseline. Inflating the balloon occluder should reduce HA blood flow to zero. Similarly, occlusion of the HA via the string at the T-junction should also produce a reading of zero HA flow if all the branches of the common HA have been ligated.

Portal venous blood pressure was monitored via a cannula threaded down a small

cecal vein into the portal vein. The cannula was advanced until its tip was situated approximately 1.5 cm from the hilum of the liver. Using a commercial intravenous needle unit (Jelco, Critikon Canada Inc.) a portal infusion line for glucagon was inserted into the portal vein and held in place by surgical tissue glue (Histoacryl).

A short length of the superior mesenteric artery (SMA) was gently cleared of its surrounding nerves and tissue. An electromagnetic flow probe(Empco, Carolina Medical Electronics size 8) was placed on the SMA to monitor blood flow. The surgical preparation is illustrated in figure 15. In this preparation SMA flow did not equal portal venous blood flow. Rather, SMA flow was used only as a rough index of portal blood flow and its changes. As described in Section VI, alterations in portal blood flow will produce inverse changes in HA flow, by means of the HA buffer response. Systemic recirculation of vasodilator agents infused directly into the HA or portal vein will act directly on the SMA and produce an increase in portal flow. This change in portal flow triggers the hepatic arterial buffer response to constrict the HA. The resultant state of the HA will be a combination of the direct action of the dilator agent and the constrictor effect of the hepatic arterial buffer response (see Section VI and Lautt et al., 1988a). To obviate this complication in these experiments, only those doses and responses that did not cause changes in SMA flow were analyzed.

After surgery was completed, the cat was allowed at least 30 minutes to stabilize before any procedures were conducted. At the end of each experiment the flow probes were calibrated in situ. The procedure for calibration is identical to that described in section VI, except that only the HA was calibrated.

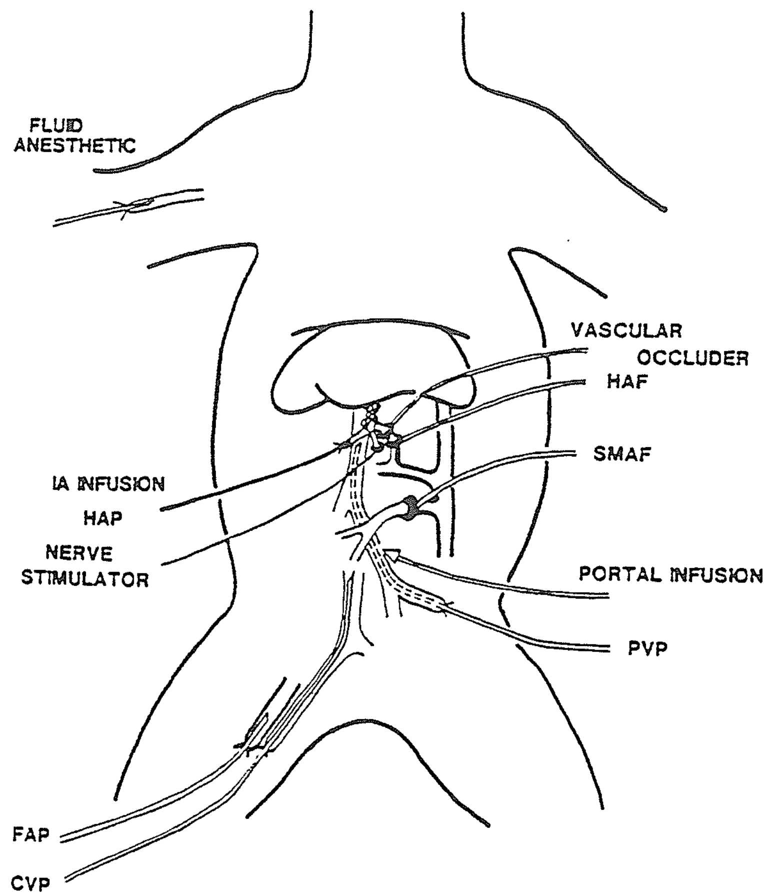


Figure 15: Preparation used to assess the pharmacology and modulatory effect of glucagon on nerve- and norepinephrine (NE)-induced constrictions of the HA. Constriction of the HA were produced by NE infused into the HA, and stimulation of the hepatic anterior nerve plexus. Glucagon was infused into the portal vein. HA and SMA flows (HAF, SMAF) were measured by electromagnetic flow probes on the common hepatic artery and SMA, respectively. SMA flow was monitored for systemic recirculation of glucagon. FAP, femoral arterial pressure; HAP, HA pressure; CVP, inferior vena caval pressure; PVP, portal venous pressure. See text for details.

Glucagon (E. Lily & Co.) was dissolved in the injectable solvent containing 1.6% glycerine and 0.2% phenol which was then diluted in Ringer's solution. Infusion volumes ranged between 0.0203-1.03 ml/min. A stock solution of norepinephrine was diluted accordingly with Ringer's solution.

All pressures were monitored using Statham pressure transducers. Pressures and flows were recorded on a Beckman Dynograph Recorder. Infusion of drugs was accomplished using a Harvard Apparatus constant infusion pump.

PROTOCOLS

Intra-Arterial and Intraportal Dose-Response Curves

Intra-arterial and intraportal venous dose-response curves were obtained using a constant infusion of glucagon and increasing to the next dose in a stepwise manner. The high dose was taken as the dose prior to that which produced recirculation, as evidenced by a rise in the SMA flow. The purpose of determining these curves was two-fold: 1) to compare the effect of equal doses of glucagon on the HA when administered through these different routes, 2) to select a low (just perceptible vasodilation), mid and high dose (highest dose that did not alter systemic hemodynamics) of glucagon from the portal dose-response curve to antagonize the nerve and norepinephrine-induced vasoconstriction.

Effect of Glucagon on Nerve and Norepinephrine-Induced Vasoconstriction

Two doses of norepinephrine, selected according to the responses of several doses initially tested in each cat, were infused directly into the HA via the gastroduodenal artery.

The anterior plexus of the hepatic nerves was stimulated at a low (average frequency 2.2 Hz) and high (6 Hz) frequency (1 msec duration, 15v square wave). Each constrictor stimulus was applied for 3 minutes. The nerve and norepinephrine responses were tested in random order. A complete test procedure consisted of: control responses for all 4 constrictor stimuli; repetition of constrictor stimuli in the presence of a low dose of glucagon; second control responses for all constrictor stimuli; repetition of constrictor stimuli in the presence of a mid dose of glucagon; third control responses for all constrictor stimuli; repetition of all constrictor stimuli in the presence of a high dose of glucagon; final control responses for all constrictor stimuli.

Calculations

Vascular responses to glucagon and the constrictor stimuli were calculated in terms of conductance (Lautt, 1989). HA conductance was calculated as follows: HA conductance = HA blood flow/ (HA blood pressure minus portal venous blood pressure). Approximations of portal blood glucagon concentrations were calculated as (dose of glucagon)/SMA flow and corrected for an estimated hematocrit of 32% (Lautt, 1976).

Peak vasoconstriction was calculated as the percent change in HA conductance from the pre-stimulation level to the initial peak of vasoconstriction (within one minute), not necessarily the lowest point of the response. In all of the control responses the peak constriction occurred within one minute from the onset of the stimulus. To determine if the degree of antagonism by glucagon was related to the stimulus intensity or type, the responses were standardized by calculating the percent inhibition of vasoconstriction: (control response

minus test response during glucagon infusions)/control response, and expressed as percent.

The pharmacodynamic estimates of Rmax (maximal dilation) and ED_{50} (dose of glucagon required to produce 50% of the Rmax) for glucagon on the HA were calculated by nonlinear regression using the Graphpad computer program (ISI software). The use of nonlinear regression to estimate the Rmax and ED_{50} has been discussed previously (Lautt, 1990), but will be briefly reviewed. To conduct pharmacological assessment of dose-response data, vascular tone must be expressed in terms of conductance, not resistance. Conductance has been shown to be superior to resistance as an index of vascular tone under in vivo conditions (Lautt, 1989) and has been discussed in section VI of this thesis. Drugs to be assessed must be infused to attain a steady-state concentration of the drug at the receptor site. Under these conditions, the dose at steady-state is assumed to be linearly related to the concentration of the drug at the receptors. In this investigation (and all others to follow) glucagon was administerd as a constant infusion and the vascular response in the HA was allowed to stabilize (or plateau) before the dose of glucagon was increased, ie., steady-state for glucagon concentrations at the receptors is assumed to have occurred. Vascular tone in the HA was calculated during the plateau response for each dose of glucagon. Only those doses of glucagon not producing systemic recirculation were used to estimate the Rmax and ED_{50}, thereby maintaining the assumption of a linear relationship between the infused dose of glucagon and the concentration of this agent at the receptor sites. It is also emphasized that a reasonable estimate of the Rmax (and consequently the ED_{50}) can be obtained if the maximal dose of the drug being assessed produces a vascular response that is at least 70% of the estimated Rmax, and if nonlinear regression analysis

shows a good fit to the data (r^2 value > 0.8) (Lautt, 1990). Lautt has substantiated these claims in several publications (Lautt, 1989, 1990; Lautt and McQuaker, 1989)

The algebraic equation of the nonlinear regression used to fit the dose-response data in this and the following studies in the thesis was:

$$Y = (A \circ X)/(B + X)$$

Y is designated as the value on the y-axis of the plot (dependent variable) or the measured response. X is designated as the value on the x-axis of the plot (independent variable) or the dose of the drug, in this situation. Y initially has a value of zero and increases to an estimated maximum plateau value of A (Rmax) with B designated as the dose of the drug required to produce 50% of the Rmax (ED_{50}). Once the estimates of Rmax (A) and ED_{50} (B) have calculated, the specific percentage of the Rmax produced by a given dose of the drug can be estimated from the equation or the curve itself.

Mean and standard errors of the mean are reported throughout. Control responses are taken as the mean of the responses measured before and after the test responses (during glucagon infusion). Comparisons are made using paired t-tests and blocked ANOVA (repeated measures analysis of variance) with multiple comparisons by Duncan's test.

RESULTS

Mean control femoral arterial pressure was 134 $\pm$ 5 mmHg; portal venous and central venous pressures were 7.9 $\pm$ 0.6 mmHg and 4.4 $\pm$ 0.5 mmHg respectively; SMA flow was 17 $\pm$ 2.1 ml/min/kg body weight and SMA conductance was 0.138 $\pm$ 0.02 ml/min/kg/mmHg; HA flow was 14.2 $\pm$ 1.9 ml/min/kg body weight and HA conductance was 0.114 $\pm$ 0.02

ml/min/kg/mmHg (n=12).

The two constrictor doses of norepinephrine for intra-arterial infusion into the HA were selected from a range of 0.125 to 1.25 μg/kg/min. The mean value for the low dose was 0.30 $\pm$ 0.04 μg/kg/min; the mean high dose was 0.8 $\pm$ 0.16 μg/kg/min. The mean low nerve frequency was 2.2 Hz (range 2-3 Hz). The high nerve stimulation frequency used was 6 Hz for all cats. The norepinephrine doses and nerve stimulation frequencies are referred to throughout as low and high for the respective stimuli.

Dose-Response Curves

The direct intra-arterial and intraportal dose-response curves indicate that glucagon is capable of dilating the HA in a dose-dependent manner. The maximum dose of glucagon not producing systemic recirculations varied between cats. The highest dose achieved in all cats, however, was 0.24 μg/kg/min and was the same for both routes of infusion. The mean change in HA conductance at this dose for intra-arterial glucagon was 62 $\pm$ 11% (n=7) and 65.2 $\pm$ 16% (n=7) for the portal infusions. The pooled values for the intra-arterial ED_{50} and Rmax, calculated by non-linear regression, were 0.113 $\pm$ 0.0035 μg/kg/min and 91.2 $\pm$ 1.33% respectively (r^2 >0.99). Pooled values of ED_{50} and Rmax for the portal administration of glucagon were 0.085 $\pm$ 0.019 μg/kg/min and 90.5 $\pm$ 9.03% (r^2=0.98) The glucagon dose of 0.24 μg/kg/min (maximal dose analyzed for the arterial and portal venous dose-response curves) was found to estimate the ED_{68} and ED_{74} for the arterial and portal venous dose-response curves. Paired comparisons of the ED_{50} and Rmax for the individual intra-arterial and intraportal curves showed no significant differences. Thus, the response of the HA to

the infusion of glucagon by either route appeared to be identical based on comparisons of the ED_{50}, Rmax and the plot of the curves (figure 16).

Effect of Glucagon on Peak Vasoconstriction

All three doses of glucagon that were selected from the portal dose-response curve to antagonize the vasoconstriction of the HA were able to produce dose-dependent dilations of this vessel. For each dose of glucagon, the portal blood glucagon concentration was estimated using SMA flow and not total portal flow since the spleen and inferior mesenteric artery were left intact and their relative contributions to portal flow were not measured. Calculated values for portal blood glucagon concentrations are therefore somewhat overestimated. The low dose of glucagon (0.047 $\pm$ 0.014 µg/kg/min) produced an estimated portal blood glucagon concentration of 3.16 $\pm$ 0.19 ng/ml, the mid dose (0.13 $\pm$ 0.04 µg/kg/min) produced an estimated portal blood glucagon concentration of 10.63 $\pm$ 0.89 ng/ml and the high dose (0.31 $\pm$ 0.07 µg/kg/min) produced an estimated portal blood glucagon concentration of 18.29 $\pm$ 1.52 ng/ml.

Figures 17 and 18 depict the control and test responses during glucagon infusions for nerve- and norepinephrine-induced vasoconstrictions. Blocked ANOVA indicated that the control responses, represented by the mean of the control responses before and after each test response, did not change with time for the nerve- or norepinephrine-induced constrictions. Blocked ANOVA of the test responses showed that glucagon did not significantly inhibit the nerve- or norepinephrine-induced constrictions at any dose. Rather, glucagon, at some doses, had a tendency to potentiate the vasoconstrictions, albeit to a small degree. No dose of

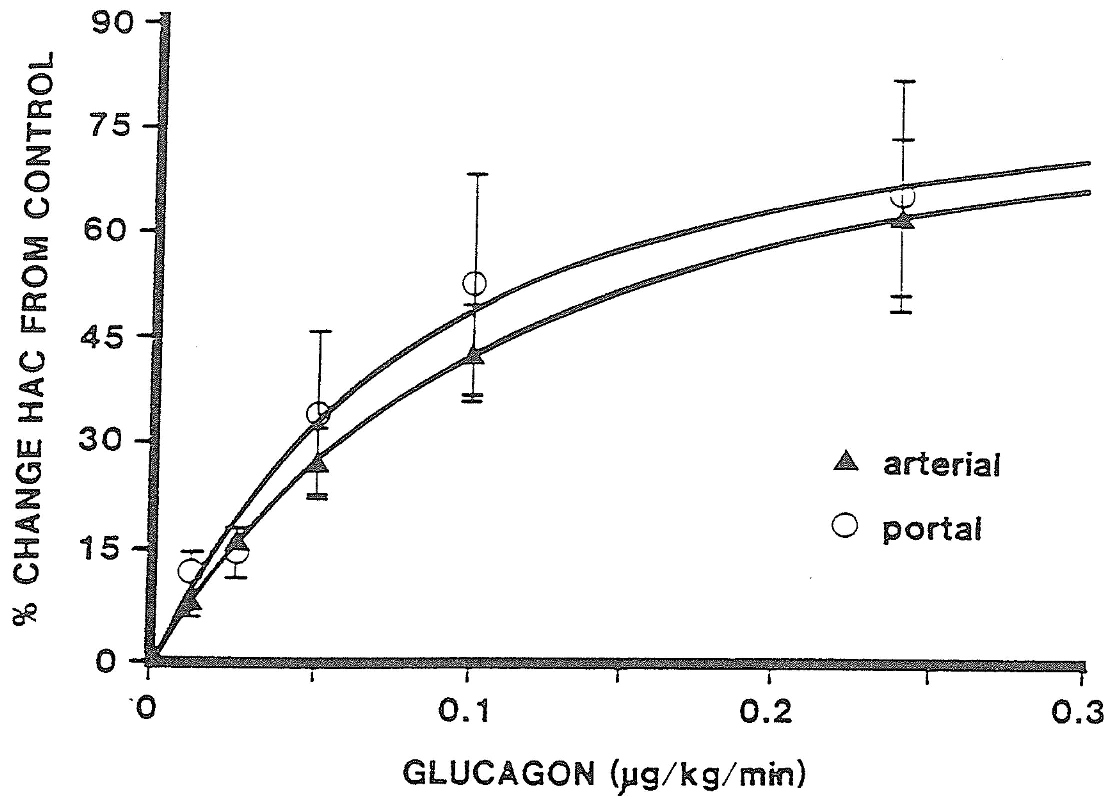


Figure 16: Intra-arterial and intraportal dose-response curves for glucagon on the percent change in hepatic arterial conductance (HAC). Responses are reported as the mean ± SE (n=7). Glucagon was administered as a constant infusion and was increased to the next dose in a stepwise manner. Only those doses that did not produce systemic recirculation are reported here, with the maximum dose being 0.24 µg/kg/min. The Rmax and ED_{50} for the intra-arterial curve were 91.2 ± 1.33% and 0.113 ± 0.0035 µg/kg/min (r^2 >0.99), respectively and 90.5 ± 9.03% and 0.085 ± 0.019 µg/kg/min for the portal curve (r^2=0.98).

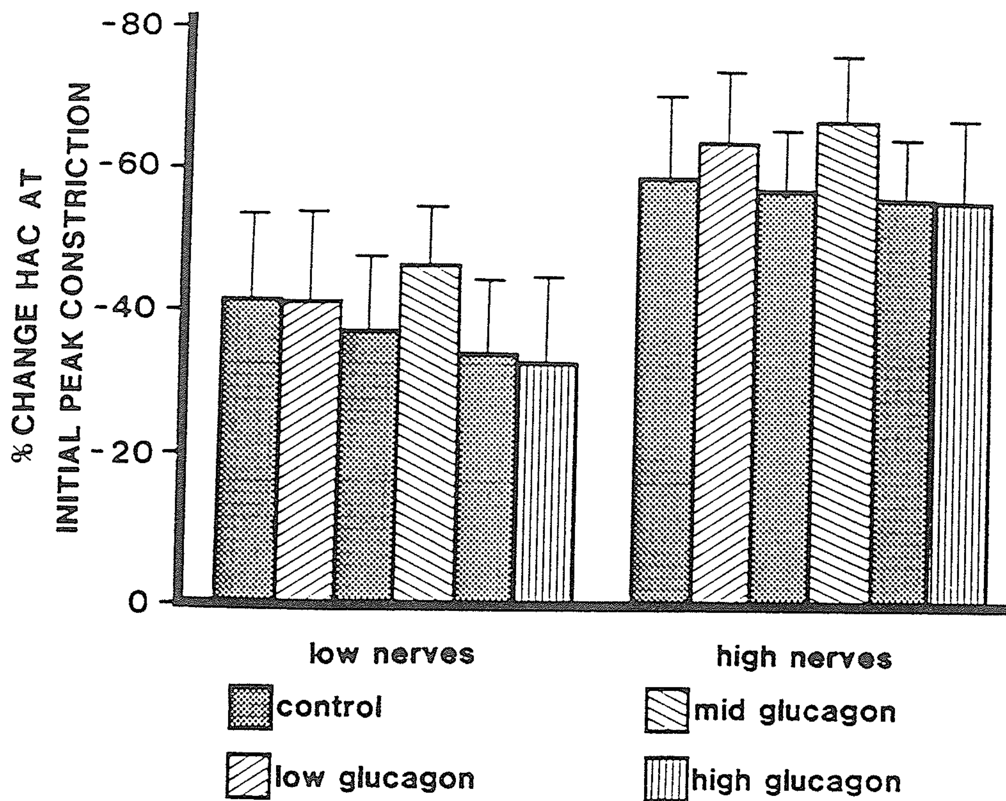


Figure 17: Effect of low (0.047 ± 0.014 µg/kg/min), mid (0.13 ± 0.036 µg/kg/min) and high (0.31 ± 0.07 µg/kg/min) doses of intraportal glucagon on the peak constriction of the HA, induced by direct nerve stimulation at low (2.2 Hz) and high (6 Hz) frequencies. Responses are reported as the mean ± SEM (n=5) of the percent changes in hepatic arterial conductance (HAC) from the pre-stimulation baselines. Responses are negative values, indicating a decrease in conductance. Control bars are the mean of the control vasoconstrictions before and after the test responses. Blocked ANOVA of the control and test responses showed that glucagon did not inhibit the vasoconstrictions at any dose. Statistical analysis of the pooled data indicated that the mid dose of glucagon produced a significant potentiation of the nerve-induced vasconstriction (29 ± 10.2%).

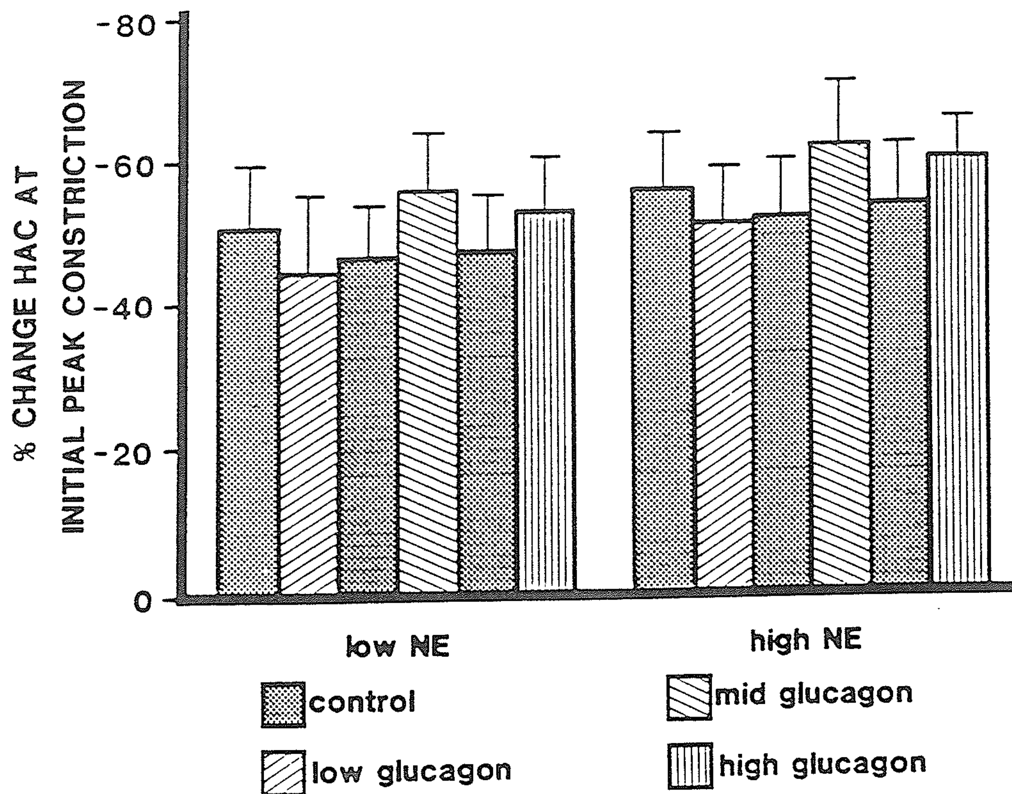


Figure 18: Effects of low (0.047 ± 0.014 μg/kg/min), mid (0.13 ± 0.036 μg/kg/min) and high (0.31 ± 0.07 μg/kg/min) doses of intraportal glucagon on the peak constrictions of the HA, induced by low (0.30 ± 0.04 μg/kg/min) and high (0.80 ± 0.16 μg/kg/min) doses of norepinephrine (NE). Responses are reported as mean ± SE(n=5) of the percent change in hepatic arterial conductance (HAC) from the pre-stimulation baselines. Responses are negative values, indicating a decrease in conductance. Control bars represent the mean of the control responses before and after each test response. Blocked ANOVA of the control and test responses showed that glucagon did not significantly inhibit the vasoconstrictions at any dose. Statistical analysis of the pooled data indicated that the mid dose of glucagon significantly potentiated the responses for norepinephrine-induced constrictions (20.6 ± 10.2%).

TABLE 2

Percent inhibition of nerve- and norepinephrine-induced vasoconstrictions in the hepatic artery by intraportal glucagon

Constrictor Stimulus	% inhibition by doses of glucagon (µg/kg/min)		
	Low 0.045 ± 0.01	Mid 0.13 ± 0.04	High 0.31 ± 0.07
Low Nerve Stim (2.2 Hz)	-1.9 ± 7.4	-40.1 ± 15.6	1.8 ± 33.5
High Nerve Stim (6 Hz)	-17.3 ± 15.6	-18.4 ± 4.6	-4.9 ± 11.6
Low NE (0.3 ± 0.04)	13.2 ± 14.0	-20.8 ± 8.8	-13.5 ± 8.7
High NE (0.8 ± 0.16)	9.1 ± 4.6	-20.4 ± 7.6	3.7 ± 17.7

Note: Norepinephrine (NE, µg/kg/min), was infused intra-arterially. Negative values indicate a potentiation of responses, positive values indicate an inhibition of vasoconstriction. Values are the mean ± SEM (n=5). Percent inhibition = [(control response-test response)/(control response)] x 100. Percent inhibitions were not significantly different by blocked ANOVA.

glucagon, however, potentiated vasoconstriction more than any other dose of this peptide, as indicated by blocked ANOVA of the calculated percent inhibition of constriction (table 2). From table 2 it is apparent that the only consistent trend was that the mid dose of glucagon weakly potentiated the vasoconstriction for both constrictor stimuli as indicated by the negative value for percent inhibition. Statistical analysis of the pooled, percent change in HA conductance data, for the mid dose of glucagon indicated that the potentiation produced by this dose of glucagon was 20.6 $\pm$ 10.2% ($P<.05$) for the norepinephrine responses and 29.2 $\pm$ 10.2% ($P<.01$) for the nerve-induced constrictions.

DISCUSSION

The aim of this study was to assess the pharmacology of glucagon on the hepatic artery (HA) of the cat and to determine whether glucagon could modulate the vasoconstrictor responses of the HA induced by direct stimulation of the hepatic sympathetic nerves and by norepinephrine infusions into the HA.

Dose Response Curves

Glucagon dilated the HA in a dose-dependent manner when administered directly into the HA. Only those doses and responses that did not produce systemic recirculation of glucagon were analyzed. Recirculation was monitored by changes in SMA flow. Previous studies in the dog (Bashour et al., 1973; Richardson and Withrington, 1976a, 1976b, 1977) and cat (reported in section VI of this thesis) reported similar dose-dependant dilations of the HA in response to intra-arterial infusions, although Ross (1970) reported HA constrictions

in the cat in response to intra-arterial glucagon. In the present study, the highest doses not producing systemic hemodynamic effects increased HA conductance by 62%. Bashour et al. (1973) and Richardson and Withrington (1976a, 1976b, 1977) reported decreases in hepatic arterial vascular resistance between 17% and 37%, which, when recalculated in terms of conductance from their available data, was approximately equal to the rise in conductance obtained in the current investigation. These investigators, however, tended to use large doses of glucagon which caused systemic perturbations in blood flow and pressure, the significance of which will be discussed later.

Intraportal infusions of glucagon also produced dose-dependent dilations of the HA, with a maximal increase of 65% in HA conductance. By expressing vascular responses as conductance (rather than the more commonly used resistance), classical pharmacodynamic analysis of the dose-response curve can be carried out using non-linear regression to estimate Rmax and ED_{50} (Lautt, 1989). Comparisons of the Rmax (maximal dilation) and ED_{50} (dose of glucagon required to produce 50% of the Rmax) for the intraportal and intra-arterial dose-response curves indicated that the responses were nearly identical for both routes of infusion. Furthermore, the glucagon dose of 0.24 µg/kg/min, which was the highest dose analyzed for both routes of infusion, was calculated to be the ED_{68} and ED_{74} for the intra-arterial and intraportal dose-response curves. Furthermore, the r^2 value for both curves, which is an indication of how well the regression curve fits the data, are well above 0.80. Thus, the Rmax for both of these curves should be a reasonable estimate of the maximal response in the HA to intra-arterial and intraportal venous glucagon (Lautt, 1990).

Despite the fact that glucagon produced equal responses in the HA whether infused

into the HA directly or into the portal vein, Richardson and Withrington (1978a), obtained equivocal responses with intraportal infusions. Other vasoactive compounds have shown less effective HA dilation when administered via the portal vein (Lautt et al., 1984), which is in contrast to the equal effectiveness shown in this study. In that investigation, systemic recirculation of the infused agents was also monitored, thereby eliminating this as a potential technical artifact. At present, the reason for the unexpected sensitivity of the HA to portal infusions of glucagon in the cat is unknown. Because there was no systemic recirculation of glucagon, the results suggest that this peptide, infused into the portal vein reaches the HA via a transhepatic route. Furthermore, glucagon may freely diffuse between the portal venules and hepatic arterioles.

As mentioned earlier, only those doses that did not produce systemic recirculation of glucagon were analyzed. Such doses produced dose-dependent dilations of the HA. Large doses of glucagon (or any vasoactive agent), infused directly into the portal vein or hepatic artery, can escape metabolism by the liver, enter the general circulation (recirculation) and produce alterations in blood flow and blood pressure in other vascular beds. More specifically, recirculation of vasodilator agents can dilate the mesenteric vasculature, thereby increasing the portal venous flow supplying the liver. It was shown in the previous section that these changes in portal flow can activate the adenosine-mediated mechanism of the hepatic arterial buffer response (Lautt et al. 1985) to produce a constriction of the HA in opposition to the direct dilating effects that the agent has on this same vessel. Results demonstrating this effect for glucagon were reported in section VI of this thesis. The resultant state of the HA will be a combination of the direct action of the dilator agent and the constrictor effect of the

hepatic arterial buffer response. Thus, to accurately assess the pharmacodynamics of intra-arterially administered vasoactive agents in the HA, it is imperative that systemic recirculation of these compounds be minimized.

Glucagon and Peak Vasoconstriction

Glucagon did not inhibit the nerve and norepinephrine- induced vasoconstriction of the HA. The mid dose of glucagon consistently potentiated the initial peak vasoconstrictions for both constrictor stimuli (table 2), however, the low and high doses of glucagon showed no significant or consistent effects on the constrictions. Our results suggest that when glucagon potentiates the vasoconstrictions, it is likely operating at a postsynaptic site, since the nerves and norepinephrine-induced constrictions were potentiated to a similar extent. This mild potentiation is not an artifact of altered baselines induced by vasodilation, since the high dose of glucagon produced a larger dilation than the mid dose, yet showed no modulation of the constrictor responses.

The finding that glucagon did not antagonize vasoconstriction at any dose and potentiated it at some doses is in conflict with earlier reports. Richardson and Withrington (1976b, 1977, 1978a) administered glucagon via the HA and portal vein, by injection and constant infusion, and found that both routes and modes of administration were able to inhibit vasoconstriction induced by direct nerve stimulation and intra-arterial injections of norepinephrine and angiotensin in a dose-dependent and competitive manner. Vasopressin-induced constrictions were also inhibited in a dose-dependant manner, but the antagonism by glucagon was non-competitive. The conflicting results from the present investigation may

reflect a species difference for the actions of glucagon since the inhibitions occurred in the dog and this investigation examined the cat. Technical differences, however, may also partially account for the discrepancy in results. In the earlier investigations, norepinephrine and glucagon injected into the HA vasculature may have gained access to the systemic circulation, thereby possibly altering the responses in the HA due to the actions of the buffer response (see previous discussion).

The blood glucagon concentrations used to inhibit the constrictions in our study and the cited dog studies, represent levels well in excess of basal (0.025-0.300 ng/ml) and pathophysiological levels (0.400-1.500 ng/ml)(Richardson and Withrington, 1981; Smitherman et al., 1975). Enhancement of glucagon secretion in the dog, by rapid hemorrhage (Lindsey et al., 1975), arginine infusion (Blackard et al., 1974), and arginine and CCK-PZ infusions (Rojdmark et al., 1978), have been shown to raise the portal blood concentration of glucagon 5 to 7 fold (0.516-1.063 ng/ml), which is still below the levels others and ourselves have used. Portal blood glucagon concentrations in the dog studies were increased to the range of 3.2 to 59.5 ng/ml whereas in the current investigation, portal blood glucagon concentrations were raised by an estimated 3.16 to 18.29 ng/ml during the low and high dose infusions of glucagon, all studies clearly reaching pharmacological levels. Thus, despite our observations that glucagon was able to mildly potentiate the vasoconstrictions, it is unlikely that physiological levels of this peptide would produce such effects.

The low dose infusion of glucagon was approximately double that of pathophysiological concentrations of this peptide. This dose produced only very slight dilations of the HA and had no significant effects on norepinephrine or nerve-induced

responses. It has been hypothesized that glucagon may represent a physiological mechanism to protect the hepatic arterial vasculature from the effects of circulating constrictor substances (Richardson and Withrington, 1976a). Our data would suggest that such a protective mechanism, in the hepatic vascular beds, is unlikely.

SUMMARY

In summary, using methodologies developed specifically for pharmacological analysis of vasoactive agents in the HA, the pharmacodynamic estimates of Rmax and ED_{50} for glucagon in this vessel were calculated using nonlinear regression of the dose response data. This is the first time nonlinear regression analysis has been used to calculate the Rmax and ED_{50} for glucagon in the HA. It was found that glucagon displays equal potency and efficacy on HA conductance whether infused into the portal vein or directly into the HA. Thus, glucagon may diffuse freely between the portal venules and hepatic arterioles. It should be stressed that the techniques used to study the effect of glucagon in the HA are such that the confounding effect of the HA buffer response will not interfere with the direct effect of glucagon on this artery. Finally, in contrast to earlier reports in the dog, we found that glucagon is not an inhibitory modulator of the peak vasoconstrictor response to nerve stimulation or norepinephrine infusions into the HA of the cat.

SECTION VII.2. Glucagon Pharmacodynamics and Modulation of Sympathetic Nerve and Norepinephrine-Induced Constrictor Responses in the Superior Mesenteric Artery of the Cat

INTRODUCTION

In the previous section, pharmacodynamic estimates of maximal dilation (Rmax) and the ED_{50} were calculated for glucagon in the hepatic artery (HA). Glucagon did not have inhibitory effects on peak vasoconstriction in the HA, induced by nerve stimulation of the hepatic anterior nerve plexus or norepinephrine infusions into the HA. This was in contrast to previous reports that glucagon was capable of inhibiting constrictions of the HA in response to nerve stimulation and norepinephrine, angiotensin and vasopressin administration (Richardson and Withrington, 1976b, 1977, 1978).

Intravenous administration of glucagon causes a preferential dilation of the mesenteric vasculature (Kock et al., 1970b). Furthermore, like the previous studies on the HA, investigators have also reported that glucagon can antagonize the responses induced by several constrictor stimuli in the mesenteric vascular beds (Kock et al., 1971; Tibblin et al., 1970). The object of this current investigation was to conduct a comparative study in the superior mesenteric artery (SMA) of the cat to determine if glucagon had any modulatory effect on vasoconstrictor responses in this vessel. The hypothesis, based on the results obtained from the HA, was that glucagon would not effectively antagonize nerve- and norepinephrine-induced vasoconstrictions of the superior mesenteric artery in the cat. The effect of glucagon on the vascular escape phenomenon was also assessed and will be detailed in section VII.4.

METHODS and MATERIALS

Cats of either sex were fasted overnight and anesthetized with pentobarbital-sodium (32.5 mg/kg) by intraperitoneal injection. Anesthesia was maintained throughout the experiment by supplemental doses (6.5 mg/kg) of the anesthetic through a brachial vein cannula. Body temperature was maintained at 37.5°C by means of a rectal probe and a thermal control unit which regulated heating rods in the surgical table.

Systemic arterial blood pressure was monitored from a catheter in the right femoral artery. Central venous pressure was monitored from a cannula inserted, via the femoral vein, into the inferior vena cava. A tracheal cannula was inserted to maintain a patent airway. Abdominal surgery was initiated by midline incision from the xiphoid plexus to the umbilicus.

After the inferior mesenteric artery was ligated, a small branch of the superior mesenteric artery (SMA), usually the first branch leading to the cecal region, was cannulated toward the SMA to allow direct intra-arterial infusions of drugs into the mesenteric vascular bed. A short length of the SMA, just proximal to the cecal artery cannulation, was exposed and the SMA nerve plexus was gently separated from this vessel. The nerve bundle was ligated, transected, and the peripheral end was placed in a snug fitting circular bipolar stimulating electrode. An electromagnetic flowprobe (EMPCO, Carolina Medical Electronics, size 8) was placed on the cleared segment of the SMA. A cannula was inserted in a small cecal vein and advanced into the portal vein to measure portal venous pressure.

After surgery was completed the cat was allowed at least 30 minutes to stabilize before any procedures were carried out. At the end of each experiment the flowprobe was calibrated in situ according to the procedure previously outlined in this thesis. The only difference in

this calibration procedure is that only the SMA was calibrated since hepatic arterial flow was not measured.

Glucagon (E. Lilly & Co.) was dissolved in the injectable solvent containing 1.6% glycerin and 0.2% phenol which was then diluted in Ringer's solution(infusion volume range, 0.203 to 1.03 ml/min). Norepinephrine was prepared from a stock solution and diluted with Ringer's solution(infusion volume range, 0.0068 to 0.68 ml/min). Infusions were carried out using a Harvard Apparatus constant infusion pump. All pressures were monitored using Statham pressure transducers. Pressures and flows were recorded on a Beckman dynograph recorder.

PROTOCOLS

Intra-arterial Dose-Response Curves for Glucagon and Norepinephrine

Intra-arterial dose-response curves for glucagon and norepinephrine were obtained in the SMA. The purpose of determining these curves was to estimate the Rmax (maximal response) and ED_{50} (dose of drug required to produce 50% of Rmax) for glucagon and norepinephrine in the SMA. These estimates were calculated by nonlinear regression using the GraphPAD INPLOT computer program (ISI Software). The dose-response curves were also used to select a low and high dose of norepinephrine to be used as constrictor stimuli and to select low, mid, and high doses of glucagon to antagonize the nerve and norepinephrine-induced vasoconstrictions. Glucagon was administered as a constant infusion (range, 0.01-5.0 µg/kg/min) and increased to the next dose in a stepwise manner. The norepinephrine dose-response curve was obtained using single dose infusions for a 3 minute

duration. This method was used instead of a constant infusion to obviate the problem of changing baselines during vascular escape which occurs during a maintained stimulus in the SMA of the cat.

Effect of Glucagon on Nerve- and Norepinephrine-Induced Vasoconstriction of the Superior Mesenteric Artery

The low and high doses of norepinephrine selected from the dose-response curve were infused directly into the SMA via a cannula in a small cecal artery. The superior mesenteric plexus of the SMA was stimulated at a low (2 Hz) and high (6 Hz) frequency (1 ms duration, 15 V square wave). The low frequency stimulation (2 Hz) has been shown to estimate the Hz_{50} (frequency of nerve stimulation required to produce 50% of the maximal constriction induced by direct nerve stimulation) for the SMA as determined by nonlinear regression of frequency-response curves (Lockhart et al., 1988). The 6 Hz frequency can be shown to be a good estimate of the Hz_{75}, as determined by the nonlinear regression curve (Lockhart et al., 1988) and by using the mathematical equation for the curve (GraphPAD INPLOT).

Each constrictor stimulus was applied for 3 minutes. The nerve and norepinephrine responses were tested in random order. The test procedure was identical to that for the hepatic arterial study and consisted of obtaining control responses for all four constrictor stimuli, repetition of constrictor stimuli in the presence of glucagon and a second set of control responses for all constrictor stimuli. This procedure was continued until the constrictor responses had been repeated in the presence of all three glucagon doses with control responses before and after each test response.

Calculations

Vascular responses to glucagon and the constrictor stimuli were calculated in terms of conductance (Lautt, 1989). SMA conductance = SMA blood flow/(systemic arterial pressure - portal venous pressure). Approximation of SMA blood glucagon concentration was calculated as follows: dose of glucagon/SMA flow, and corrected for an estimated hematocrit of 32% (Lautt, 1976).

Peak vasoconstriction was calculated as the percent change in SMA conductance from the pre-stimulation level to the initial peak of vasoconstriction (within 1 minute). To determine if the degree of antagonism by glucagon was related to the stimulus intensity or type, the responses were standardized by calculating the percent inhibition of vasoconstriction: (control response - test response during glucagon infusions)/control response, and expressed as percent.

Mean and standard error of the mean are reported throughout. Control responses are taken as the mean of the responses measured before and after the test responses except when otherwise stated. Comparisons are made by paired analysis and blocked ANOVA (repeated measures analysis of variance) with multiple comparisons by Duncan's test.

RESULTS

The mean control femoral arterial pressure was 141.8 $\pm$ 8.4 mmHg; portal venous and central venous pressures were 7.6 $\pm$ 0.6 and 4.6 $\pm$ 0.4 mmHg, respectively; SMA flow was 21.0 $\pm$ 3.5 ml/min/kg body weight and SMA conductance was 0.154 $\pm$ 0.02 ml/min/kg/mmHg.

Dose-Response Curves

The Rmax for glucagon, calculated from the curve in figure 19 (pooled data) and based on the percent change in SMA conductance was 162.8 $\pm$ 13%. The ED_{50} for the pooled data was 0.98 $\pm$ 0.23 µg/kg/min (1 µg glucagon=0.282 nmole). The mean of the Rmax and ED_{50} for the individual glucagon curves from each experiment were 156.4 $\pm$ 34% and 0.86 $\pm$ 0.04 µg/kg/min and are in close agreement with the pooled data. The doses of glucagon selected from the dose-response curves to antagonize the constrictor responses were: low glucagon, 0.114 $\pm$ 0.024 µg/kg/min; mid glucagon, 0.30 $\pm$ 0.06 µg/kg/min; high glucagon 0.61 $\pm$ 0.11 µg/kg/min. The low dose of glucagon produced an estimated blood-glucagon level of 20.5 $\pm$ 5.7 ng/ml; the mid and high doses of glucagon produced estimated blood-glucagon levels of 52.9 $\pm$ 14.4 ng/ml and 82.3 $\pm$ 20.53 ng/ml, respectively. All 3 doses were capable of producing dose-dependent dilations of the SMA.

The Rmax and ED_{50} for norepinephrine estimated from the dose-response curve for the pooled data were -110.3 $\pm$ 6% and 0.38 $\pm$ 0.06 µg/kg/min (1 µg norepinephrine=5.9 nmole), indicating that norepinephrine was capable of producing an intense vasoconstriction of the SMA (figure 20). The mean of the Rmax and ED_{50} for the individual norepinephrine curves from each experiment were -99.9 $\pm$ 0.01% and 0.50 $\pm$ 0.15 µg/kg/min. The mean values of the low and high norepinephrine doses selected from the curves were 0.27 $\pm$ 0.04 and 0.79 $\pm$ 0.16 µg/kg/min. These doses were chosen at the time of experimentation, before estimates of the ED_{50} were calculated. After nonlinear regression of the pooled dose-response data for norepinephrine was completed, it was determined that the low and high doses of norepinephrine were equal to the ED_{42} and ED_{68} for this agent in the SMA. The low and

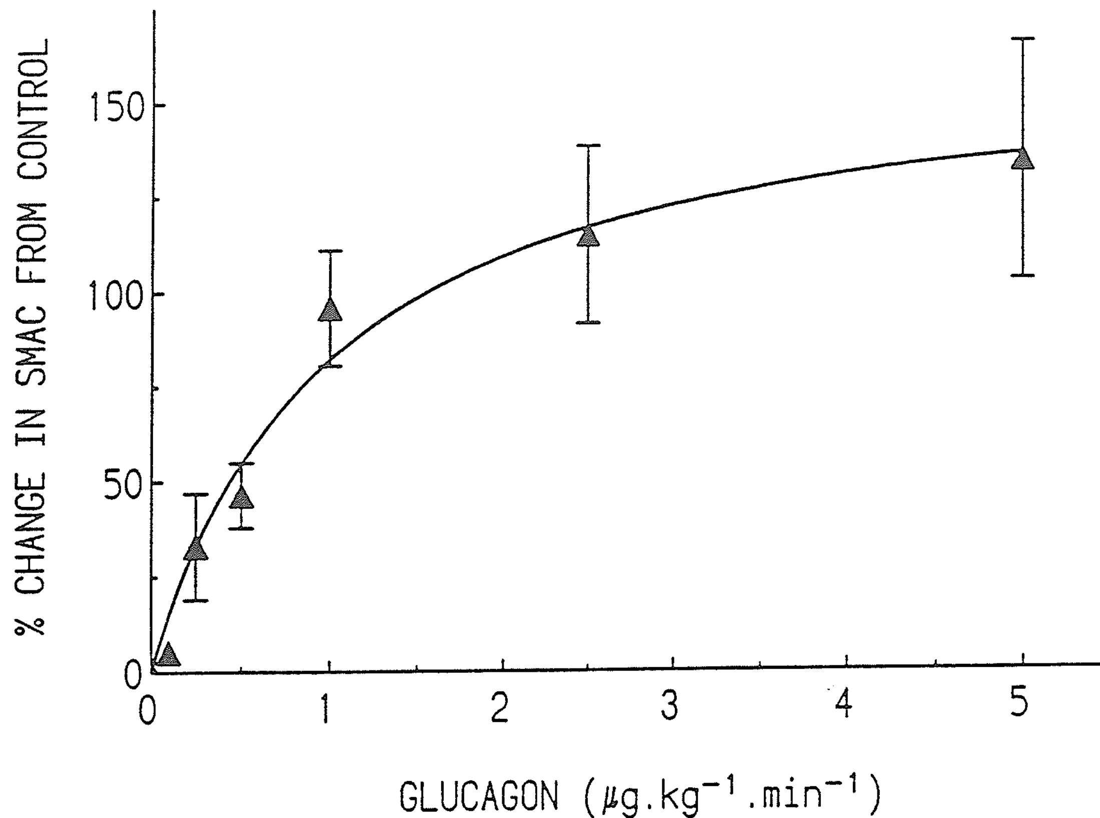


Figure 19: Intra-arterial dose-response curve for pooled glucagon responses in the superior mesenteric artery in terms of percent change in superior mesenteric arterial conductance (SMAC). Responses are reported as the mean $\pm$ SEM (n=5). The absolute basal conductance before glucagon infusion was 0.160 $\pm$ 0.022 ml/min/kg/mmHg. Glucagon was administered as a constant infusion and increased to the next dose in a stepwise manner. The Rmax and ED_{50} for glucagon, estimated by nonlinear regression from the pooled data, were 162.8 $\pm$ 13% and 0.98 $\pm$ 0.22 µg/kg/min, respectively. The Rmax and ED_{50} estimated from the individual dose-response curves from each experiment were 156.4 $\pm$ 34% and 0.86 $\pm$ 0.04 µg/kg/min and are in close agreement with the values from the pooled data.

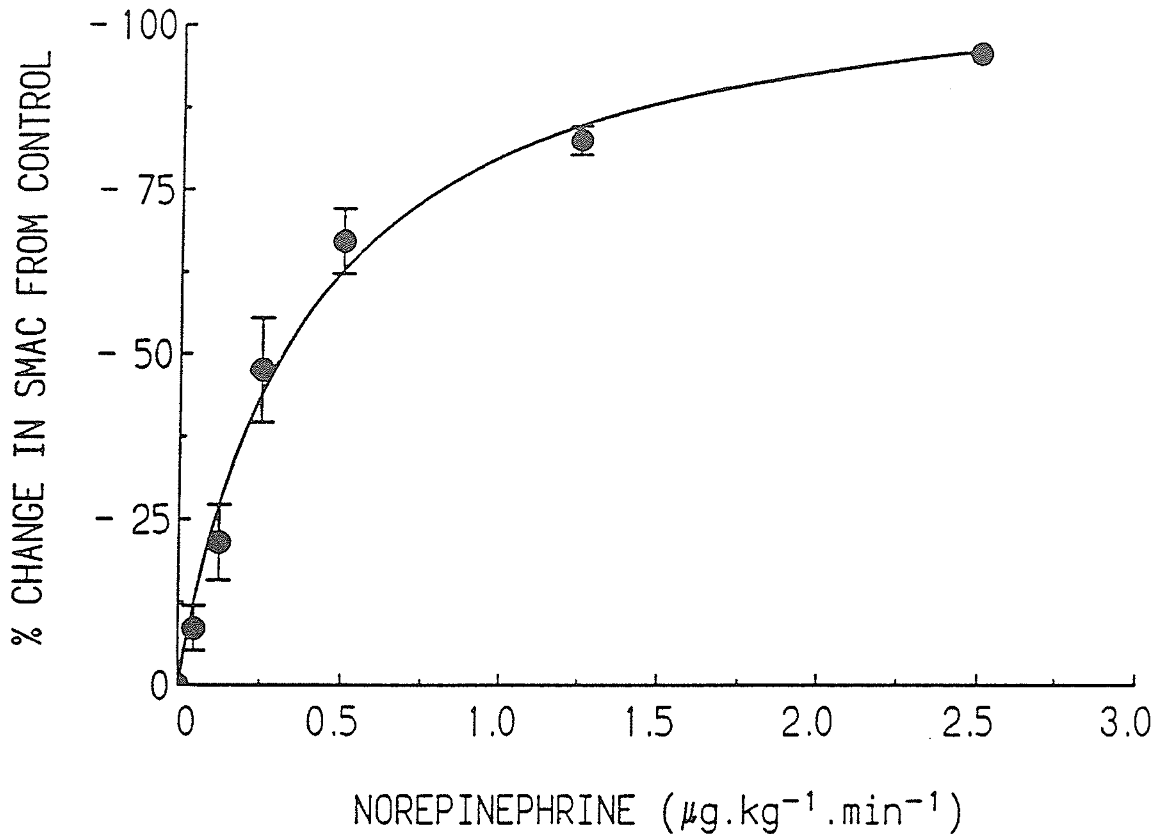


Figure 20: Intra-arterial dose-response curve for pooled norepinephrine (NE) responses in the superior mesenteric artery in terms of percent decrease in superior mesenteric arterial conductance (SMAC). Data points represent the mean $\pm$ SEM (n=7). The absolute basal conductance before NE infusion was 0.230 $\pm$ 0.039 ml/min/kg/mmHg. NE was administered as a constant infusion of a single dose for a 3 minute period (range 0.025-2.5 µg/kg/min) rather than increasing to the next dose in a stepwise manner. The Rmax and ED_{50} for NE, estimated by nonlinear regression from the pooled data, were -110.3 $\pm$ 6% and 0.38 $\pm$ 0.06% µg/kg/min respectively and were in close agreement with the estimates from the individual dose-response curves from each experiment.

high frequency nerve stimulations used were 2 Hz and 6 Hz.

Effect of Glucagon on Peak Vasoconstriction

Figures 21 and 22 depict the control and test responses in the SMA during glucagon infusions for the norepinephrine- and nerve-induced peak vasoconstrictions. Paired analysis of the control peak constrictor responses indicated that the high constrictor stimuli produced significantly larger constrictions compared to the low stimuli. Blocked ANOVA of the test responses showed that for the low norepinephrine constrictions (figure 21), the high dose of glucagon was able to significantly inhibit the peak constriction by 39.8 ± 11.8% ($P<0.01$). The low and mid doses of glucagon produced only a 10.9 ± 5.9% and 2.7 ± 12.6% insignificant inhibition of the peak responses, respectively. Glucagon had no statistically significant effect on the peak constriction produced by high norepinephrine; the high dose of glucagon produced only a 15.8 ± 5.6% inhibition of the high norepinephrine peak response.

Glucagon had similarly weak effects on the nerve-induced peak responses (Figure 22). The low nerve peak constrictor responses were significantly inhibited by the mid and high doses of glucagon; the mid dose of glucagon produced an 18.6 ± 8.8% inhibition ($P<0.05$) and the high dose produced a 34.4 ± 8.5% inhibition ($P<0.01$) of the peak response. The high nerve peak constrictor responses were not affected to any significant extent by any dose of glucagon as indicated by blocked ANOVA of the percent change in SMA conductance.

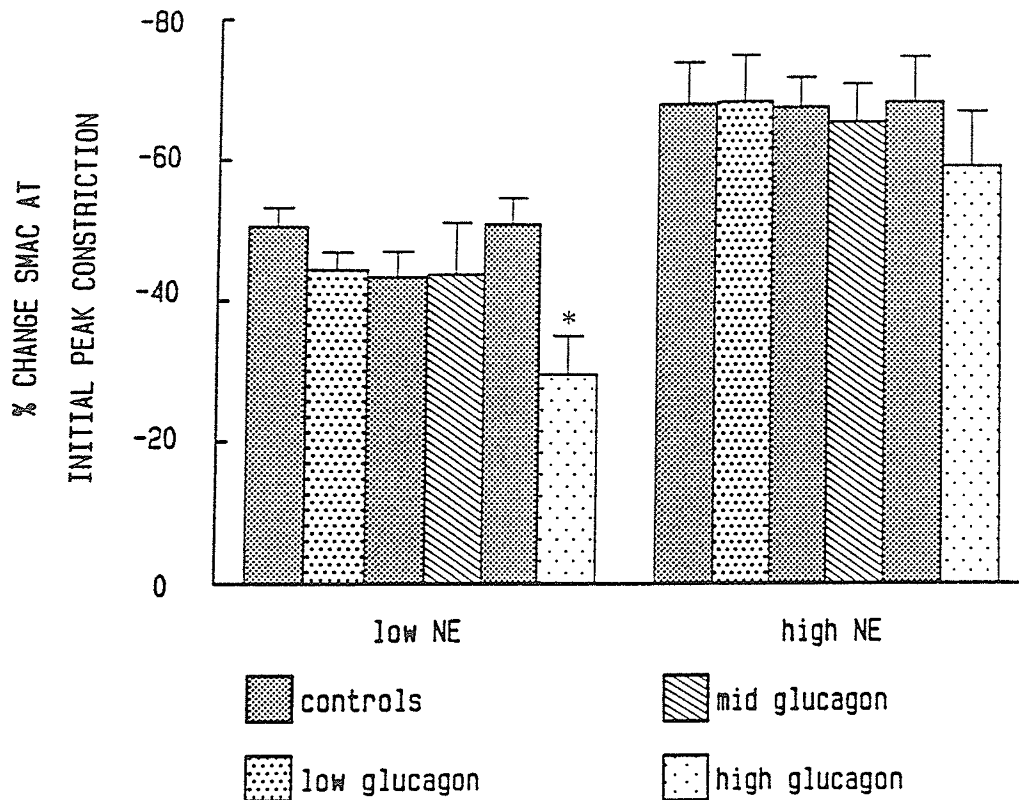


Figure 21: Effects of low (0.114 ± 0.024 μg/kg/min), mid (0.30 ± 0.06 μg/kg/min), and high (0.61 ± 0.11 μg/kg/min) doses of intra-arterial glucagon on the peak constrictions of the SMA induced by low (0.27 ± 0.04 μg/kg/min) and high (0.79 ± 0.16 μg/kg/min) doses of norepinephrine (NE). Responses are reported as the mean ± SEM (n=7) of the percent change in superior mesenteric arterial conductance (SMAC) from the pre-stimulation baseline. Responses are negative values indicating a decrease in conductance. Control bars represent the mean of the control responses before and after each test response. Blocked ANOVA of the control and test responses showed that only the high dose of glucagon significantly inhibited the vasoconstriction (39.8 ± 11.8%). Glucagon had no significant inhibitory effect on peak constrictions induced by high NE infusion.

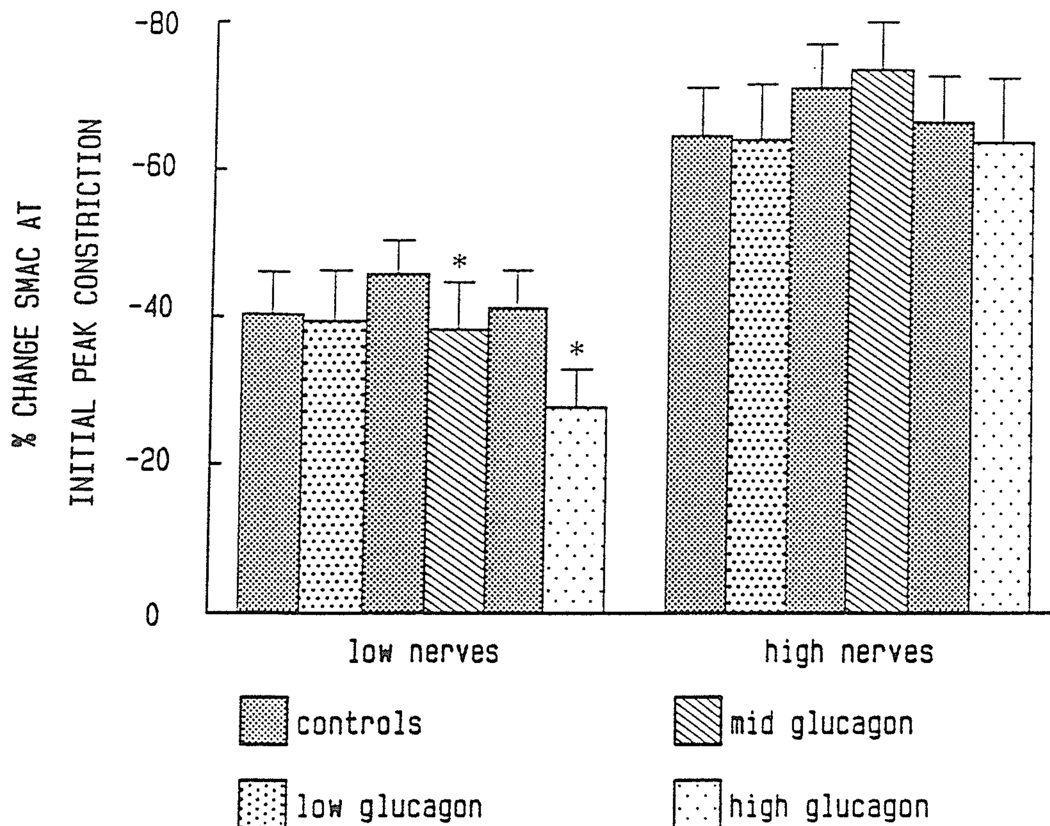


Figure 22: Effect of low (0.114 ± 0.024 µg/kg/min), mid (0.30 ± 0.06 µg/kg/min), and high (0.61 ± 0.11 µg/kg/min) doses of intra-arterial glucagon on the peak constriction of the SMA induced by direct nerve stimulation at low (2 Hz) and high (6 Hz) frequencies. Responses are reported as the mean ± SEM (n=7) of the percent changes in superior mesenteric arterial conductance (SMAC) from the pre-stimulation baselines. Responses are negative values indicating a decrease in conductance. Control bars are the mean of the control constrictions before and after the test responses. Blocked ANOVA of the control and test responses showed that the mid and high doses of glucagon significantly inhibited the peak constrictor responses to low nerve stimulation (18.6 ± 8.7% and 34.4 ± 8.5% inhibition, respectively). Glucagon was unable to significantly inhibit the peak constrictor responses induced by the high nerve stimulation.

DISCUSSION

In investigations and reviews of the pharmacological actions of endogenous vasoactive agents in the splanchnic vascular bed, it is still widely cited that glucagon has a significant inhibitory effect on sympathetic-induced constrictions in the hepatic artery (HA) and superior mesenteric artery (SMA). These findings, however, have not been reproduced in the HA of the cat, as reported in section VII.1. of this thesis. The present investigation was a comparative study in the mesenteric vascular bed to assess the status of glucagon as an inhibitory modulator of nerve- and norepinephrine-induced vasoconstrictions of the SMA in the cat. Pharmacodynamic estimates of the Rmax and ED_{50} for glucagon and norepinephrine in the SMA were also calculated, based on analysis of dose-response curves for these agents conducted during each experiment.

Dose-Response Curves

As expected, glucagon, infused directly into the SMA, produced a dilation of this vessel. Shoemaker et al. (1959) were the first to report the effects of glucagon on the splanchnic and hepatic vasculature, although only an increase in total hepatic flow was reported. Since then, many investigators have detailed this same observation (Kock et al., 1970a, 1970b; Tibblin et al, 1970). Glucagon has also been shown to dilate the hepatic artery in a dose-dependent manner upon intravenous, intra-arterial, and intraportal venous infusions (Richardson and Withrington, 1976b; Bashour et al., 1973 and sections VI and VII of this thesis).

SMA conductance, rather than the more commonly used resistance, was calculated

to express the vascular responses. Thus, an increase in conductance would imply that the vessel had dilated and a decrease in conductance indicates that a constriction occurred. As discussed earlier, by using conductance, classical pharmacodynamic analysis of the dose-response data could be carried out by nonlinear regression to estimate the Rmax and ED_{50} (Lautt, 1989). The present paper represents the first time pharmacodynamic estimates derived by nonlinear regression have been reported for glucagon and norepinephrine in the SMA in vivo. The Rmax (162%) and ED_{50} (0.98 µg/kg/min) estimated from the pooled glucagon data (Figure 18) were in close agreement with the mean of the estimates calculated from the individual experiments (Rmax, 158%; ED_{50}, 0.86 µg/kg/min). Tibblin et al., (1971) reported decreases in resistance of approximately 63% which when recalculated in terms of conductance from their available data (171%) was very similar to our estimate of Rmax. These investigators, however, administered bolus intravenous injections of glucagon (10 µg/kg) whereas our doses were constantly infused into the artery.

In the study reported in section VII, the HA had an Rmax of 91% and an ED_{50} of 0.113 µg/kg/min for glucagon. The pharmacodynamic data thus indicates that the HA is more sensitive to glucagon than the SMA, while glucagon is capable of producing greater dilations in the SMA. It has been observed previously that intravenously infused glucagon dilated the hepatic artery earlier and more rapidly than the SMA (see figure 14, section VI).

Effect of Glucagon on Vasoconstriction

Glucagon has been reported to inhibit or reduce norepinephrine, epinephrine, phenylephrine, direct nerve stimulation, and hemorrhage-induced vasoconstrictor responses

in the SMA of the dog (Kock et al., 1971; Tibblin et al., 1971). Inspection of the nerve stimulation data (Kock et al., 1971) indicates that glucagon administration (10 µg/kg) produced an upward shift in the blood flow baseline. However, the nerve-induced peak constrictor response for flow and blood pressure appear to be intact and of comparable size to the control response. The norepinephrine, epinephrine, phenylephrine and hemorrhage responses do appear to be inhibited, however extremely large doses of glucagon were used to produce these effects.

In the current study, it was found that only the mid and high doses of glucagon were able to antagonize the low frequency nerve stimulation and low dose norepinephrine-induced peak constrictor responses. This would suggest that glucagon is acting at a post-synaptic site. Figures 21 and 22 illustrate that the high dose of glucagon can only inhibit the low norepinephrine peak constrictor response by 39% and the mid and high dose could only inhibit the 2 Hz nerve responses by 18% and 35%. Peak constrictor responses induced by the high norepinephrine and high nerve frequency stimuli were not significantly affected by glucagon at any dose. The blood glucagon concentrations used to inhibit the constrictions in our investigation and the cited dog studies were well over basal (0.025-0.300 ng/ml) and pathophysiological levels (0.400-1.500 ng/ml) (Richardson and Withrington, 1981; Smitherman et al., 1978). Estimates of glucagon levels in the dog studies using their standard dose of 10 µg/kg were approximately 270 ng/ml. We increased the glucagon level by an estimated 20.5 and 82.3 ng/ml for the low and high doses of glucagon.

Recently, in the cat, hepatic arterial or intraportal infusions of glucagon were shown to mildly (but not significantly) reduce the maximal response (Rmax) of the liver capacitance

vessels (blood volume expulsion) to hepatic nerve stimulation (Lautt et al., 1991). The ED_{50} for the nerve-induced capacitance response was significantly increased from 3.5 Hz to 5.6 Hz. The weak suppression of the capacitance response was only accomplished using very large doses of glucagon which is consistent with the very large doses that were required to mildly inhibit the constrictor responses in this study.

Portal hypertension and cirrhosis, are known to be associated with elevated levels of glucagon. It has also been suggested that glucagon may play a major role in the intestinal hyperemia in these states (Benoit et al., 1986). Other investigators (Cerini et al., 1989), however, concluded that glucagon plays only a minor role in the hyperdynamic splanchnic circulation in rats with biliary cirrhosis. In our present study the lowest dose of glucagon produced an average dilation of only 34%, and had no effect on any aspect of the constrictor responses, despite producing very high levels of this peptide. Our results would suggest that glucagon is unlikely to have major vascular effects in the mesenteric vascular bed even in concentrations seen in pathological states.

SUMMARY

In summary, this is the first report of the use of nonlinear regression to obtain estimates of the Rmax and ED_{50} for glucagon and norepinephrine in the SMA in vivo. Glucagon was capable of dilating the mesenteric vascular bed of the cat but only at non-physiological doses of the peptide. Calculation of the Rmax and ED_{50} for glucagon in the SMA suggests that this peptide is capable of dilating the SMA to a greater extent than the hepatic artery, whereas the hepatic artery is more sensitive to glucagon. At high doses, glucagon was able to partially

inhibit the peak constrictor responses to low constrictor stimuli. It must be stressed, however, that the inhibitions of the constrictor responses were very mild and supraphysiological doses of glucagon were required to obtain such effects. These results are essentially in accord with the study in the HA (section VII.A.) and support the hypothesis that even at physiological or pathophysiological doses, glucagon is not an effective inhibitory modulator of constrictor responses to nerve stimulation or norepinephrine infusion in the mesenteric vascular bed of the cat. Although the results from this thesis and those from the earlier dog studies may represent species-specific differences, hopefully it will bring to light that a foregone conclusion may, in some cases, merely represent our ignorance.

Section VII.3. The Phenomenon of Vascular Escape: Quantification and Expression of Results

INTRODUCTION

In the hepatic and mesenteric vascular beds of the cat, maintained sympathetic stimulation (norepinephrine infusion or direct stimulation of vascular nerves) results in an initial peak vasoconstriction of the hepatic artery (HA) and superior mesenteric artery (SMA) which occurs within 1-1.5 minutes after commencement of stimulation. With continued stimulation, the blood flow response of these vessels (in an intact preparation) returns toward the baseline despite the maintained stimulus. This response eventually plateaus after approximately 3 minutes of stimulation and is known as vascular escape. Vascular escape, therefore, is that phenomenon whereby a tachyphylaxis occurs in the vasoconstriction of an arteriole to a constant sympathetic stimulation (Greenway, 1984a) and has been discussed in the general introduction of this thesis.

Traditionally, vascular tone and vascular responses have been expressed as resistance and percentage change in resistance. As noted in section VI of this thesis, conductance, which is the inverse of resistance, is a more appropriate means of expressing arterial vascular tone, and, in this laboratory, has become the index of choice for reporting the results of hemodynamic studies conducted in arterial vascular beds. The arguments for the use of conductance will be briefly reviewed. Resistance is calculated as perfusion pressure/blood flow and is nonlinearly related to blood flow. Conductance, is calculated as blood flow/perfusion pressure. Conductance, therefore, is directly and linearly related to blood

flow. In recent years, it has been suggested that vascular tone, calculated as resistance, can often distort the results of vascular and hemodynamic studies (Lautt, 1989). This has been ascribed to the nonlinear relationship between resistance and blood flow in which even simple mathematical manipulation of the data (such as calculation of arithmetic means) can be rendered inaccurate. Because of the linear relationship between conductance and blood flow, such artifacts do not occur, thereby allowing mathematical manipulation of the data without incurring gross distortion of the data (Lautt, 1989). O'Leary (1991) has also reported that the use of conductance, not resistance, is a better index to determine the relative importance of responses in blood pressure regulation.

Vascular escape is calculated as an "escape index", which in turn has been calculated using an index of vascular tone, traditionally resistance. In light of the previous discussion, it is possible that the use of resistance may be introducing mathematical artifacts into the escape index calculation. Thus, the question arises as to what is the best index with which to calculate vascular escape. In an intact preparation, after reaching its lowest level at the peak constriction, blood flow rapidly returns toward the pre-stimulation levels. Blood pressure may also rise as the response progresses from peak constriction to the escape phase. Thus, while vascular escape is primarily a blood flow event in the intact preparation, expressing escape in terms of blood flow, without consideration of the changes in blood pressure, however, would not accurately represent the changes in vascular tone (determined by the moment to moment state of blood flow and blood pressure in the vessel) which ultimately describes the responses of the arterioles undergoing vascular escape.

In this investigation, the issue of which index of vascular tone, resistance or

conductance, is the most appropriate to calculate vascular escape is addressed. Based on the linear relationship between blood flow and conductance and the fact that in an intact preparation vascular escape is primarily a blood flow event, the hypothesis of this investigation is that conductance, rather than resistance, will be a superior means of expressing vascular escape. To this end, hypothetical (ideal) data were used to predict which index of vascular tone would be most appropriate to describe vascular escape. These data were then compared to actual experimental escape responses obtained in the mesenteric vascular bed of the cat to determine if the hypothetical relationships between blood pressure and blood flow are consistent with the biological relationships. This investigation will prove that conductance is indeed the most appropriate index of vascular tone with which to report vascular escape. In doing so, the results from this study also validate the methods which will be used to assess the effect of glucagon on vascular escape in the forthcoming sections.

MATERIALS AND METHODS

Cats of either sex (n=7) were fasted overnight and anesthetized with pentobarbital-sodium (32.5 mg/kg) by intraperitoneal injection. Anesthesia was maintained throughout the experiment by supplemental doses (6.5 mg/kg) of the anesthetic through a brachial vein cannula. Body temperature was maintained at 37.5°C by means of a rectal probe and a thermal control unit which regulated heating rods in the surgical table.

Arterial blood pressure was monitored from a catheter in the right femoral artery. Central venous pressure was monitored from a cannula inserted, via the femoral vein, into the inferior vena cava. A tracheal cannula was inserted to maintain a patent airway. After

an abdominal incision from the xiphoid process to the umbilicus was made, the inferior mesenteric artery was ligated. A small branch of the superior mesenteric artery (SMA), usually the first branch leading to the cecal region, was cannulated toward the SMA to allow direct intra-arterial infusions of drugs into the mesenteric vascular bed. A short length of the SMA, just proximal to the cecal artery cannulation, was exposed and the SMA nerve plexus was gently separated from this vessel. The nerve bundle was ligated, transected, and the peripheral end was placed in a snug fitting circular bipolar stimulating electrode. An electromagnetic flowprobe (EMPCO, Carolina Medical Electronics, size 8) was placed on the cleared segment of the SMA. A cannula was inserted in a small cecal vein and advanced into the portal vein to measure venous pressure.

After surgery was completed the cat was allowed at least 30 minutes to stabilize before any procedures were carried out. At the end of each experiment the flowprobe was calibrated in situ according to the procedure outlined in section VI. Norepinephrine was prepared from a stock solution and diluted with Ringer's solution (infusion volume range, 0.0068 to 0.68 ml/min). Infusions were carried out using a Harvard Apparatus constant infusion pump. All pressures were monitored using Statham pressure transducers. Pressures and flows were recorded on a Beckman dynograph recorder.

PROTOCOLS

A low and high dose of norepinephrine was selected from an intra-arterial norepinephrine dose-response curve obtained at the beginning of each experiment. In keeping with the other investigations reported in this thesis, the Hz_{50} (2 Hz frequency, 1 ms

duration, 15 V square wave) and the Hz_{75} (6 Hz frequency) nerve frequencies were used to stimulate the SMA nerve plexus. Each constrictor stimulus was repeated 4 times per cat (28 responses in total per stimulus group) and was applied for 3 minutes. The nerve and norepinephrine responses were tested in random order.

Calculations

Vascular responses to the constrictor stimuli were calculated in terms of conductance and resistance. SMA conductance = SMA blood flow/(systemic arterial pressure - portal venous pressure), SMA resistance = (systemic arterial pressure-portal venous pressure)/SMA blood flow. Peak vasoconstriction was calculated as the percentage change in SMA conductance, resistance and blood flow from the pre-stimulation level to the initial peak of vasoconstriction (within 1 minute). Vasoconstriction reaches a peak approximately 1 minute after the stimulus is applied. After this time the phenomenon of vascular escape occurs in which the blood flow returns toward the baseline and reaches a plateau level by about 3 minutes. The escape index is calculated as: (plateau escape conductance - peak response conductance)/(control conductance - peak response conductance) x 100%. Escape was calculated in resistance as: (peak response resistance - plateau response resistance)/(peak response resistance - control response resistance) x 100%. Plateau escape conductance was measured after 3 minutes of stimulation. Weak constrictor responses tended to make accurate measurements of vascular escape very difficult. Thus, to avoid the enormous errors that may have been incorporated into our calculations, we established an inclusion criterion that escapes would be quantitated only if the original vasoconstriction was greater than 15%.

Experimental and hypothetical data were analyzed by linear and nonlinear regression analysis (GraphPAD INPLOT computer program, ISI Software).

Mean and standard error of the mean are reported throughout. Comparisons are made by paired analysis and blocked ANOVA with multiple comparisons by Duncan's test.

RESULTS

The mean control femoral arterial blood pressure was 141.8 $\pm$ 8.4 mmHg; portal venous and central venous pressures were 7.6 $\pm$ 0.6 and 4.6 $\pm$ 0.4 mmHg, respectively; SMA flow was 21.0 $\pm$ 3.5 ml/min/kg body weight and SMA conductance was 0.15 $\pm$ 0.02 ml/min/kg/mmHg. The mean values for the low and high norepinephrine doses selected from the dose-response curves were 0.27 $\pm$ 0.04 and 0.79 $\pm$ 0.16 μg/kg/min. Sample size for the nerve stimulations was the full compliment of 28 responses for each stimulus. Some of the norepinephrine responses were unusable due to technical problems with the response. These problems were unstable or highly fluctuating flow responses during the norepinephrine infusion, and peak constrictions of less than 15% change in vascular tone. The sample size for the low and high norepinephrine infusion groups was reduced to 19 responses.

Peak Vasoconstrictions

Peak vasoconstriction was calculated in terms of flow, conductance, and resistance (table 3). Paired analysis of the percentage change during the peak constrictor responses indicated that, for all three methods of describing vasoconstriction, the high constrictor stimuli produced significantly larger constrictions compared to the low stimuli for both the

TABLE 3

Percent change in flow, conductance, and resistance at peak vasoconstriction in the superior mesenteric artery

Stimulus	% Change at peak vasoconstriction			
	n	Flow	Conduct	Resist
Low NE (0.27 ± 0.04)	19	-44.7±2.2	-48.1±2.2	100±10
High NE (0.79 ± 0.16)	19	-63.9±3.5*	-68.4±3.9*	360±77*
Low Nerve Stim (2 Hz)	28	-39.9±2.6	-42.9±2.6	85.9±8.8
High Nerve Stim (6 Hz)	28	-64.5±3.3*	-67.9±3.1*	302±38*

Abbreviations: Conduct, conductance (ml/min/kg/min); Resist, resistance (mmHg/ml/min/kg); NE, norepinephrine (NE). Values are the mean ± SEM; n=number of responses from 7 cats. Units of flow, ml/min/kg. Norepinephrine was infused directly into the superior mesenteric artery. Statistics between the high and low constrictor stimuli responses were by paired comparisons, *$P<0.05$.

nerves and norepinephrine. In addition, table 3 illustrates that the changes in blood flow at peak constriction were paralleled by the changes in conductance whereas the changes in resistance were greater than the changes in flow.

Regression analysis of vascular escape data

a) Hypothetical data

In this first step of analysis, SMA flow-escape responses were plotted against the escape responses from the norepinephrine and nerve stimulations calculated as conductance and resistance. It follows that the index which accurately represents SMA blood flow-escape should: a) be linearly related to the flow-escape responses, b) have a regression line with a slope approaching 1.0 and c) this line should pass through the origin of the graph without being forced.

These criteria were tested using hypothetical (ideal) data (table 4, figure 23) where blood flow decreased to the same level at the peak constriction (5 ml/min) but rose to different levels during the escape phase thereby producing different degrees of escape. To properly reproduce the hemodynamic responses occurring during sympathetic stimulation, arterial pressure was raised to a similar extent as to that occurring under experimental conditions (20%). According to these data, the conductance-flow escape relationship is linear, has a slope of 0.8 (as determined by linear regression) and passes through the origin. The slope of 0.8, as opposed to 1.0 (a perfect correlation), can be accounted for by the 20% increase in arterial pressure (table 4). As a result, conductance (calculated as blood flow/perfusion pressure) will be affected by the simultaneous changes in flow and pressure

TABLE 4

Hypothetical data predicting resistance-flow escape and conductance-flow escape relationships

Basal Parameters		
SAP (mmHg)	PVP (mmHg)	Flow (ml/min/kg)
100	5	30

Parameters During Sympathetic Stimulation						
Blood Pressure (mmHg)		Blood Flow (ml/min/kg)		% Escape		
SAP	PVP	Peak	Escape	Flow	Conduct	Resist
120	5	5	5	0	0	0
120	5	5	11.25	25	20	64
120	5	5	17.5	50	40	85
120	5	5	30	100	80	96

Abbreviations: SAP, superior mesenteric arterial pressure; PVP, portal venous pressure; Conduct, conductance; Resist, resistance; Peak, blood flow at peak constriction; % Escape, escape indexes calculated for flow, conductance and resistance.
Calculations: Conductance = Blood Flow/(SAP-PVP), Resistance = (SAP-PVP)/Blood Flow, % Escape: %Flow Escape = (escape flow - peak flow)/(control flow - peak flow) x 100, %Cond Escape = (escape cond - peak cond)/(control cond - peak cond) x 100, %Resist Escape = (peak resist - escape resist)/(peak resist - control resist) x 100

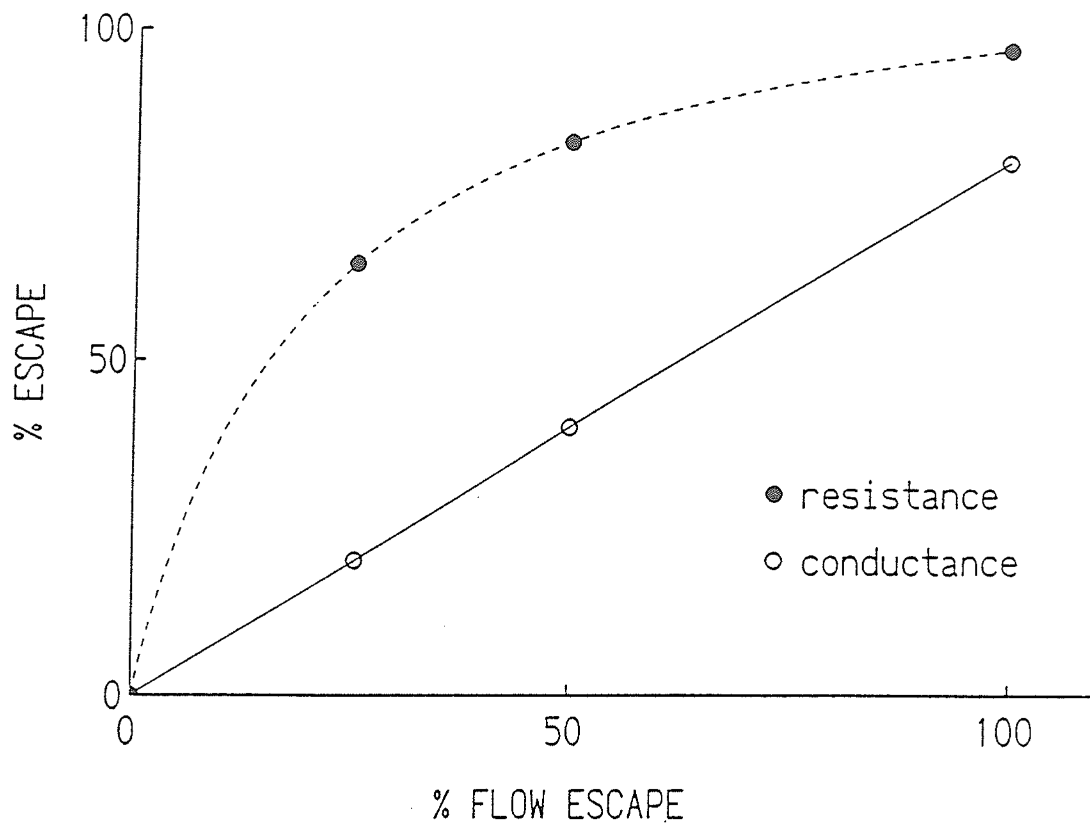


Figure 23: Graphic representation of the hypothetical (ideal) data from table 4. The ordinate represents the percent escape calculated in conductance and resistance (see methods for equations), and the abscissa represents the percent flow-escape. These results clearly illustrate that the conductance-flow escape relationship is linear (○ open circles) and the resistance-flow escape relationship is nonlinear (● closed circles).

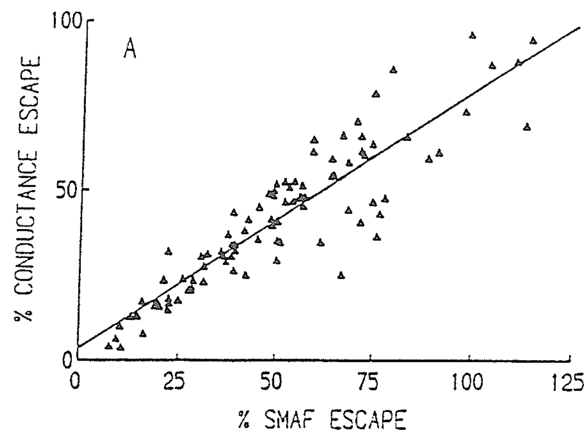
and slightly underestimate, but consistently represent the flow-escapes when arterial pressure rises.

The hypothetical data predicted that the resistance-flow escape relationship would be nonlinear (figure 23). Whereas conductance consistently estimates the flow-escape, resistance inconsistently estimates flow-escape as well as grossly overestimates these escapes over the majority of the curve (table 4). The hypothetical data therefore suggest that conductance would be a better index of escape than resistance.

b) Experimental data

Escape responses from the norepinephrine and nerve constrictions were not significantly different (blocked ANOVA). Therefore, these responses were pooled and the experimental conductance-flow escape and resistance-flow escape data were submitted to linear regression (figure 24). The conductance-flow data, as predicted, produced a linear relationship (figure 24a). This regression line had a slope of 0.75 ± 0.04 ($r^2=0.84$) and x- and y-intercepts of -4.7% (x when y=0) and $3.5 \pm 2.1\%$ (y when x=0) respectively. Although these intercepts are significantly different from the origin, the regression line passes extremely close to this point. When linear regression is conducted on the same data but forcing the regression line through the origin, the slope of the line becomes 0.81 ± 0.04 and is almost indistinguishable from the unforced regression line.

The linear regression line for the resistance-flow escape data had a slope of 0.69 ± 0.05 (figure 24b). Although the slope of the regression line is not greatly different from the conductance-flow line, the fit of the curve to the data ($r^2=0.65$) is poorer than the



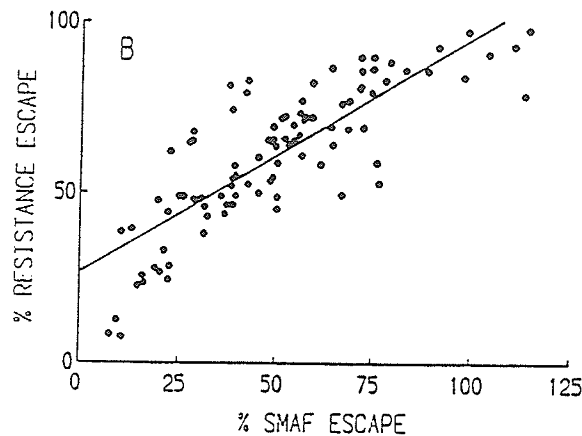


Figure 24: Comparison of linear regressions between superior mesenteric arterial blood flow (SMAF) vascular escape and escape responses calculated using conductance (figure 24A) or resistance (figure 24B). Escape responses for the norepinephrine and nerve stimuli were not significantly different, therefore the results were pooled (n=95). According to this analysis, that index which best expresses vascular escape should be linear with flow-escapes, have a slope approaching 1.0 and pass through the origin without being forced. The conductance-flow escape data (figure 2A) has a slope of 0.75 ± 0.04 (r^2= 0.84) and x and y intercepts of -4.7% (when y=0) and 3.5 ± 2.1% (when x=0). The resistance-flow data (figure 24B) has a slope of 0.69 ± 0.05 (r^2=0.65) and x and y intercepts of -36.9% (when y=0) and 25.5 ± 2.9% (when x=0).

conductance-flow data and the intercepts (x=-36.9% when y=0; y=25.5 $\pm$ 2.9% when x=0) are a considerable distance from the origin (figure 24b). This was not unexpected since the hypothetical data suggested that this relationship would be curvi-linear. Thus, we re-analyzed the same resistance-flow escape data using nonlinear regression (figure 25). This curve describes the data more appropriately than the linear regression analysis; when flow-escape is zero, resistance-escape is also zero rather than 25.5% (y-intercept). Additionally, the r^2 value increased to 0.73. Figure 25 also illustrates that the experimental resistance-escapes overestimate flow-escapes as predicted from the hypothetical data. Accordingly, to see if resistance introduced any significant error into our results, vascular escape in terms of blood flow was compared to escapes calculated using conductance and resistance. The mean values for the percentage escape in SMA blood flow, conductance and resistance from low and high norepinephrine and nerve stimulations are detailed in table 5. As predicted, conductance-escapes slightly underestimated flow-escapes and, like the flow responses, conductance escapes were not dose- or frequency-dependent. Conversely, resistance-escapes (in most cases) overestimated the degree of flow-escape. More importantly, however, resistance-escapes for the high frequency nerve stimulations (65.1 $\pm$ 3.0%) were significantly larger than the escape responses from the low frequency stimulations (56.0 $\pm$ 3.7%, paired analysis, $p<0.01$), suggesting frequency-dependent escape responses.

Changes in Perfusion Pressure

The effects of sympathetic nerve stimulation and norepinephrine infusions on arterial pressure, portal venous pressure and perfusion pressure in the mesenteric arterial bed are

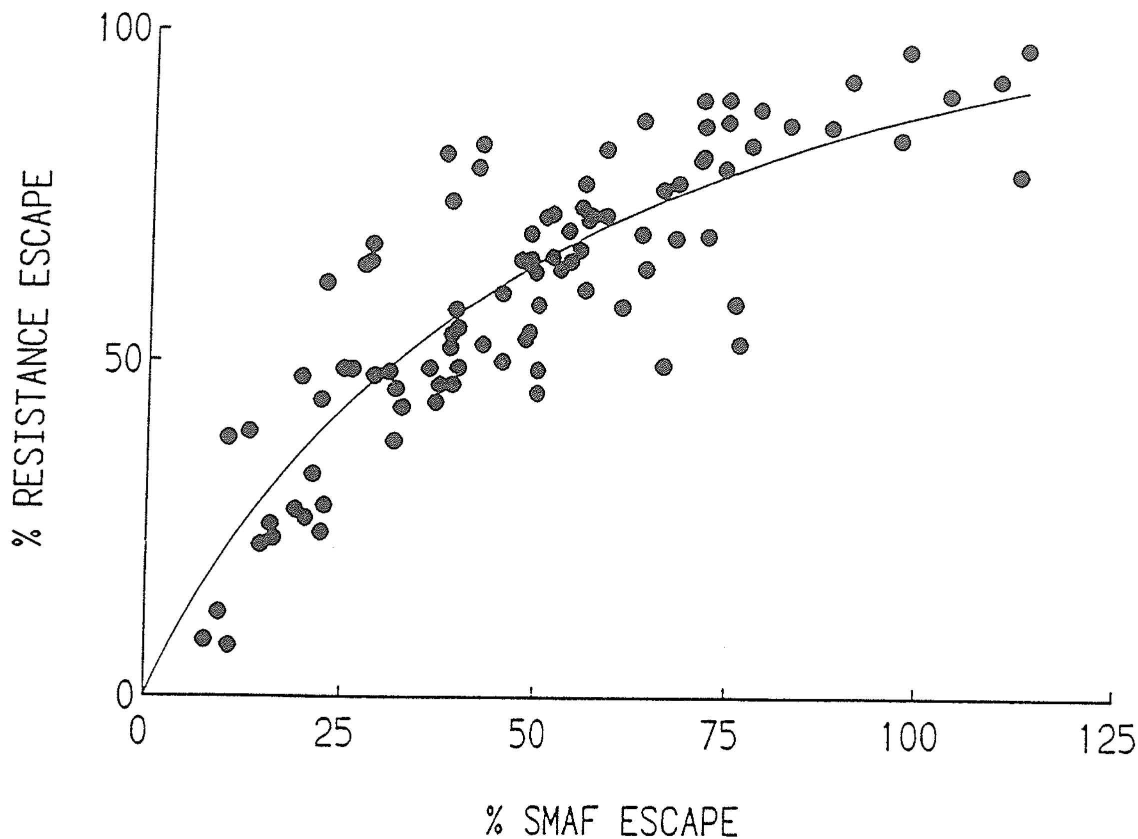


Figure 25: Nonlinear regression of pooled SMA flow-escape and resistance-escape responses from norepinephrine and nerve stimulation. As indicated by the predicted data (table 3 and figure 23), the resistance-flow escape data were best described as being nonlinear. These data were, therefore, submitted to nonlinear regression. This curve fits the data with an r^2 of 0.73 compared to 0.65 when analyzed with linear regression. Despite the better fit, this curve is not linear and will not consistently reflect the escape in blood flow and therefore would not be an appropriate index of vascular escape.

TABLE 5

Vascular escape calculated in terms of flow,
conductance and resistance

Stimulus	% Vascular escape			
	n	Flow	Conduct	Resist
Low NE (0.27 ± 0.04)	19	60.2±27.6	46.3±6.1	58.3±6.1
High NE (0.79 ± 0.16)	19	54.1±5.0	39.7±3.7	65.1±4.8
Low Nerve Stim (2 Hz)	28	48.2±3.8	43.5±3.5	56.0±3.7
High Nerve Stim (6 Hz)	28	42.9±4.3	37.9±4.0	65.1±3.0*

Abbreviations: Conduct, conductance (ml/min/kg/min); Resist, resistance (mmHg/ml/min/kg); NE, norepinephrine. Values are the mean ± SEM; n=number of responses from 7 cats. Norepinephrine was infused directly into the superior mesenteric artery. Statistics between low and high constrictor stimuli responses was by paired comparisons, *$P<0.05$.

TABLE 6

Average arterial, portal venous, and perfusion pressure in the superior mesenteric artery before and during the escape phase from constrictor responses induced by norepinephrine and nerve stimulation

Stimulus	Response	FAP	PVP	PP
Low NE (0.27±0.04)	Control	112.6±3.7	9.0±0.3	103.6±3.5
	Escape	126.4±4.9*	10.6±0.4*	115.8±4.7*
High NE (0.79±0.16)	Control	114.4±3.4	9.2±0.2	105.2±3.3
	Escape	141.7±5.6*	11.9±0.6*	129.8±5.2*
Low Nerve (2 Hz)	Control	113.0±2.9	9.2±0.2	103.8±2.8
	Escape	118.5±3.0*	9.2±0.2	109.3±3.0*
High Nerve (6 Hz)	Control	110.9±2.3	8.9±0.1	101.9±2.2
	Escape	122.1±2.8*	9.0±0.2	113.1±2.8*

Abbreviations: FAP, femoral arterial blood pressure; PVP, portal venous blood pressure; PP, perfusion pressure (FAP-PVP); NE, norepinephrine (µg/kg/min), infused into the superior mesenteric artery. Values represent the mean ± SEM from 7 cats. Statistics between the control and escape responses for each stimulus were by paired comparisons, *$P < 0.001$.

noted in table 6. Arterial pressure and perfusion pressures (FAP-PVP) during the escape phase of the constrictor responses were significantly elevated from the pre-stimulation pressures for all stimulation groups. Portal venous blood pressures were slightly, but significantly elevated during the escape phase of the norepinephrine infusion data. Portal pressures were not significantly affected by nerve stimulation. The nerve and norepinephrine stimulations produced significant dose- and frequency-dependent increases in perfusion pressure. Norepinephrine responses tended to produce larger increases in perfusion pressure compared to the nerve responses, however only the high norepinephrine perfusion pressure (129.8 $\pm$ 5.2) was significantly larger than that during the low and high nerve stimulations. Perfusion pressures produced by norepinephrine were also more variable (range, -1.15 to 55.0% increase in perfusion pressure) than the nerve responses. The mean of the percentage change in perfusion pressure for the pooled nerve and norepinephrine responses was 12.1 $\pm$ 1.3 % (n=94).

DISCUSSION

Vascular escape has been an enigmatic phenomenon. The mechanisms of this response are yet to be clarified despite some new and interesting hypotheses recently presented. The object of this aspect of the investigation into vascular escape was not related to the mechanism, but rather the quantification and validation of the techniques required to study the response. A full discussion of vascular escape will follow in section VII.D., when the effect of glucagon on this phenomenon is reported.

Conductance vs. Resistance for Calculating Escape Responses

It should be noted that although conductance- and resistance-escapes were compared to flow-escapes, flow-escapes, per se, are not an accurate index by which to describe vascular escape due to changes occurring in blood pressure. Expressing escape in terms of blood flow does not indicate the changes occurring in vascular tone, which is the index in which we are interested. However, because blood flow (not blood pressure) is the parameter which undergoes the largest changes during escape in the intact preparation, vascular tone should represent these changes in blood flow. Flow-escapes are merely an indication of the vascular response which is best described by an index of vascular tone. The aim of this analysis was, therefore, to determine which index, resistance or conductance, is the most appropriate to use when assessing changes in vascular tone during escape responses.

Using theoretical examples, Lautt (1989) recently argued that conductance, rather than resistance is a more appropriate calculation to use to express vascular tone when the primary changing factor is blood flow, as in vascular escape. This is because conductance is linearly related to blood flow, the variable parameter in the flow-pressure relationship. Resistance is nonlinearly related to flow, but is linearly related to pressure, the relative invariant in the flow-pressure relationship. It should follow that the index of vascular tone used to describe vascular escape should be directly and linearly related to that factor undergoing the greatest changes. The implication of this is that using conductance to calculate vascular escape should consistently represent the degree to which blood flow-escapes and vascular tone changes, regardless of the absolute changes in flow occurring at the initial constriction and during escape. When using resistance, however, vascular escape can be seriously

misrepresented depending on the changes in flow at peak constriction and during the escape phase. The most obvious demonstration of this distortion is seen if the initial constriction causes flow to approach zero. In this case, resistance, will approach infinity. As a result, any calculation incorporating this resistance value, such as vascular escape, will become unrealistic and skew the calculated escape value.

Using a similar theoretical argument, O'Leary (1991) modeled how a change in regional vascular resistance or conductance affected mean arterial pressure when a vascular bed receives a varying percentage of cardiac output. O'Leary found that the effect of a change in resistance on blood pressure is highly dependent on the baseline resistance, whereas the effect of a change in conductance on blood pressure is independent of the baseline conductance. Consequently, if one reports the effects of a given change in resistance on blood pressure, the results will be as variable as the baseline blood flow (cardiac output) and calculated resistance. If one uses conductance, the variability in the results due to differences in baseline blood flow and vascular tone is avoided.

In the present investigation it was tested to see if the theoretical arguments could be verified using hypothetical and experimental data. In short, the experimental results fully supported the theoretical predictions. The hypothetical data demonstrated that conductance- and flow-escapes were linearly related, resistance- and flow-escapes were non-linearly related (figure 23, table 4) and that conductance appeared to be a better index to describe vascular escape. In respect to the three criteria necessary to be a good index of escape, conductance-escapes from the experimental data were linearly related to flow-escapes; this relationship had a slope approaching 1.0 (0.75) and the regression line passed very close to the origin of

the graph. This last criteria is necessary since at zero flow-escape, there cannot be any vascular escape whether calculated in conductance or resistance. The linear regression line of the resistance-flow escape data had x and y intercepts which were quite distant from the origin (x=-36.9% when y=0; y=25.5% when x=0) and therefore did not meet the final criteria. Nonlinear regression of the resistance-flow escape responses forced the curve through the origin and provided a better fit to the data.

Conductance-escapes indexes were consistently less than flow-escapes indexes whether using the hypothetical data or the experimental data. This can be attributed to the fact that perfusion pressure increases during sympathetic stimulation. Because conductance is calculated as blood flow/perfusion pressure, any changes in perfusion pressure will cause a conductance-flow regression line to deviate from a perfect correlation (or slope) of 1.0, depending on the degree and direction of change in pressure. In the hypothetical data, perfusion pressure was raised consistently by 20% thereby producing a slope of 0.80 rather than 1.0. The conductance-flow escape regression line for the experimental data had a slope of 0.75 despite highly variable elevations in blood pressure during the constriction. It should also be noted that in the experimental situation blood flow and blood pressure were not controlled to any extent whereas the hypothetical data was kept within specific limits. Despite this, the experimental data completely supported the results obtained by the hypothetical data. The artifact induced by using resistance to estimate vascular tone may account for discrepancies in the literature related to vascular escape. Earlier investigations (Folkow et al., 1964a) suggested that the extent of vascular escape (calculated using resistance) may be dependent on the degree of initial constriction. In this study, escapes

calculated in resistance (table 5) also suggested that a frequency-dependent relationship exists for the nerve data. Conductance escapes, however, paralleled the flow-escapes and neither flow- nor conductance-escapes showed dose or frequency-dependence, despite dose and frequency-dependent initial peak constrictions. These findings are also consistent with previous results from our laboratory (Lautt et al., 1988b).

Practical Considerations

The practical consequence of these results is that conductance-escapes will consistently and accurately describe vascular escape even in the non-ideal situation where arterial pressure undergoes changes in response to local vasoconstriction. Because it is not linearly related to flow-escape, resistance-escape will not consistently represent vascular escape. In practice, the use of conductance will eliminate the error introduced by resistance when blood flow approaches zero flow and calculated resistance increases to infinity. This is especially important when assessing the action of pharmacological agents on vascular escape and its possible mechanism(s). For example, changes in the blood flow response during the peak constriction as a result of a drug can have enormous effects on calculated resistance. This, in turn, can have drastic effects on calculated vascular escape, despite the fact that the blood flow may have escaped to the same extent as in the control response. If conductance had been utilized, the error introduced by resistance is circumvented and the erroneous conclusion that this drug affected vascular escape would have been avoided.

SUMMARY

Although vascular escape is primarily a blood flow event, calculating the escape index in terms of flow does not truly represent the changes in vascular tone which the arterioles have undergone. The aim of this investigation was to determine which index of vascular tone, conductance or resistance would be the most appropriate to express vascular escape. Because of the linear relationship between conductance and blood flow and the exponential (nonlinear) relationship between resistance and blood flow in vascular responses, conductance proved to be the most appropriate index of vascular tone to express vascular escape. It is possible that the exponential relationship between resistance and blood flow may account for some of the discrepancies in the literature regarding vascular escape. Indeed, it was demonstrated that vascular escape indexes calculated in terms of resistance grossly misrepresented blood flow-escape indexes, and, according to statistical analysis, would have led to erroneous conclusions about vascular escape. Thus, not only does this investigation confirm that conductance is the most suitable index of vascular tone with which to express vascular escape, it also validates the methodologies used to assess the action of pharmacological agents on vascular escape and the possible mechanisms controlling this phenomenon.

Section VII.4. Modulatory Effect of Glucagon on Vascular Escape in the Hepatic and Mesenteric Vascular Beds of the Cat

INTRODUCTION

In sections VII.1. and VII.2., the results of studies investigating the effects of exogenous glucagon on the nerve- and norepinephrine-induced peak vasoconstrictor responses in the hepatic artery (HA) and superior mesenteric artery (SMA) were reported. Section VII.3. demonstrated and discussed the advantages and necessity of using conductance rather than resistance for expressing vascular escape. Section VII.4. will now detail the results of the pharmacological action of glucagon on vascular escape in the hepatic artery (HA) and superior mesenteric artery (SMA), using conductance as the index of vascular tone to calculate the escape index.

The results to be presented in this section were originally obtained as a part of the investigations reported in sections VII.1. and VII.2., but are reported separately for the sake of organization.

METHODS AND MATERIALS

The surgical protocol for the HA series and SMA series have already been detailed and can be found in sections VII and VIII, respectively. Low and high constrictor stimuli for the HA series were 0.30 ± 0.04 and 0.80 ± 0.16 µg/kg/min infusions of norepinephrine into the HA via the gastroduodenal artery and 2.2 and 6 Hz frequency stimulations of the hepatic arterial anterior nerve plexus. Norepinephrine doses in the SMA series were 0.27

$\pm$ 0.04 and 0.79 $\pm$ 0.16 μg/kg/min and were infused directly into the SMA via a small branch off the main trunk of the SMA. Stimulation frequencies were 2 and 6 Hz and were applied directly to the superior mesenteric nerve plexus. Each stimulus was applied for 3 minutes to obtain a peak constriction and vascular escape response.

The three doses of glucagon selected from the glucagon dose-response curves conducted in the HA series to antagonize the constrictor and escape responses were 0.047 $\pm$ 0.014 (low), 0.13 $\pm$ 0.04 (mid) and 0.31 $\pm$ 0.07 μg/kg/min (high) and were infused into the portal vein. The glucagon doses used in the SMA study were 0.11 $\pm$ 0.02, 0.30 $\pm$ 0.06 and 0.61 $\pm$ 0.11 μg/kg/min and were infused into the SMA directly.

PROTOCOLS

The nerve and norepinephrine responses were tested in random order. A complete test procedure consisted of: control responses for all 4 constrictor stimuli; repetition of constrictor stimuli in the presence of a low dose of glucagon; second control responses for all constrictor stimuli; repetition of constrictor stimuli in the presence of a mid dose of glucagon; third control responses for all constrictor stimuli; repetition of all constrictor stimuli in the presence of a high dose of glucagon; final control responses for all constrictor stimuli.

Calculations

HA conductance was calculated as follows: HA conductance = HA blood flow/ (HA blood pressure minus portal venous blood pressure).

Vasoconstriction reaches a peak approximately one minute after the stimulus is applied. After this time the phenomenon of vascular escape occurs in which the response returns towards the baseline and reaches a plateau level by about 3 minutes. Vascular escape is calculated as an escape index: (plateau escape conductance minus peak response conductance)/(control conductance minus peak response conductance) x 100%. Peak vasoconstriction was calculated at the initial peak of constriction (within one minute), not necessarily the lowest point of the response. Plateau escape conductance was measured after 3 minutes of stimulation. In addition to percent escape, the percent inhibition of escape caused by glucagon was also calculated: (control response escape minus test response escape)/(control response escape)x 100. Weak vasoconstrictor responses tended to make accurate measurements of vascular escape very difficult. Thus, to avoid the enormous errors that may have been incorporated into our calculations, an inclusion criteria was established such that escapes would only be quantitated if the original vasoconstriction was greater than 15%.

Glucagon (E. Lily & Co.) was dissolved in the injectable solvent containing 1.6% glycerine and 0.2% phenol which was then diluted in Ringer solution. A stock solution of norepinephrine was diluted to the appropriate concentration for infusion with normal Ringer solution.

Mean and standard error of the mean are reported throughout. Control responses are taken as the mean of the responses measured before and after the test responses(during glucagon infusion). Comparisons are made using blocked ANOVA with multiple comparisons by Duncan's test.

RESULTS

Calculated in terms of vascular conductance, the mean escape from vasoconstriction of the control responses before and after each test response did not change significantly with time. Paired analysis of the escape index data indicated that there was no significant difference between escape responses for the low and high constrictor stimuli.

Glucagon and Vascular Escape in the Hepatic Artery

Glucagon was able to inhibit the vascular escape responses induced by all constrictor stimuli. Percent inhibition of escape indicates the extent to which each dose of glucagon inhibits the vascular escape of the test response compared to its own control escape response. Blocked ANOVA of the low and high nerve and norepinephrine responses, in terms of the percent inhibition of escape, showed that for each dose of glucagon the extent of inhibition was not different for any of the constrictor stimuli. Thus, the percent inhibition data was pooled and is presented in figure 26. Glucagon appears to inhibit the vascular escape of both stimuli in a dose-dependent manner. The effect of the high dose of glucagon represents a significant inhibition (80 $\pm$ 23%, $P<.05$) of the escape response to the nerve-induced constriction as indicated by statistical analysis of the original percent escape data. Similarly, the mid and high doses of glucagon significantly inhibited the escape response to the norepinephrine-induced constrictions by completely abolishing escape (109 $\pm$ 34.8% and 115 $\pm$ 34.8% inhibition respectively, $P<.05$). While there is a large degree of variability in the inhibitions, these doses of glucagon appear to allow a slightly greater amount of vasoconstriction during the escape stage of the constrictor response than at the initial peak

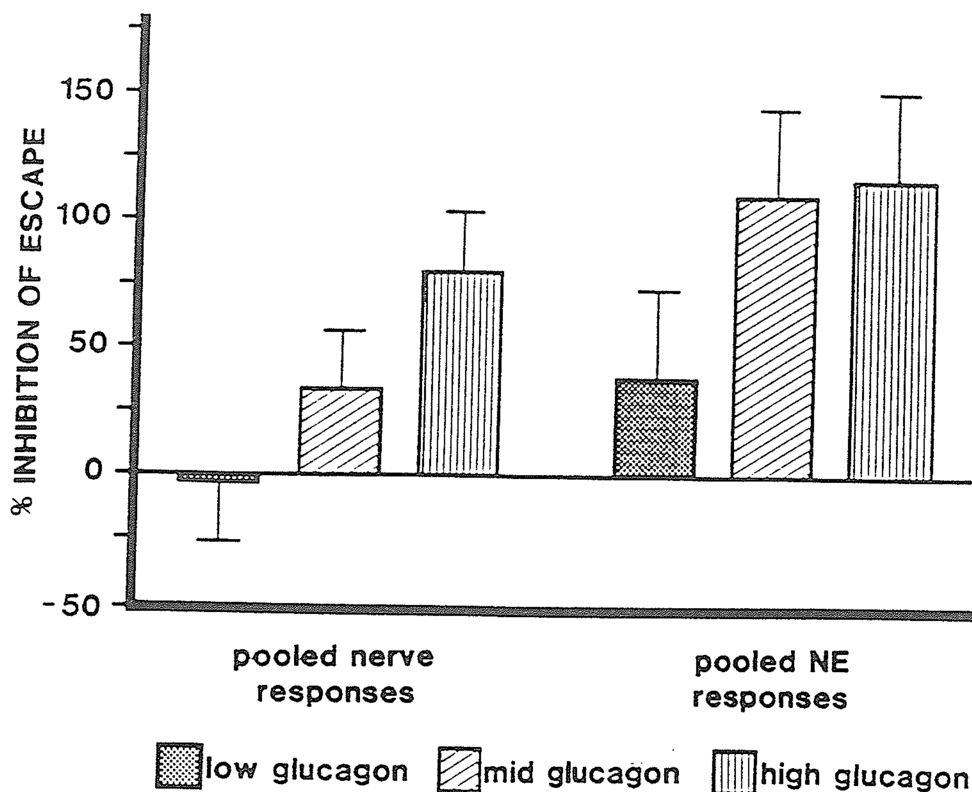


Figure 26: Percent inhibition of escape for pooled nerve and norepinephrine responses in the hepatic artery during low, mid and high doses of glucagon (see text for the escape equation). Responses are the mean $\pm$ pooled SE (n=8). Glucagon inhibited the vascular escape for both constrictor groups in a dose-dependant manner, with the high dose of the peptide significantly decreasing the escape to the nerve responses (80% inhibition) and the mid and high doses of glucagon completely abolishing the vascular escape to the norepinephrine-induced constriction (109% and 115% respectively). Inhibition of greater than 100% reflects the observation that constriction slowly continued to develope over the 3 minute stimulation period.

of the response.

Figure 27 depicts an example of the inhibitory effect of glucagon on vascular escape from a 6 Hz nerve-induced vasoconstriction taken from one experiment. The test response (middle panel), carried out during a constant infusion of 0.2 µg/kg/min of glucagon into the portal vein, represents a 50% inhibition of escape as compared to the mean of control 1 and control 2 responses, which were produced in the absence of glucagon.

Glucagon and Vascular Escape in the Superior Mesenteric Artery

Vascular escape responses in the SMA were also calculated using conductance. The escape index of the control responses before and after each test response did not change significantly with time. Paired analysis of all control escape responses indicated that there was no significant difference between the escape responses from the low and high constrictor stimuli.

Unlike the effect glucagon had on vascular escape in the hepatic arterial bed, blocked ANOVA indicated that glucagon had no significant modulatory effect on vascular escape from any constrictor stimuli (table 7).

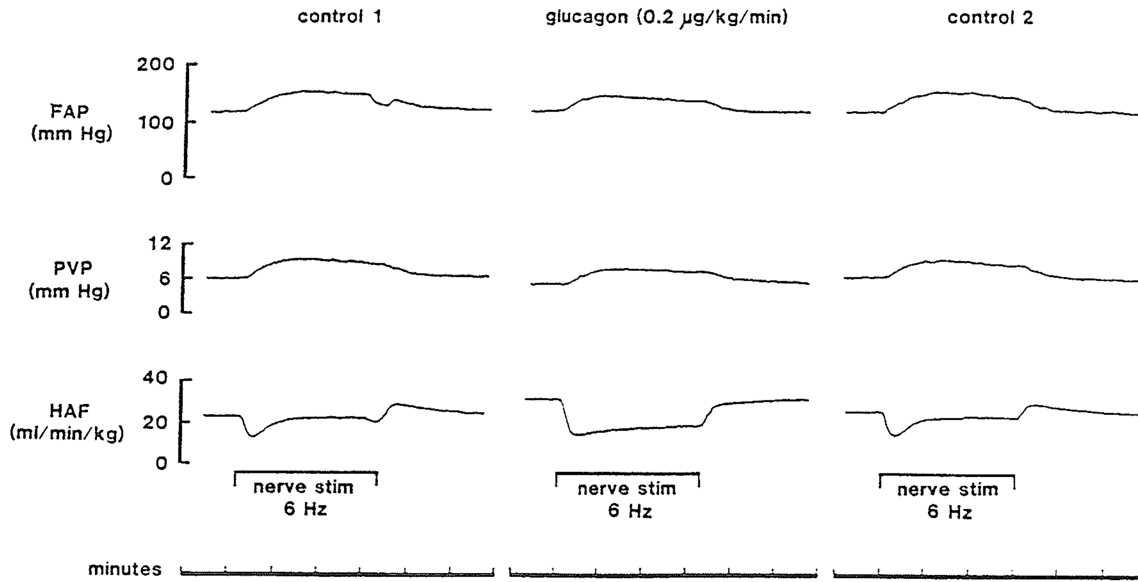


Figure 27: Tracing taken from one cat representing the inhibitory effect of glucagon on vascular escape from a 6 Hz nerve-induced vasoconstriction of the hepatic artery. Abbreviations: FAP, femoral arterial blood pressure; PVP, portal venous blood pressure; HAF, hepatic arterial blood flow. Vascular escape is the phenomenon in which blood flow returns towards its baseline following the peak vasoconstriction elicited during a sustained constrictor stimulus. In this example glucagon inhibited vascular escape by 50% (middle panel) compared to the mean escape from the two control responses.

TABLE 7

Percent escape from initial peak constriction in the presence and absence of glucagon in the superior mesenteric artery

Stimulus	% Escape					
	Control$^{\Phi}$	Low Glucagon	Control	Mid Glucagon	Control	High Glucagon
Low NE (0.27±0.04)	42.6±12.6	51.0±10.9	32.6±13.0	40.4±9.2	43.7±14.6	33.6±21.2
High NE (0.79±0.16)	30.7±7.3	40.2±8.3	36.3±10.0	30.6±8.1	35.3±7.5	37.6±7.9
Low Nerves (2 Hz)	44.1±5.9	50.0±8.8	43.3±8.1	51.1±8.8	42.0±7.3	54.1±11.7
High Nerves (6 Hz)	34.6±6.7	39.9±9.2	39.9±9.2	42.6±5.7	39.9±6.8	48.9±9.3

NE, norepinephrine (µg/kg/min). Glucagon doses, low (0.114 ±0.024 µg/kg/min), mid glucagon (0.30±0.06 µg/kg/min), high glucagon (0.61±0.11 µg/kg/min). $^{\Phi}$Control values represent the means of the control values before and after the test escape.

DISCUSSION

The previously reported aspects of these investigations demonstrated that glucagon was capable of mildly inhibiting the peak constrictor effects induced by nerve stimulation and norepinephrine in the mesenteric arterial vascular bed, yet was incapable of doing so in the hepatic arterial vascular bed. Rather, glucagon mildly (but significantly) potentiated the peak responses in the HA. It should be noted, however, that the inhibitions of the constrictor responses in the mesenteric artery required glucagon levels to be well beyond pathophysiological levels and even at these concentrations, the extent of inhibition was very weak. Furthermore, only the constrictions induced by the low frequency nerve stimulation and low dose of norepinephrine were affected. It was concluded from these studies that, in opposition to earlier reports, glucagon likely did not have any physiological role as a protective mechanism against excessive constriction, nor did it have any major vascular effects at physiological concentrations. These earlier studies, however, did not examine the effects of glucagon on the vascular escape phase of the constrictor response to a maintained pressor stimulus.

Vascular Escape

Vascular escape, as defined by Greenway (1984a), has the following characteristics: 1) It occurs only in arteriolar smooth muscle, but not in venous smooth muscle. 2) It occurs during reflex activation as well as during direct electrical stimulation of the sympathetic nerves. 3) It occurs at all frequencies of stimulation. 4) Blood flow may recover to above the control level. 5) Vascular escape occurs during constant pressure or

constant flow perfusion of the vascular bed being studied. 6) Escape is unaltered after the administration of beta-receptor blocking agents. True vascular escape has been demonstrated in the small intestine of cats (Greenway et al., 1976; Folkow et al., 1964a) and dogs (see Greenway 1984a), in the renal vascular bed of cats (Lutz and Henrich, 1973) and in other mesenteric preparations in rats, cats and humans (see Greenway 1984a). It has also been clearly demonstrated in the HA vascular bed of cats (Lautt, 1977b; Greenway and Oshiro, 1972), but does not occur in the hepatic arterial vasculature of the dog (Greenway and Oshiro, 1972), nor in skeletal muscle (Folkow et al., 1964b) or adipose tissue (Oberg and Rossel, 1967). As well documented as this phenomenon is, no definitive mechanism(s) has been found to explain vascular escape although theories do exist. The two most likely mechanisms are: 1) Release of an unknown vasodilator substance secondary to sympathetic nerve stimulation and, 2) A cellular mechanism rendering the vascular smooth muscle unable to maintain its contraction in response to the neurotransmitter released upon stimulation of the sympathetic nerves (see Greenway, 1984a). Recently, Chen and Shepherd (1991) suggested that vascular escape may be due to the increase in blood-H^+ ion concentration which develops during a maintained sympathetic nerve-induced constriction of the mesenteric vascular bed. They propose that escape results, in part, due to the ability of increased H^+ ions to impair norepinephrine stimulation of postjunctional α_2 receptors in the intestinal circulation. Remak et al. (1990) proposed that neurogenically induced escape responses may be due to afferent nerve fibres releasing vasodilatory peptides during stimulation of the nerves, per se.

Effect of Glucagon on Vascular Escape in the Hepatic Artery and Superior Mesenteric Artery

Data from these studies show that glucagon had an inhibitory effect on the escape response from nerve- and norepinephrine-induced vasoconstrictions in the hepatic vascular bed, but not in the mesenteric vessels. That is, glucagon prevented the dilation of the HA vasculature during the escape phase of the constrictor response. At some doses, glucagon abolished escape completely and allowed a greater tone in the vessel to develop compared to its initial peak response. The current results suggest that in the HA, the effect of glucagon on vascular escape is consistent with a post-synaptic site of action and that glucagon is acting in a dose-dependent manner.

Other agents have been shown to have variable effects on vascular escape. For example, adenosine deaminase has been shown to mildly inhibit escape in the porcine mesenteric vasculature (Crissinger et al., 1988), whereas 8-phenyl-theophylline, a selective adenosine receptor antagonist, did not alter escape in the mesenteric vascular bed of the cat (Lautt et al., 1988b). Beta-adrenergic receptor blockade, by definition, should not affect vascular escape, however Ross (1967, 1971) reported that propranolol reduced the degree of escape from NE infusions, but not from nerve stimulation in the cat mesenteric vasculature. Thus, Ross may not have been affecting vascular escape, but some other part of the constrictor response. Lautt et al. (1988b), observed no effect of propranolol (8-phenyl-theophylline and oubain) on escape from norepinephrine- or neurogenic-induced constrictions in this same vascular bed. Similarly, Ross and Kurrasch (1969) found that in the cat, propranolol had no effect on escape from NE-induced constriction of the HA. Part of the

confusion in this area of research is that some investigators have reported their data in terms of resistance. As noted in the previous section, the use of resistance can introduce mathematical artifacts into the calculation of escape indexes due to its nonlinear relationship with blood flow. This is particularly relevant when assessing the action of a pharmacological agent on vascular escape. Any alteration of the peak constrictor response by the agent, rather than the escape response itself, can appear as an effect on vascular escape.

Despite the degree of confusion in the literature regarding the modulation of vascular escape, the findings from the study in the hepatic vascular bed represent the first successful modulation of vascular escape by physiological or pharmacological means in the HA. It must also be stressed that all analysis of vascular escape was calculated using conductance, thereby reducing the possibility of an arithmetic artifact in the results.

The mechanism by which glucagon exerts its inhibitory effect is unclear. If the theory by Chen and Shepherd (1991) is correct, it suggests that glucagon is capable of preventing an increase in the H^+ ion concentration or interferes with the interaction of the H^+ ions and the α_2- receptors. In an investigation by Madden, Ludewig and Wangensteen (1971) glucagon was administered intravenously to dogs, and, among other parameters, the pH of arterial, venous and portal venous blood was measured. Although the pH in all blood samples had dropped by approximately 0.02 units 30 minutes after the glucagon injection, the pH in arterial and portal venous blood had risen 0.02 and 0.03 units, respectively, one minute after glucagon administration. The latter changes in the pH were, however, not significantly increased from control levels. There were also no blood samples taken between

the one minute mark and 30 minutes post-glucagon injection. It is interesting to speculate that if the transient increase in pH was the result of the "injection" of glucagon (resulting in a transient rise in glucagon), the constant infusion of glucagon in the present hepatic arterial study may have produced a maintained elevation in the pH of the blood. If such an elevation in pH were to occur, it could counteract the action of the H^+ ions at the α_2-receptors and inhibit vascular escape. At the moment, however, I am not aware of any investigation that has reported the blood pH during a constant infusion of glucagon. However, Davis et al. (1985), reported that infusions of glucagon in fasted dogs produced a 30% rise in lactate in arterial blood, reaching a peak 15 minutes after the start of the glucagon infusion. Blood pH was not measured in this study. However, these authors also note that under certain conditions ie., animals that have not fully recovered from surgery, the liver consumed lactate rather than produced this metabolite.

The selectivity of glucagon for the hepatic vasculature over the mesenteric artery may be related to the greater potency this compound has in the HA compared to the SMA (discussed in sections VII.2. of this thesis). Because glucagon had inhibitory effects on HA escape and not SMA escape, the liver itself may have contributed to this selective inhibition of vascular escape. It is possible that glucagon acted on the liver, which in turn, released a substance or modified the blood such that vascular escape was inhibited.

SUMMARY

In summary, glucagon was found to selectively inhibit vascular escape from neurogenically-mediated and norepinephrine-induced constrictions of the HA, but not in the

mesenteric vascular bed. The selective effect of glucagon on the escape responses in the HA may be related to the greater sensitivity the HA appears to have to glucagon, compared to the SMA. This selective effect may also be due to a glucagon-liver interaction such that the liver releases an inhibitory substance or somehow affects vascular escape. Although the mechanism by which glucagon is working is not clear, it appears to do so in a dose-dependent manner and at a post-synaptic site. All data from the investigations in the hepatic and mesenteric arterial vascular beds were calculated in terms of conductance, in accordance with the results obtained in section VII.3. Thus, the observation that glucagon inhibited vascular escape in the HA but not in the SMA is not due to a mathematical artifact.

SECTION VIII

ASSESSMENT OF CHRONIC BILE DUCT-LIGATION AS A MODEL OF PORTAL HYPERTENSION IN THE CAT

INTRODUCTION

In the previous sections (VI and VII) methodological, pharmacological and functional aspects of glucagon were investigated in the hepatic and splanchnic arterial vascular beds. Additionally, features of the intrinsic (hepatic arterial buffer response) and extrinsic (nervous and humoral) regulation of the hepatic artery (HA) were assessed with respect to glucagon. In this next section, the emphasis of the investigation is focused, not on the arterial vessels, but the portal and hepatic venous vasculature.

In recent years, major advances have taken place in the understanding of the hepatic venous and portal venous system in respect to the maintenance of intrahepatic blood pressure, which in turn, has repercussions on the microcirculation of the liver and systemic circulation in general. This is particularly obvious when one considers the cardiovascular dysfunction that is associated with some chronic liver diseases, such as cirrhosis and portal hypertension (PHT). These issues have been thoroughly discussed in the general introduction of this thesis. Thus, it was an interest of mine to assess the function of the hepatic venous system under a diseased state.

The effect of PHT on this system was the most relevent condition to study. The majority of hemodynamic studies conducted in portal hypertensive animals, however, has involved the partial portal vein stenosis model. This is a form of pre-hepatic PHT,

presumably without effect on the hepatic venous sphincters and therefore would be inappropriate for studying the hepatic venous system. Chronic bile duct-ligation (CBDL) has been reported to produce hepatic cirrhosis closely mimicking that of alcohol-induced cirrhosis in man, and has been used as an experimental model of PHT in dogs (Bosch et al., 1983) and rats (Franco et al., 1979). CBDL has been reported to produce a postsinusoidal form of PHT and, therefore, should be a reasonable means of affecting the hepatic venous system. Furthermore, CBDL has not been used in the feline species other than for studies of obstructive jaundice. Thus, the object of this investigation was to assess the technique of CBDL as a model of PHT in the cat and determine the effect this condition may have on the hepatic venous system, in particular, the site(s) of increased resistance leading to PHT.

METHODS AND MATERIALS

Cats of either sex were randomly assigned as sham-operated controls or bile duct-ligated test animals. The test animals were further divided into three groups of varying duration of bile duct-ligation, 10 days, 14 days, or 21 days. All sham operated cats were studied 10 days after the initial surgery.

Surgical and Postoperative Procedures

a) Bile Duct-Ligation Surgery

Cats were fasted overnight and anesthetized with sodium pentobarbital (32.5 mg/kg) by intraperitoneal injection. An intravenous infusion line was started in a brachial vein to draw a blood sample and to administer 62.5 mg of ampicillin washed in with 10 ml of

warmed 5% dextrose Ringers solution. The IV was then removed. Ampicillin (62.5 mg) was also given intramuscularly (IM) into the hind limb muscles prior to surgery. Under sterile conditions, a mid-line incision from the xiphoid process to the umbilicus was performed and a laparotomy conducted. A 1 cm length of the common bile duct, approximately 1 cm from the duodenum, was cleared and separated from the surrounding tissue. The bile duct was ligated twice (2.0 silk sutures) and sectioned between the ligatures. The cystic duct leading to the gallbladder was also ligated using 2.0 silk sutures. The abdominal muscles were closed with 2.0 chromic gut sutures using a continuous running stitch. The skin layer was closed with 2.0 chromic gut sutures using a continuous hidden mattress stitch. The sham-operated cats underwent the identical surgical procedure except that their bile duct and cystic duct were not ligated. Animals were transferred to a recovery room where they were individually housed in stainless steel cages until they had recovered from the anesthesia. Animals were kept warm with blankets and hot water bottles or circulating hot water mattresses. Animals were checked frequently during the recovery period. Only one animal died during this time. Each animal received 62.5 mg of ampillicin daily for the first two days following surgery. No analgesics were necessary for postoperative care.

These experiments were carried out over two separate time periods. In the first period all animals were housed individually in stainless steel cages but were given ample time daily for exercise and play outside the cages. During the second period of experiments, the cats were housed communally in a special postoperative room (approximately 6.5 m x 2.5 m) equipped with ramps and shelves for climbing and exercise. No more than 6 cats

were housed simultaneously which afforded the animals abundant space. In both cases, cats were monitored for behavioral changes and general health status. All medical attention and requirements were dealt with immediately in collaboration with the on-staff veterinarian. Abdominal incisions were checked daily for the first week. No cats appeared to suffer from any surgically-related infections. Several cats in all groups did suffer from mild to moderate upper respiratory tract infections and were treated with Amoxicillin (an ampicillin analogue). Eye infections were also common among the animals and were treated effectively with a glucocorticoid antibiotic according to the manufacturer's directions (Gentocin Durafilm).

In the treatment groups, some animals became anorectic as their conditions progressed. When deemed necessary, these animals were fed a balanced carbohydrate, protein and vitamin milk-based diet (Pro-Balance Maximum Feline Nutrition, Norden Laboratories). Test animals that became nauseated or vomited were treated with 5-10 mg of dimenhydrinate IM (Squibb Canada Inc.). Hydration status of the cats was monitored. Any cats showing the first signs of dehydration were given subcutaneous sterile saline infusions (back of neck) according to standard veterinarian techniques.

b) Acute Experimental Surgery

On the assigned day (10, 14, or 21 days post-ligation), cats were anesthetized with pentobarbital sodium (32.5 mg/kg) by intraperitoneal injection after an overnight fast. Anesthesia was maintained throughout the experiment by supplemental doses (6.5 mg/kg) of the anesthetic through a brachial vein cannula. Body temperature was maintained at 37.5°C by means of a rectal probe and a thermal control unit regulating heating rods in the

surgical table. Systemic arterial pressure was measured from a cannula in the right femoral artery (FAP). Inferior vena caval pressure (IVCP) was measured from a cannula inserted into the femoral vein and advanced well into the inferior vena cava. A test blood sample was taken from this cannula early in the surgical preparation and used to measure post-ligation serum biochemistries and hematocrit. A tracheal cannula was inserted to maintain a patent airway and for artificial ventilation. A laparotomy was performed on the same site as in the initial surgery. The inferior mesenteric artery was ligated. The common hepatic artery and anterior plexus of the hepatic nerves were located. The nerves were carefully separated from the common hepatic artery, ligated, sectioned, and the proximal end placed in a circular bipolar stimulating electrode. The gastroduodenal artery, which branches from the junction of the common hepatic artery and hepatic artery proper, was also ligated. In some of the test animals, the common bile duct was grossly expanded and prevented access to the gastroduodenal artery. The spleen was removed and the splenic artery ligated.

Portal venous pressure (PVP) was measured from a cannula inserted in a small cecal vein and passed into the portal vein. The end of the cannula was located approximately 1.5 cm from the hilum of the liver.

A commercial i.v. unit (Jelco 23G) was inserted in the superior mesenteric vein and glued in place (Histoacryl) to allow for portal venous infusions of norepinephrine. The cat was placed on the respirator and an incision was made into the thoracic cavity at the level of the 6th intercostal space to expose the vena cava and heart. The jugular vein was located and a PE90 sealed tip cannula, with side-holes located approximately 3 and 7 mm from the tip, was inserted into this vein. The cannula was manipulated past the heart and into the

thoracic vena cava. The cannula was then directed by the surgeon's fingers into the hepatic vein. The cannula was advanced into the liver until it reached a wedged position. The cannula was then pulled back slightly from this wedged position (approximately 0.5 cm) and was used to measure intrahepatic, or lobar venous pressure (LVP). In most cases the location of the cannula could be verified to be in the liver by feeling it in one of the liver lobes. However, to determine whether this cannula was located in the proper position, that is, beyond the hepatic venous sphincter sites, a test bolus of norepinephrine was injected into the portal vein. LVP and PVP should rise to a similar extent with IVCP remaining unaffected. Animals were allowed a minimum of 30 minutes to stabilize before any experimental protocol was performed.

All pressure transducers were set to zero relative to the midpoint of the inferior vena cava at the hepatic outlet site. Calibration of the transducers was done using a water manometer for venous pressures and a mercury manometer for arterial pressures.

PROTOCOLS

Plasma Biochemistries

The control blood sample taken before bile duct-ligation surgery and the test blood sample taken on the day of acute experimentation were immediately centrifuged and the plasma drawn off and frozen. The plasma was later analyzed for glucose, lactate, triglycerides, albumin, total protein, direct and total bilirubin, cholesterol, high density lipoproteins (HDL), lactate dehydrogenase, gamma glutamyl transpeptidase (GGPT), alanine aminotransferase (ALT; SGPT), aspartate aminotransferase (AST; SGOT), and alkaline

phosphatase. All compounds were measured using an Abbott VP Supersystem Photometric Analyzer (Abbott Laboratories).

Pressure Profiles

After cats had been given time to stabilize following surgery, the lobar venous catheter was withdrawn in 0.5 cm increments. After each incremental withdrawal the pressures were allowed to stabilize and were noted. As this procedure continued, LVP progressively decreased toward IVCP until LVP was equal to or similar to IVCP, or until withdrawal of the cannula failed to produce any further changes in LVP. The cannula was then re-inserted into the liver. These hepatic venous pressure profiles were then repeated during constriction of the hepatic vascular bed imposed by a 0.5 µg/kg/min constant infusion of norepinephrine into the portal vein or a continuous 8 Hz frequency stimulation (1 msec, square pulse, 15v) of the hepatic anterior plexus. During a nerve stimulation of the anterior plexus, PVP and LVP initially rise with PVP becoming larger than LVP. PVP then undergoes an escape phenomenon whereby this pressure decreases towards and plateaus at or near to the elevated LVP, despite the continued nerve stimulation. Only after this response had plateaued (approximately 3 minutes) was the hepatic venous pressure profile conducted. Norepinephrine infusion also raises PVP and LVP, but the escape phenomenon does not occur. The hepatic venous pressure profile was conducted after PVP and LVP responses had plateaued (approximately 3 minutes). PVP and LVP responses were measured at the peak of the PVP response. FAP responses were also measured at the peak of the arterial pressure response.

The hepatic venous resistance sites were characterized in respect to their length and the percent of the total drop in LVP which occurred over this length. This was assessed during the pressure profiles. As the LVP cannula was withdrawn, a pressure drop could be measured. A pressure drop of $\geq$ 0.5 mmHg in one incremental withdrawal (0.5 cm) was the criteria for considering that the cannula tip was passing through an area of increased resistance. For each cat, the number of 0.5 cm increments over which a $\geq$ 0.5 mmHg drop in LVP occurred was recorded and this length was considered to be the extent of the resistance site. The pressure drop occurring over this region of resistance was then calculated as a percent of the total drop in LVP over the entire hepatic venous profile.

After completion of the experiment, a large hemostat was used to clamp the vena cava at the level of the diaphragm thereby securing the hepatic venous catheter in the liver. The location of the hepatic venous catheter within the liver was determined at this point.

Histology

The liver, kidneys, and heart were removed, weighed, and inspected for gross changes and pathology. The liver was prepared for histological and pathological analysis. Blocks of tissue were cut transversely from each lobe and immersed in labelled bottles containing 10% neutral formal saline for fixation. Tissue blocks were embedded in paraffin wax and were cut in 7 µm thick sections. Two stains were used: Delafield's hematoxylin-eosin regressive method in which nuclei stain deep blue and other cellular structures stain pink; Mason's trichrome method, which clearly differentiates fibrous connective tissue from liver parenchyma, with collagen staining green and other cellular structures staining magenta. All

slides were analyzed by an examiner without prior knowledge of the treatment to avoid observer bias.

Absolute values of FAP, PVP, LVP, and IVCP, as well as pressure gradients between PVP and IVCP (PVP-IVCP gradient), PVP and LVP (PVP-LVP gradient) and LVP and CVP (LVP-IVCP gradient) are reported as the mean ± the standard error of the mean. Because the gradient values were small, the percent change in these gradients and any calculations utilizing these gradients used the mean values of the respective pressures incorporated in the gradient rather than the mean of the percent change in the gradients taken from the individual experiments. This method of calculation obviated grossly exaggerated percent change values that can occur based on changes in a very small initial values. Correlations between plasma biochemistries and hemodynamic parameters were calculated using a linear regression curve fitting computer program (GraphPAD Inplot Graphics).

Statistical comparisons were selected according to the type of data and included paired and unpaired t-tests, unblocked and blocked ANOVA's, with multiple comparisons by Duncan's test.

RESULTS

Cats recovered from anesthesia generally within 24 hours after surgery. All cats ate and drank readily at this point. Sham-operated (referred to as sham) animals did not show any signs of ill effects introduced by the surgery. In the bile duct-ligated groups (referred to as test groups), jaundice became evident by the fourth day post-bile duct-ligation (range 2-6 days) and became progressively worse with time. It was noted, however, that in some

of the 21 day test cats, the degree of jaundice appeared to stabilize or even decrease slightly during the third week after surgery.

Sham cats did not exhibit any obvious changes in behavior throughout the postoperative period. Cats were generally bright, active, and alert and maintained appetites, regular bowel movements, and voiding of urine. The effect of bile duct-ligation on test animals differed between cats even within the same test group. Most test cats became anorectic to some extent, lost weight, and showed varying degrees of lethargy with the condition of several cats in the 21 day group becoming quite severe. This condition, however, was not restricted to the 21 day group and some cats in each test group became very ill.

Sham cats (n=9, weight 3.27 $\pm$ 0.35 kg) did not lose weight during the 10 day recovery period (3.28 $\pm$ 0.31 kg). Cats in the three test groups lost a significant amount of weight compared to pre-ligation weight: 10 day test, 3.38 $\pm$ 0.28 kg versus 2.84 $\pm$ 0.21 kg (n=8, p=0.001); 14 day test, 3.94 $\pm$ 0.20 kg versus 3.21 $\pm$ 0.19 kg (n=7, $p<0.001$); 21 day test, 4.13 $\pm$ 0.43 kg versus 3.33 $\pm$ 0.33 kg (n=6, p=0.001). The pre-operative weight was not significantly different between the sham and the three test groups nor was the amount of weight lost by the three test groups.

Gross Inspection of Liver and Viscera

a) Sham Group

In all cases the sham animals did not display any signs of liver or biliary system pathology upon initial observation. Peripheral vessels (brachial vein, femoral vein, and

femoral artery) were normal in appearance, as were vessels in the abdomen.

b) Test Groups

The extent of obvious liver and biliary tract pathology was variable between animals and did not necessarily appear to be related to the duration of bile duct-ligation. Some animals in all test groups displayed varying degrees of liver injury, including general and localized regions of jaundiced liver tissue, varying degrees of pebbling on the liver surface, congestion, swelling of liver lobes, and rounding of lobe edges. The liver colour varied between the normal shade of brownish-red to a very congested deep red colour as well as having very mottled colorations on the surface of the liver. Vessels in the periphery (brachial vein, femoral vein and artery, jugular vein) and internal vessels (abdominal vessels and ascending aorta) were jaundiced. It should be cautioned, however, that, as mentioned above, these particular liver conditions were found to varying degrees, with extreme cases of each appearing in all three test groups.

The biliary system in most test animals was excessively dilated, including the common bile duct and hepatic ducts leading from the liver. In one animal the common bile duct was measured to be 3 cm wide. The presence of white bile was found in the biliary system of the test animals. White bile is a clear, colourless, mucoid-like fluid (although it does not contain mucopolysaccharides) found in the biliary tree of animals with chronic biliary obstruction. It is free of bile pigments and bile salts and has an alkaline pH (8-10). The protein content has been reported to be less than that of normal bile (Harber and Rees, 1963). The existence of white bile in man is reportedly rare (Flint, 1937), but very few studies have been conducted on this fluid. The mechanism of production of white bile is

poorly understood.

Histological Changes

Progressive histopathological changes characteristic of development of biliary cirrhosis occurred in cats following varying periods of bile duct-ligation. Extrahepatic obstruction of the bile ducts resulted in inflammatory changes of hepatic parenchymal cells, proliferation and dilation of small bile ducts, vascular dilation and hepatic fibrosis. According to the categorization of Sherlock (1989), animals with the most severe pathology appeared to be in stage III of cirrhosis development, which was indicated by the presence of septal and bridging fibrosis. Regenerative nodule formation was absent, indicating that full-blown cirrhosis (stage IV) had not yet developed.

a) Hepatic Fibrosis

Hepatic fibrosis was only qualitatively assessed, therefore, time-dependent changes in the fibrosis were not analyzed statistically. Nevertheless, progressive hepatic interlobular fibrosis developed in all cats as a consequence of bile duct-ligation. fibrosis and bile duct proliferation developed in most triad spaces. Concentric patterns of collagen fibres containing accumulations of leucocytes, encapsulated the interlobular veins, hepatic arteries and bile ducts. An increase of fibrous connective tissue interconnected contiguous triad spaces between hepatic lobules. Tracks of fibrous strands extended from interlobular areas into hepatic sinusoids. Dense connective tissue lesions were more abundant around greatly dilated lobar bile ducts. In some animals, these fibrous lesions extended into interlobular

spaces in a spider-spread pattern. Increased amounts of isomorphous connective tissue were observed to lie adjacent to the endothelial lining of dilated intralobular portal and hepatic veins. Despite the extensive fibrosis, the basic architecture of the polyhedral lobular histology was maintained in all test group cats.

b) Hematological Inflammatory Changes

Chronic inflammatory changes occurred around periportal and intralobular spaces. Dense accumulations of mononuclear and polymorphonuclear leucocytes were present at triad spaces and interlobular areas. The greatest concentrations of leucocytes were observed in triad spaces with bile duct hyperplasia. Small accumulations of leucocytes were found, on occasion, in hepatic sinusoids and less frequently in bile ducts. There was no evidence of Kupffer cell proliferation.

c) Vascular Dilation

Hepatic veins, central veins and portal veins in the triads, were often increased in size. Dilations of this type were non-uniformly distributed in the liver. There was no evidence of structural changes in the arterial vessels.

e) Bile Duct Hyperplasia

Bile duct hyperplasia was evident in all test cats. The new ducts were differentiated from vascular structures by the presence of low columnar epithelium circularly arranged to form a duct. Increased portal fibroblast activity occurred in only those triads with increased

numbers of bile ducts. The hyperplasia did not occur uniformly in all triad spaces.

Blood and Plasma Analysis

Pre-ligation hematocrit and hematocrit on the day of acute experimentation were measured and are as follows for the individual groups. Sham group: 29.8 ± 1.8% versus 29.4 ± 2.3% (n=7, not significant,ns); 10 day test, 28.9 ± 2.8% versus 26.4 ± 3.0% (n=5, ns); 14 day test, 32.5 ± 2.3% versus 26.7 ± 2.0% (n=7, ns); 21 day, 29.3 ± 1.5% versus 28.5 ± 3.1% (n=6, ns). There was no significant difference between any pre-ligation hematocrit value and the values after ligation as determined by paired analysis of the data.

Analysis of the various blood chemistries before and after ligation for the sham and test groups are detailed in tables 8A, 8B and 8C. These results were analyzed statistically in three ways. Paired analysis was used to compare serum values before ligation and at the time of acute experimentation for each group. The pre-ligation values for each group were compared by unblocked ANOVA to determine if there were any differences in the basal values of the chemistries. The post-ligation values for each group were also compared by unblocked ANOVA to determine if a time-dependent effect of bile duct-ligation on these chemistries exist.

The chemistries that were analyzed included the standard indices of liver and tissue damage, ALT (SGPT), AST (SGOT), GGTP, and alkaline phosphatase as well as indices of liver function and metabolism such as albumin and cholesterol (levels). The AST levels between the sham control and sham test samples were significantly different but were still within normal levels. Whether or not this increase within the sham group represents an

TABLE 8A

Plasma values from sham-operated controls, and after 10, 14, and 21 days of bile duct-ligation

		SHAM (n=9)	10 DAY (n=7)	14 DAY (n=7)	21 DAY (n=6)
LACTATE	CONT	21.89 ± 4.49	17.36 ± 2.54 NS	17.28 ± 2.74 NS	14.93 ± 1.57 NS
		NS	NS	NS	**
	TEST	20.08 ± 3.31	20.99 ± 10.14 NS	12.29 ± 1.51 NS	7.81 ± 9.13 NS
TRIGLYCERIDES (6-58 mg/dl)	CONT	22.87 ± 3.14	26.34 ± 3.43 NS	27.25 ± 5.44 NS	24.93 ± 3.40 NS
		NS	*	**	NS
	TEST	34.98 ± 10.60	78.34 ± 19.80 *	80.69 ± 9.98 *	48.67 ± 8.97 NS
GLUCOSE (70-150 md/dl)	CONT	86.77 ± 5.75	83.35 ± 3.96 NS	95.54 ± 6.10 NS	92.21 ± 3.72 NS
		**	*	NS	*
	TEST	340.57 ± 50.97	257.37 ± 54.85 NS	187.04 ± 34.61 *	117.68 ± 10.11 **
ALBUMIN (2.2-3.5 gm/dl)	CONT	2.81 ± 0.14	2.74 ± 0.15 NS	3.04 ± 0.10 NS	3.10 ± 0.15 NS
		**	NS	NS	**
	TEST	2.13 ± 0.17	3.10 ± 0.28 *	3.16 ± 0.27 *	3.55 ± 0.18 **
TOTAL PROTEIN (5.5-7.5 g/dl)	CONT	6.81 ± 0.28	7.09 ± 0.31 NS	7.26 ± 0.22 NS	7.34 ± 0.41 NS
		*	NS	NS	NS
	TEST	5.78 ± 0.20	7.61 ± 0.50 *	7.94 ± 0.54 *	7.52 ± 0.43 NS

See table 8C for common abbreviations and statistical procedures.

TABLE 8B

Plasma values from sham-operated controls, and after 10, 14, and 21 days of bile duct-ligation

		SHAM (n=9)	10 DAY (n=7)	14 DAY (n=7)	21 DAY (n=6)
DIRECT BILIRUBIN	CONT	0.036 ± 0.006	0.029 ± 0.03 NS	0.206 ± 0.110 NS	0.133 ± 0.041 NS
		NS	**	***	**
	TEST	0.041 ± 0.007	13.00 ± 3.30 **	15.46 ± 2.40 ***	12.60 ± 2.10 **
TOTAL BILIRUBIN (0-0.8 mg/dl)	CONT	0.221 ± 0.019	0.283 ± 0.038 NS	0.419 ± 0.123 *	0.328 ± 0.038 NS
		NS	**	***	**
	TEST	0.197 ± 0.017	17.51 ± 4.48 *	20.91 ± 3.13 ***	17.65 ± 2.84 **
GGTP (1.8-183 IU/L)	CONT	2.06 ± 0.36	1.24 ± 0.36 NS	0.65 ± 0.198 *	0.75 ± 0.38 NS
		NS	**	**	***
	TEST	1.13 ± 0.13	17.33 ± 3.49 **	18.57 ± 4.18 **	22.56 ± 2.91 **
AST (10-60 IU/L)	CONT	16.07 ± 1.26	19.53 ± 2.02 NS	14.18 ± 1.88 NS	16.10 ± 3.29 NS
		*	*	*	***
	TEST	28.80 ± 4.25	214.14 ± 57.21 **	215.16 ± 61.90 **	153.30 ± 21.60 *
ALT (10-60 IU/L)	CONT	33.19 ± 9.67	19.91 ± 5.30 NS	38.28 ± 8.73 NS	31.54 ± 4.16 NS
		NS	**	**	**
	TEST	31.52 ± 5.15	495.00 ± 104.30 **	466.49 ± 84.89 **	530.50 ± 82.00 NS

See table 8C for statistical procedures. Abbreviations: GGTP, gamma glutamyl transpeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

TABLE 8C

Plasma values from sham-operated controls, and 10, 14, and 21 day duration of bile duct-ligation

		SHAM (n=9)	10 DAY (n=7)	14 DAY (n=7)	21 DAY (n=6)
LDH (10-200 IU/L)	CONT	79.86 ± 10.69	115.46 ± 27.75 NS	70.35 ± 14.63 NS	68.27 ± 9.47
		NS	NS	NS	NS
	TEST	153.47 ± 33.25	274.98 ± 116.35 NS	305.07 ± 119.72 NS	96.67 ± 22.02 NS
Alkaline Phosphatase (<30 IU/L)	CONT	26.55 ± 5.67	19.67 ± 2.15 NS	44.48 ± 14.02 NS	40.90 ± 12.53 NS
		NS	*	NS	*
	TEST	39.94 ± 14.15	113.66 ± 35.90 NS	160.78 ± 47.12 NS	187.50 ± 50.02 NS
CHOLESTEROL (116-126 mg/dl)	CONT	77.21 ± 10.70	81.89 ± 11.46 NS	80.77 ± 11.46 NS	83.75 ± 15.90 NS
		NS	*	**	**
	TEST	67.24 ± 7.54	187.30 ± 25.34 ***	172.06 ± 16.32 **	148.00 ± 15.90 *
HDL	CONT	17.58 ± 8.05	18.50 ± 8.26 NS	30.89 ± 8.52 NS	28.20 ± 11.30 NS
		NS	**	NS	NS
	TEST	17.07 ± 8.29	24.03 ± 8.50 NS	29.83 ± 6.51 NS	31.20 ± 9.10 NS

Values in brackets are for normal cats (taken from Bentinck-Smith et al., 1989). All reported values are the mean ± SEM. Common abbreviations, CONT, blood sample taken before biliary obstruction; TEST, blood sample taken on day of acute experimentation after biliary obstruction. Statistics: paired comparisons were made between the control sample (set-up surgery) and test samples (day of experiment) for all groups (significance noted between test and control values). Sham and test groups were compared by unblocked ANOVA (significance from sham group noted to the right of test values). Abbreviations: LDH, lactate dehydrogenase; HDL, high density lipoprotein.

influence from the surgery alone or is a normal flux in this enzyme activity is not clear.

AST and ALT levels in the ligated test groups were massively elevated from the sham control and sham test levels. ALT levels, which are more specific for liver tissue injury, were elevated 15-16 fold from the sham test levels in all three test groups. The AST levels rose approximately 7 times in the 10 and 14 day test groups but decreased slightly (but not significantly) by the 21 day period (5 fold increase). GGTP, which is considered to be an indicator of hepatobiliary pathology, was increased in all three test groups compared to the sham group. The normal range of GGTP, however, is very wide and only the 21 day group levels were elevated above this range. Alkaline phosphatase, which also reflects impaired biliary tract function, was significantly elevated in all the tests groups compared to the sham, but not between test groups. Along with the elevated GGPT levels, these data suggest that the biliary system of the test cats was impaired by our interventions. The test levels of the alkaline phosphatase were, however, extremely variable which accounts not only for the large test values but also the low levels of significance between pre-ligation controls and test levels.

Statistically significant changes in the sham test sample and 21 day test sample occurred for albumin. All albumin levels, control and test, remained within normal levels. Cholesterol levels were mildly elevated from sham levels and were highest in the 10 day group (187.3 $\pm$ 25.34 mg/dl). Only minor changes occurred in the HDL levels. Triglyceride levels were significantly increased in the 10 and 14 day test groups compared to the sham test levels and the control levels of each of these groups. Glucose levels in all test blood samples, including the sham group, were significantly elevated with the sham test group

actually showing the largest elevation in glucose. There was a progressive decline toward normal levels. Although the 21 day group still had a significantly increased test glucose level compared to the control level, this test level was within normal limits.

The control levels of direct and total bilirubin were all well within normal levels. In all three groups, bile duct-ligation significantly increased these levels between 77 and 164 times the control levels. Direct bilirubin reached maximum levels (15.46 $\pm$ 2.4 mg/dl) at 14 days post-ligation. Total bilirubin followed a similar pattern reaching a maximum level of 20.9 $\pm$ 3.13 mg/dl in the 14 day group which represented a 50 fold increase from the control level.

Hepatic Venous Pressure Profiles

Hepatic venous pressure profiles from the sham and test groups were obtained under basal conditions and in the presence of exogenous norepinephrine (0.5 µg/kg/min), infused at a constant rate into the portal vein, and during an 8 Hz frequency nerve stimulation of the hepatic anterior plexus. An example of a typical control profile in the basal state taken from a sham cat is shown in figure 28. Several points should be noted (and will be further detailed in this section). 1) PVP and LVP are recording a very similar pressure which supports previous findings which suggest that there is very little presinusoidal resistance in the portal vascular system under basal conditions. In this tracing, the pressure difference between PVP and LVP is approximately 1 mmHg. 2) LVP is not measuring a wedged pressure since this cannula has been pulled back from the wedged position and respiratory movements are present and independent of PVP. This suggests that LVP is a true

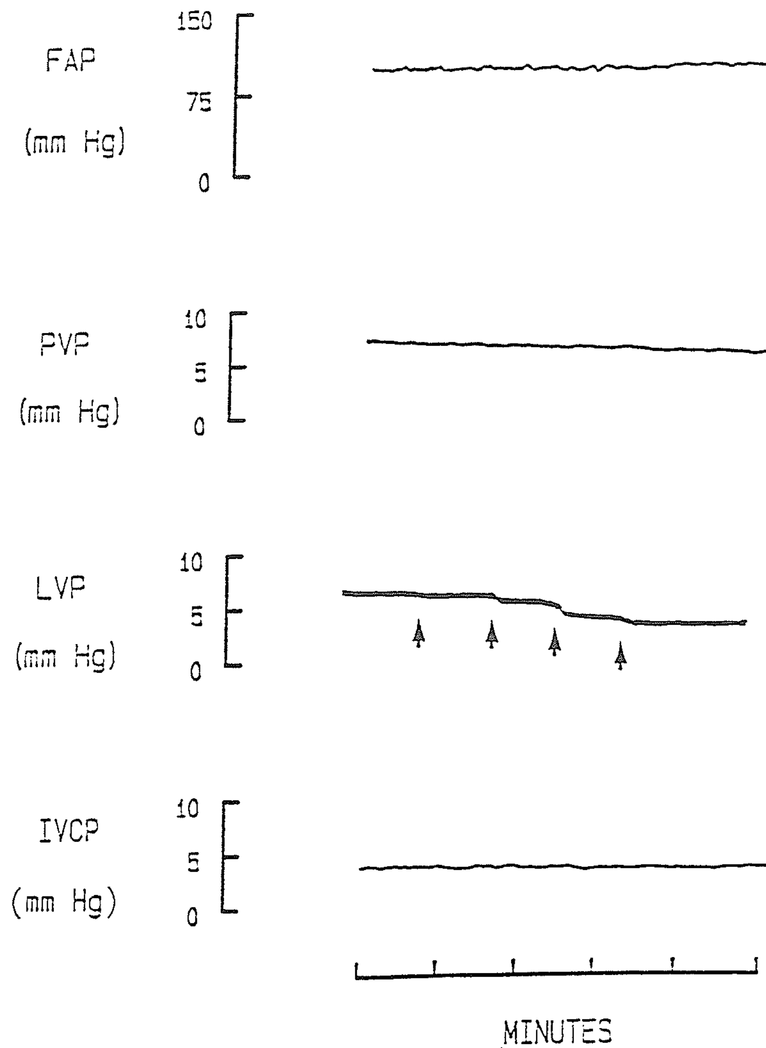


Figure 28: Tracing of a typical pressure profile in the basal state, taken from a sham-operated animal. Before the LVP cannula was withdrawn, PVP and LVP were recording a very similar pressure suggesting that there was very little presinusoidal resistance in the portal vascular system under basal conditions. Arrows indicate each sucessive 0.5 cm withdrawal of the LVP cannula. As the catheter was withdrawn, LVP dropped in stages until LVP was equal to or similar to IVCP, indicating that the tip had passed through an hepatic venous resistance site.

measurable pressure and is not measuring PVP via a static column of blood. 3) As the catheter is withdrawn in 0.5 cm increments, the LVP also drops in stages until the pressure recorded is equal to or similar to IVCP. This suggests that the catheter tip is passing through a hepatic venous resistance site. At this final point the cannula tip has passed beyond the hepatic venous resistance site and is measuring pressure from the large hepatic veins which is equal to IVCP. The LVP cannula could then be re-inserted into the liver and be shown to measure the same pressure recorded before the initial withdrawal. There is, however, no assurance that the cannula entered the same hepatic vein as before thereby precluding any type of paired statistical comparisons between the different profiles.

Hepatic venous profiles were measured under basal conditions in all groups to define the hepatic venous resistance sites and to determine if these sites were altered by bile duct-ligation. The length over which the major pressure drop occurred in LVP during the profile and the percent of the total pressure drop in LVP which occurred over this length was determined. These profiles were also measured during an 8 Hz nerve stimulation of the anterior plexus and during a constant infusion of norepinephrine (0.5 μg/kg/min) into the portal vein, to determine if these stimuli altered the resistance sites in the sham and test groups.

Under basal conditions, the lengths of the resistance sites were significantly larger in the 21 day test groups (n=19 values from 6 cats, 1.24 $\pm$ 0.12 cm; range 0.5-2.0 cm) compared to the sham (n=17 values from 6 cats, 0.85 $\pm$ 0.09 cm; range 0.5-1.5 cm), 10 day (n=9 values from 3 cats, 0.83 $\pm$ 0.19 cm; range 0.5-2.0 cm), and 14 day test groups (n=13 values from 5 cats, 0.77 $\pm$ 0.09 cm; range 0.5-1.5 cm). The percent of the total pressure

drop in LVP which occurred over the length of these resistance sites also increased significantly in the 21 day test group (86.9 ± 2.8%) compared to the sham group (68.1 ± 5.1%), but not the 10 (74.7 ± 0.67%) and 14 day test groups (74.7 ± 6.1%).

Norepinephrine and 8 Hz nerve stimulation mildly increased the length of these resistance sites in all experimental groups but these changes were not statistically significant. The percent of the total pressure drop in LVP accounted for by these resistance sites were unaltered by norepinephrine and nerve stimulation.

Pressure Measurements

Figure 29 details the basal FAP, PVP, and LVP for the four groups of cats (10 day sham, 10 day test, 14 day test, and 21 day test) measured before hepatic venous profiles. Basal FAP was 87.3 ± 3.3 mmHg. FAP for the 10, 14, and 21 day test groups was 80.6 ± 3.3 mmHg, 85.2 ± 3.2 mmHg, and 90.1 ± 3.7 mmHg, respectively, and was not significantly different from the sham group or each other (figure 29A). This value for the sham FAP was slightly lower than normal (100-120 mmHg) and may represent some influence of the initial set-up surgery or possibly an underlying state of dehydration.

The mean PVP (6.0 ± 0.4 mmHg) and LVP (5.8 ± 0.5 mmHg) were not significantly different in the sham animals (figures 29B and 29C) and supports previous findings from this laboratory. In the 10 day group PVP and LVP were 7.4 ± 0.2 mmHg and 6.9 ± 0.3 mmHg ($p=0.024$), 14 day PVP and LVP were 6.1 ± 0.6 mmHg and 5.4 ± 0.7 mmHg ($p=0.001$), and 21 day test group PVP and LVP were 9.5 ± 0.3 mmHg and 7.8 ± 0.3 mmHg ($p<0.001$). The absolute levels of PVP and LVP did not rise significantly from the sham level in the 10 and

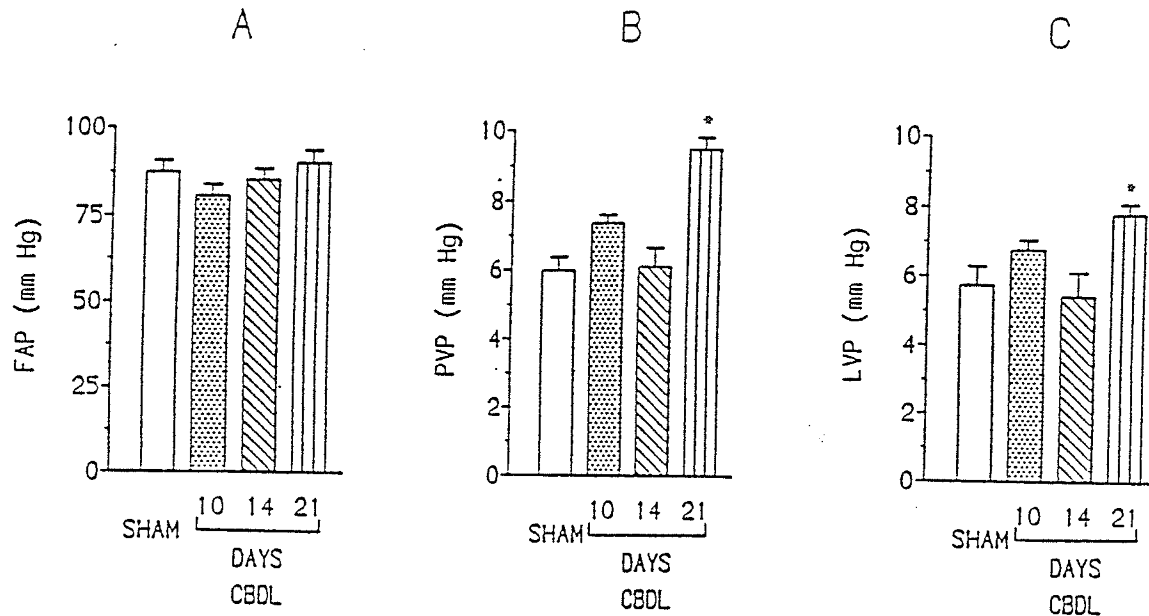


Figure 29: Basal levels of femoral arterial pressure (FAP, A), portal venous pressure (PVP, B) and lobar venous pressure (LVP, C) for the four groups of cats, sham-operated (□), 10 (▩), 14 (▧), and 21 (▥) days bile duct-ligation (CBDL). There was no significant difference in the FAP between any the groups of animals (unblocked ANOVA). FAP in the sham group was, however, lower than normal arterial blood pressure. PVP and LVP were significantly elevated from sham levels of these pressures only in the 21 day test group (unblocked ANOVA).

14 day test groups (ANOVA), although there was a slight elevation in PVP and LVP in the 10 day test group. By 21 days, however, PVP and LVP had increased such that PVP was significantly greater than the sham group ($p<0.001$), 10 day group ($p<0.01$) and 14 day groups ($p<0.001$), and LVP was significantly larger than the sham group ($p<0.05$) and 14 day group ($p<0.01$). It should be noted that PVP in the 21 day test group (9.5 mmHg) is still within normal estimates of PVP. Thus, while PVP and LVP did rise in response to bile duct-ligation they did not rise to pathological levels. CVP was unaffected by bile duct-ligation.

Pressure Gradients

Calculating the different pressure gradients between PVP, LVP, and IVCP gives us a relative indication of the degree of vascular resistance existing across the entire liver (PVP-IVCP), and whether these resistances are presinusoidal (PVP-LVP) or postsinusoidal (LVP-IVCP). The combined pre- and postsinusoidal gradients should equal the pressure gradient across the liver ([PVP-LVP] + [LVP-IVCP] = [PVP-IVCP]). The PVP-ICVP gradient is of special interest since elevations in this gradient correlate to the degree of pathology in portal hypertension and cirrhosis more so than the actual rise or absolute value of PVP. The three sets of pressure gradients of each group of animals are presented graphically in figure 30.

In sham animals, the PVP-IVCP (1.6 ± 0.3 mmHg), PVP-LVP (0.5 ± 0.2 mmHg), and LVP-IVCP gradients (1.0 ± 0.3 mmHg) were quite small which is in accordance with the low pressure/low driving pressure vascular system constituting the hepatic portal venous circulation under normal conditions.

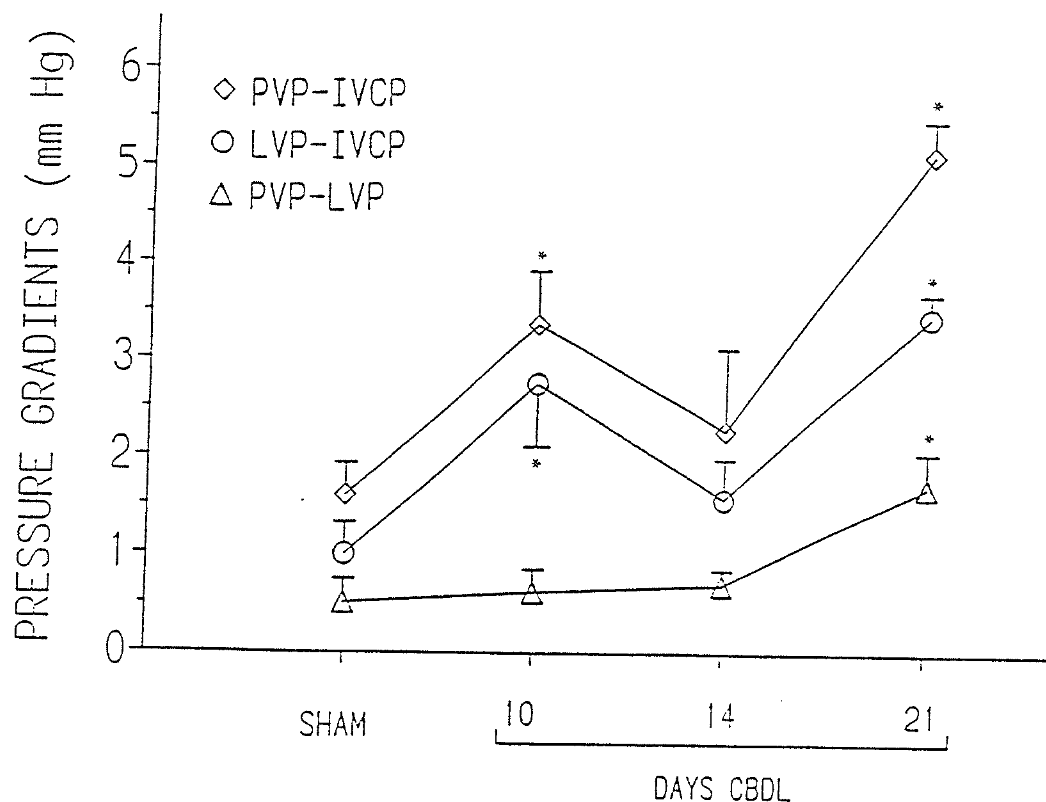


Figure 30: Basal hepatic pressure gradients calculated from PVP, LVP and IVCP for sham and test groups (CBDL). PVP-IVCP indicates the relative resistance occurring over the entire liver, PVP-LVP indicates the extent of presinusoidal resistance and LVP-IVCP indicates the relative resistance across the postsinusoidal sites. According to these data, there is a significant initial increase in the postsinusoidal resistance at 10 days, and a small amount at 14 days post-ligation (increase in the PVP-IVCP and LVP-IVCP gradient). The rise in postsinusoidal resistance fully accounts for the changes in PVP and LVP in the 10 and 14 day test groups. By 21 days post-ligation, a small, but significant degree of presinusoidal resistance has developed (increase in PVP-LVP gradient) and contributes to the rise in PVP, but not LVP (see text).

In the 10 day test group, PVP-IVCP (3.4 ± 0.6 mmHg) and LVP-IVCP (2.8 ± 0.6 mmHg) gradients were significantly increased from the sham levels. The PVP-LVP gradient for the 10 day (0.6 ± 0.2 mmHg) and 14 day test groups (0.7 ± 0.2 mmHg) were not significantly elevated from the level of the sham group (ANOVA). PVP-IVCP and LVP-IVCP gradients in the 14 day test animals returned towards, and were not significantly different from the sham gradients. However, all gradients were significantly elevated in the 21 day test groups; PVP-IVCP had increased to 5.2 ± 0.3 mmHg, the PVP-LVP gradient increased to 1.7 ± 0.3 mmHg and the LVP-IVCP gradient increased to 3.5 ± 0.2 mmHg.

The results detailed in figures 29 and 30 support previous findings that under basal conditions the main site of resistance to portal blood flow is postsinusoidal. In the sham group, postsinusoidal resistance accounted for 79.2% of the PVP-IVCP gradient (calculated as LVP-IVCP gradient/PVP-IVCP gradient x 100). Presinusoidal resistance therefore accounted for the remaining 20.8% of the PVP-IVCP gradient. At 21 days however presinusoidal resistance started to increase and the percent contribution of postsinusoidal resistance to the PVP-IVCP gradient decreased to 67.1% (presinusoidal contribution therefore increased to 32.9%). Thus, absolute values of PVP and LVP and the pressure gradient data suggest that in this model of liver disease, resistance to portal blood flow remains primarily at the postsinusoidal sites, while resistance at presinusoidal sites increases gradually and becomes significant larger at 21 days post-ligation.

Active Responses

The responsiveness of the arterial and portal venous vessels to sympathetic nerve

stimulation and norepinephrine infusion were assessed in all groups of animals. Pressures were measured at the peak of their response and are summarized in tables 9 and 10. The changes in the pressure responses for each group are illustrated in figures 31A, 32A, and 33A and summarized in tables 11 and 12.

Stimulation of the Hepatic Anterior Nerve Plexus

The absolute change in FAP in response to nerve stimulation (figure 31A, table 11) for the three test groups were significantly depressed compared to the sham but were not significantly different from each other. The 21 day group appeared to regain some responsiveness to nerve stimulation (figure 31A, table 11).

The PVP response to nerve stimulation was also significantly depressed by bile duct-ligation in the 10 and 14 day test groups compared to the sham group (table 9 and table 11, figure 32A). By 21 days, however, the responsiveness of PVP returned towards normal and the change in PVP to 8 Hz nerve stimulation was not significantly different from the sham group. The responsiveness of LVP to nerve stimulation paralleled the depressed sensitivity of the PVP and arterial responses (table 9). LVP in all test groups increased slightly during the nerve stimulation, however, except for the 21 day group these elevations were not significantly different from their pre-stimulation control levels. This finding is also reflected in the change in LVP (table 11, figure 33A). The change in LVP for the 10 and 14 day test groups are significantly less than the change in LVP for the sham group. In the 21 day test groups, the change in LVP returned towards, but, was still significantly less than the change in LVP in the sham group. Central venous pressure was unaffected by nerve stimulation.

TABLE 9

Peak responses of absolute femoral arterial pressure (FAP), portal venous pressure (PVP), and lobar venous pressure (LVP), to 8 Hz nerve stimulation in sham-operated and chronic bile duct-ligated cats

		FAP (mmHg)	PVP (mmHg)	LVP (mmHg)
Sham (n=9)	Control	83.7 ± 3.2	6.5 ± 0.4	6.2 ± 0.7
	Peak	125.2 ± 2.2 **	10.4 ± 0.7 **	8.5 ± 0.8 **
10 Days (n=4)	Control	76.1 ± 4.3	7.5 ± 0.4	6.3 ± 0.6
	Peak	86.5 ± 4.3 *	8.6 ± 1.0 NS	6.8 ± 0.8 NS
14 Days (n=6)	Control	86.6 ± 4.0	6.7 ± 1.0	5.9 ± 1.3
	Peak	98.4 ± 8.0 NS	8.2 ± 1.0 **	6.4 ± 1.2 NS
21 Days (n=8)	Control	97.6 ± 6.6	10.1 ± 0.6	8.3 ± 0.6
	Peak	114.4 ± 5.9 **	13.2 ± 0.8 ***	9.3 ± 0.8 *

All reported values are the mean ± SEM. Pre-stimulation control values and peak constriction values, within each group, were compared by paired analysis. Control values were measured just prior to nerve stimulation and are not the same values reported for the basal data. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS, not significantly different from control.

TABLE 10

Peak responses of absolute femoral arterial pressure (FAP), portal venous pressure (PVP), and lobar venous pressure (LVP), to a 0.5 µg/kg/min norepinephrine infusion into the portal vein in sham-operated and chronic bile duct-ligated cats

		FAP (mmHg)	PVP (mmHg)	LVP (mmHg)
Sham (n=5)	Control	83.6 ± 3.0	5.4 ± 0.5	5.9 ± 0.5 $^{\Phi}$
	Peak	117.7 ± 6.5 *	8.1 ± 0.6 **	7.4 ± 0.5 ***
10 Day (n=4)	Control	73.5 ± 5.1	6.9 ± 0.1	5.8 ± 0.5
	Peak	99.3 ± 6.9 *	9.9 ± 0.3 **	7.1 ± 0.1 *
14 Day (n=6)	Control	84.7 ± 6.0	6.1 ± 1.2	5.4 ± 1.4
	Peak	108.6 ± 6.0 **	8.7 ± 0.9 ***	6.7 ± 1.2 *
21 Day (n=8)	Control	97.1 ± 6.1	9.7 ± 0.5	7.8 ± 0.6
	Peak	131.0 ± 6.5 ***	14.8 ± 0.9 ***	10.6 ± 1.0 ***

All reported values are the mean ± SEM. Pre-infusion control values and peak values, measured during norepinephrine infusion, within each group, were compared by paired analysis. Control values were measured just prior to norepinephrine infusion and are not the same values reported for the basal data. Norepinephrine was administered as a constant infusion into the portal vein. $^{\Phi}$, n=3, * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS, not significantly different from control.

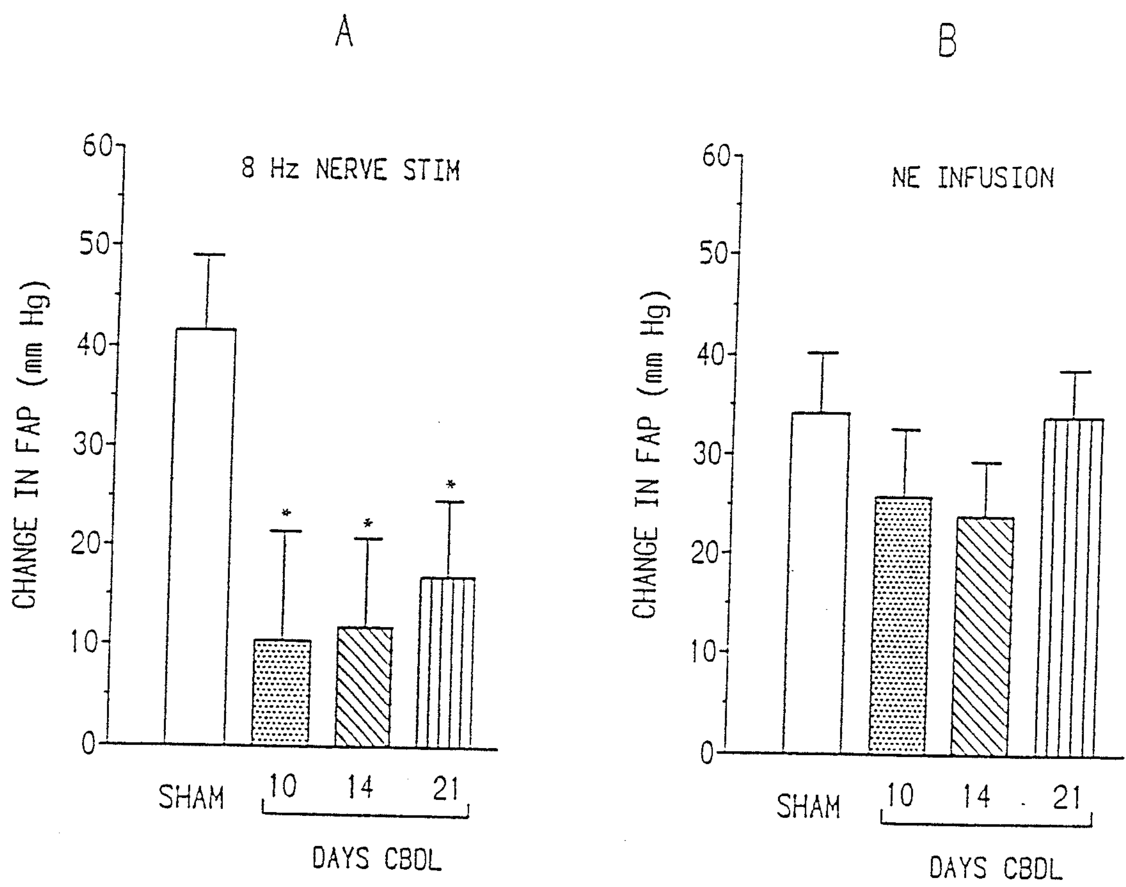


Figure 31: Changes in femoral arterial pressure (FAP) in sham-operated (□), and 10 (▨), 14 (▧), and 21 (▥) day bile duct-ligated test animals (CBDL), in response to 8 Hz nerve stimulation (A) and 0.5 µg/kg/min infusion of norepinephrine into the portal vein (B). Chronic bile duct-ligation significantly depressed the FAP response to nerve stimulation in all 3 test groups (unblocked ANOVA), but had no effect on norepinephrine-induced FAP responses.

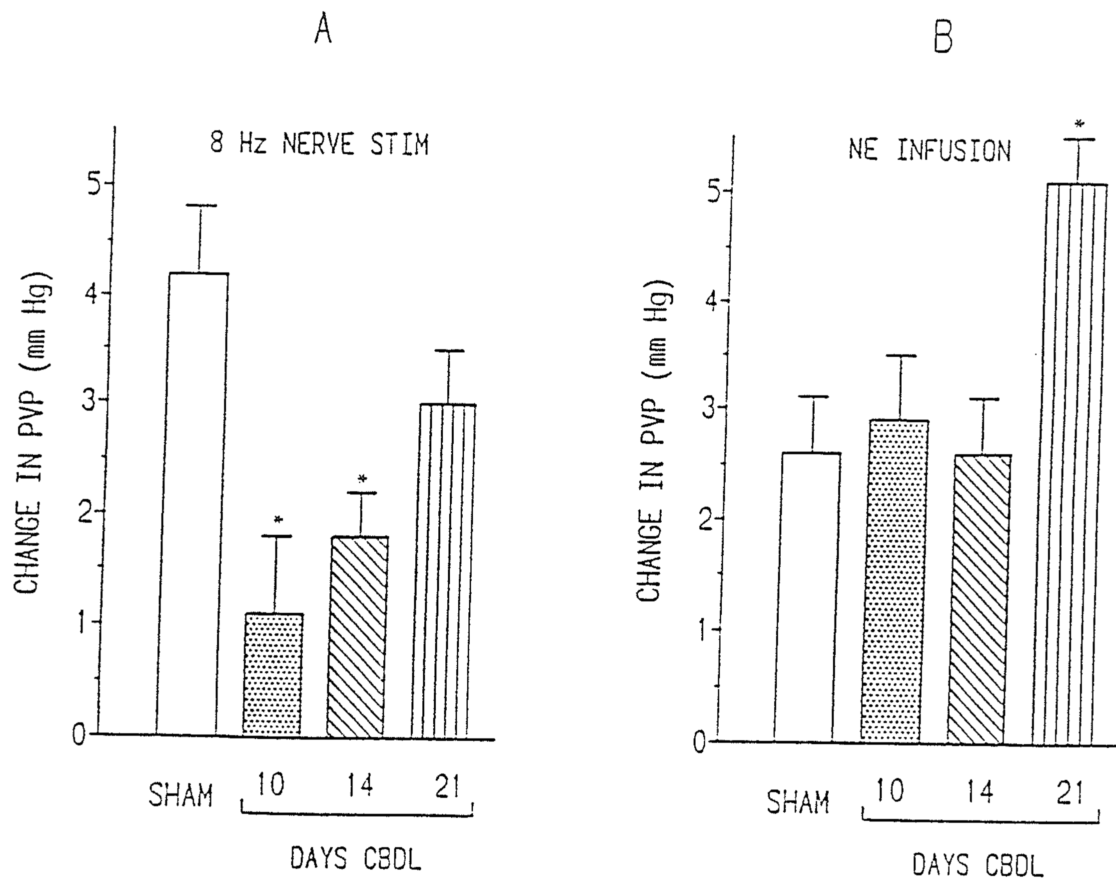


Figure 32: Changes in portal venous pressure (PVP) in sham-operated (□), and 10 (▩), 14 (▧), and 21 (▥) day bile duct-ligated test animals (CBDL), in response to an 8 Hz nerve stimulation (A) and 0.5 µg/kg/min infusion of norepinephrine into the portal vein (B). Responses to nerve stimulation were significantly depressed in the 10 and 14 day test groups. By 21 days post-ligation, PVP responses had partially returned and were not statistically different from the sham responses. PVP responses to norepinephrine (B), were unaffected by biliary obstruction in the 10 and 14 day test groups, but displayed a significantly potentiated response in the 21 day test group.

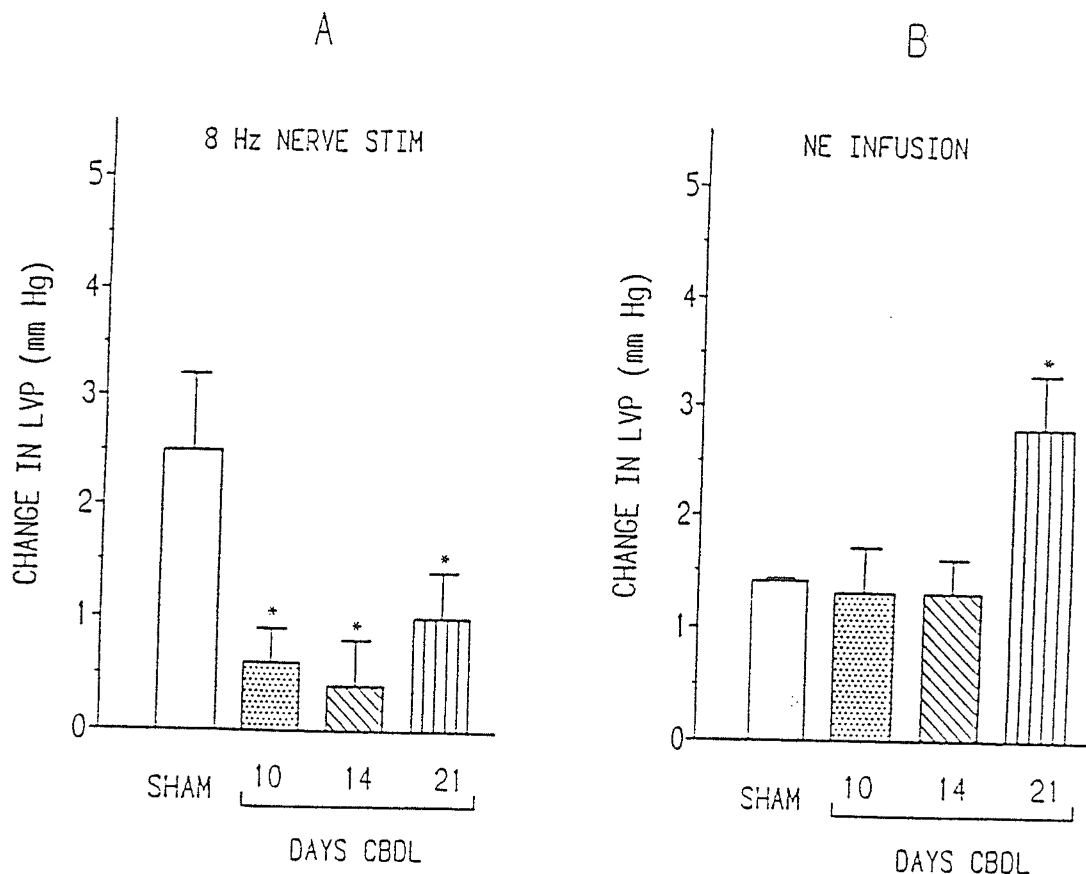


Figure 33: Changes in lobar venous pressure (PVP) in sham-operated (□), and 10 (▩), 14 (▧), and 21 (▥) day bile duct-ligated test animals (CBDL), in response to an 8 Hz nerve stimulation (A) and 0.5 µg/kg/min infusion of norepinephrine into the portal vein (B). Like FAP and PVP responses, LVP responses to nerve stimulation were significantly depressed in the 10, 14 and 21 day test groups. Unlike the PVP responses, the LVP responses did not make a significant recovery in the 21 day test group, suggestive of a prolonged, functional, postsinusoidal lesion. LVP responses to norepinephrine were also unaffected in the 10 and 14 day test groups, but demonstrated a potentiated response in the 21 day test group.

TABLE 11

Values for the changes in femoral arterial pressure (FAP), portal venous pressure (PVP), and lobar venous pressure (LVP), in response to 8 Hz frequency nerve stimulation in sham-operated and chronic bile duct-ligated cats

	FAP (mmHg)	PVP (mmHg)	LVP (mmHg)
Sham (n=9)	41.6±11.4	4.2±1.1	2.5±0.7
10 Day (n=4)	10.4±3.2*	1.1±0.7*	0.6±0.3*
14 Day (n=6)	11.8±6.3*	1.8±0.4*	0.4±0.4**
21 Day (n=8)	16.9±4.7*	3.0±0.5	1.0±0.4*

All reported values are the mean ± SEM. Values for the sham, 10, 14, and 21 day test groups, for each parameter, were compared by unblocked ANOVA and statistical significance is noted for the test group compared to the sham group. There were no significant difference between the 10, 14, and 21 day groups for any parameter. *, $P<0.05$; **, $P<0.001$.

TABLE 12

Values for the changes in femoral arterial pressure (FAP), portal venous pressure (PVP), and lobar venous pressure (LVP), in response to a 0.5 µg/kg/min norepinephrine infusion into the portal vein in sham-operated and chronic bile duct-ligated cats

	FAP (mmHg)	PVP (mmHg)	LVP (mmHg)
Sham (n=5)	34.1±8.1	2.7±0.3	$1.4 \pm 0.03^{\Phi}$
10 Day (n=4)	25.8±5.8	2.9±0.4	1.3±0.4
14 Day (n=6)	23.9±4.4	2.6±0.4	1.3±0.4
12 Day (n=8)	33.9±4.6	$5.1 \pm 0.6^{*\alpha}$	$2.8 \pm 0.5^{*\beta}$

All reported values are the mean ± SEM. Values for the sham, 10, 14, and 21 day test groups, for each parameter, were compared by unblocked ANOVA and statistical significance is noted for the test group compared to the sham group. *, $P<0.05$; $^{\alpha}$, significantly greater than sham, 10 and 14 day groups; $^{\beta}$, significantly greater than 10 and 14 day groups; $^{\Phi}$, n=3.

Effect of Exogenous Norepinephrine

Unlike the nerve stimulation responses, the responsiveness of FAP, PVP, and LVP to norepinephrine was not depressed as a result of bile duct-ligation. The peak pressure values are detailed in table 10 and the changes in pressures are reported in table 12. These responses are also illustrated in figures 31B, 32B, and 33B. The change in FAP responses for the test groups were not significantly different from the sham group and in all cases the peak FAP was significantly different from the pre-stimulation control values.

Sensitivity of the portal venous system to norepinephrine, represented by the change in PVP and LVP, was also not depressed as a result of bile duct-ligation (table 10 and 12, figures 32B and 33B). While the change in PVP and LVP were not significantly different from the respective sham groups in the 10 and 14 day test groups, the 21 day test group appeared to be supersensitive to the effects of exogenous norepinephrine. The change in PVP doubled from 2.7 ± 0.3 mmHg in the sham group, to 5.1 ± 0.6 mmHg in the 21 day group in response to the same dose of norepinephrine and was significantly larger than the sham, 10 and 14 day test group responses. The change in LVP also doubled from 1.4 ± 0.03 mmHg in the sham group to 2.8 ± 0.8 mmHg in the 21 day test group and this change was significant from the 10 and 14 day groups but not the sham group. It, therefore, appears that bile duct-ligation is affecting some aspect of the sympathetic system or hepatic vasculature such that nerve responses are initially impaired but appear to regenerate and a supersensitivity to exogenous norepinephrine develops by 21 days.

Correlations Between Serum Biochemistries and Hemodynamic Parameters

Correlations were conducted between the plasma biochemical findings and hemodynamic parameters of FAP, PVP, LVP, and the pressure gradients PVP-ICVP, LVP-IVCP, and PVP-LVP, to determine if there were significant relationships between the plasma biochemistries and the vascular disruptions. Although there were significant elevations in the serological indices of liver damage, these values did not correlate with any of the pressures or pressure gradients.

Inspection of Internal Organs

Upon completion of the experiments, the heart, kidneys, and liver of each cat were removed, blotted dry, and weighed. The mean weight of the heart from the sham, 10, 14, and 21 day test groups were 7.92 $\pm$ 0.53 g, 7.50 $\pm$ 0.43 g, 7.59 $\pm$ 0.52 g and 7.28 $\pm$ 0.76 g, respectively, and were not significantly affected by bile duct-ligation. Kidney weights were also unaffected by bile duct-ligation. Sham-operated animals had an average single kidney weight of 13.47 $\pm$ 1.27 g, 10 day tests were 12.86 $\pm$ 1.77 g, 14 day tests were 15.78 $\pm$ 1.3 g, and 21 day kidneys weighed 13.77 $\pm$ 1.75 g. Kidneys from the test animals did, however, gain a greenish-yellow coloration with some kidneys possessing dark green localized blotching on the surface which transcended through to the medullary region. No other signs of disease or pathology were observed.

Absolute liver weight and the percent of total body weight accounted for by the liver in all three test groups were significantly larger from the sham group and are listed in table 13. Liver weight and the percent of body weight were maximal in the 14 day group. The

21 day group liver weight and the percent of body weight value decreased slightly but were not significantly different from the 14 day group. This data suggests that there may have been a duration-dependent effect of bile duct-ligation on the liver that peaked at 14 days and possibly began a recuperation to some extent (although not significantly) by 21 days.

TABLE 13

Liver weights[α] and percentage of body weight for sham-operated, 10, 14, and 21 day chronic bile duct-ligated cats

	Liver Weight (g)	% of Body Weight
Sham (n=9)	71.8±3.7	2.30±0.18
10 Day (n=8)	98.4±6.5*	3.20±0.17*
14 Day (n=7)	114.8±7.0**	3.62±0.23**
21 Day (n=6)	104.5±4.5*	3.19±0.40*

All reported values are the mean ± SEM. % of body weight was calculated as (liver weight/body weight) x 100%. Statistical comparisons between the sham, 10, 14, and 21 day test groups were by unblocked ANOVA. Statistical significance is noted for the test group compared to the sham group. There were no significant difference between the 10, 14, and 21 day groups. [α], liver was weighed only after the acute experiments. *, <0.05; **, $P<0.001$.

DISCUSSION

Chronic bile duct-ligation (CBDL) in experimental animals has been characterized as a model of portal hypertension (PHT) in the dog by Bosch et al. (1983). In this investigation, dogs were reported to have liver cirrhosis, systemic hypotension and a significant degree of portal-systemic shunting, an etiology closely resembling that of cirrhosis and PHT in man. Another finding in experimental animals with liver disease and PHT is a reduced end-organ responsiveness to norepinephrine (reviewed by Bomzon, 1990). This is, however, a controversial issue, with reports of normal, potentiated and diminished vascular reactivity from a variety of vessels from different species. Part of the confusion exists due to comparisons of vascular reactivity from animals with varying periods of biliary obstruction.

Traditionally, CBDL has been conducted in dogs and rats. The cat has been used to study the serological effects of obstructive jaundice, but nothing is known about the effects of total biliary occlusion on the hepatic circulation in this species. Thus the main objective of this investigation was to determine whether CBDL could produce PHT and be an effective and acceptable research model of PHT in the cat over a 3 week period. Another objective of this study was to identify the sites of increased vascular resistance within the hepatic vasculature. A final objective was to assess the vascular reactivity of the portal and hepatic venous vascular beds in response to infused norepinephrine and direct electrical stimulation of the hepatic anterior nerve plexus. It was also of interest to re-assess the serological and histological changes occurring over the 3 week period of biliary obstruction.

Because this was an initial investigation, blood flows were not measured. By

measuring PVP and intrahepatic pressure (LVP), it was possible to identify the general location of changes in resistance occurring in the hepatic vascular bed without calculating the actual resistance. Moreover, by avoiding the extra surgery involved with measuring blood flow, the animals were subjected to less surgical trauma.

In this study, PVP and LVP were significantly elevated after 21 days of complete biliary obstruction, but were still within the normal range for these pressures; PHT did not develop over this period. These animals did, however, show signs of hepatic dysfunction, severe disruptions of normal liver structure and architecture, and a selective impairment of hepatic neural functions. These animals also demonstrated features often associated with PHT, such as a reduced vascular reactivity to nerve stimulation, despite the absence of PHT. At the same time, however, a potentiated vascular reactivity to norepinephrine occurred. Thus, although PHT did not occur, an intriguing set of serological, histological and cardiovascular lesions developed, which will be addressed in the following discussion.

Serological and Histological Alterations

The elevation of the standard serological indices of liver damage, ALT, AST, GGTP, alkaline phosphatase and cholesterol in the test animals is consistent with the development of liver injury and biliary tract hyperplasia. The elevated levels of these enzymes in the test animals were in excellent agreement with previously published values for cats that underwent biliary obstruction (Center et al., 1983). The enzymes, however, are not sensitive indicators of liver damage and are used clinically to indicate primarily the presence, not necessarily the degree of damage. Moreover, these enzymes are not necessarily specific for liver damage

and become elevated upon muscle and bone injury (McIntyre, 1983).

Direct and total bilirubin were significantly elevated in each test group compared to the sham-operated animals, but levels were not different between the test groups. Bilirubin levels had, therefore, reached a plateau level within the first 10 days after bile duct-ligation.

Glucose levels were found to be significantly greater in all post-ligation test plasma samples (taken on the day of acute experimentation) including that for the sham-operated cats, which were, in fact, the highest levels recorded. It is possible that this may represent a stress-induced hyperglycemic response caused by the acute experimental surgery since these blood samples were taken from an indwelling cannula rather than from a veno-puncture technique used in the initial ligation or sham surgery. It is also an interesting observation that the elevated glucose levels recorded in the post-ligation test samples progressively declined from the sham-operated group (highest) to the 21 day test group (lowest) suggesting that this hyperglycemic response may also be undergoing a time-dependent impairment. It is known that patients with cirrhosis have impaired gluco-regulatory functions possibly due to insulin and glucagon resistance (Yeung and Wang, 1974; Silva et al., 1988). The causes of these resistances is not known, but glucagon and insulin binding have been reported to be decreased in bile duct-ligated rats (Sakai, 1992). In a recent publication by Lee et al. (1992a), an absence of hepatic parenchymal nerves in cirrhotic human livers and a reduced innervation in pre-cirrhotic livers was found. It is reasonable to surmise that a dysfunctional or diminished hepatic nervous system may play a role in the disrupted glucose metabolism. Progressive parenchymal cell injury under conditions of CBDL may also account for the observed decline in the glucose response. Indeed, the serological data indicate that CBDL

is capable of producing liver dysfunction.

The histological changes in the livers of the test animals are consistent with those reported by other investigators (Bosch et al., 1983; Del Rio Lozana and Andrews, 1965). Although CBDL is capable of producing liver cirrhosis, histological evidence from this investigation suggests that the test animals were in a pre-cirrhotic state, evidenced by the lack of nodule formation (Sherlock, 1989). Extensive collagenization and fibrogenesis around the dilated, hypertrophied and hyperplastic bile ducts was evident, even in the 10 day test group. Qualitative assessment did indicate that fibrosis tended to be localized to portal triads in the early stages of obstruction and advanced to bridging and extensive interlobular fibrosis in the 21 day group. Because the hepatic injury was not quantitated, it was not determined statistically if the fibrosis and structural damage was a time-dependent phenomenon, although this type of pathology is clearly of a progressive nature. Thus, the serological and histological data indicate that in the cat, CBDL causes a rapid and extensive degree of hepatic structural and parenchymal damage that is comparable to that reported for dogs, rats and man with biliary obstruction. Furthermore, the hepatic insult is well developed by 10 days post-ligation although by 3 weeks the test animals were still in a pre-cirrhotic stage of pathology.

Effect of CBDL on the Hepatic and Systemic Vasculature

a) Effect on Basal Portal and Lobar Blood Pressures

In normal animals under basal conditions, PVP and LVP are not significantly different from each other, implying that the major site of resistance to portal and hepatic

blood flow is at a postsinusoidal site and that presinusoidal resistance is negligible (Lautt et al., 1986). Measurements of PVP and LVP in the sham-operated group (figures 28, 29b and 29c) and calculation of the relative contribution of postsinusoidal resistance to the overall trans-hepatic pressure gradient (79.2%) fully support this concept.

CBDL caused the basal PVP and LVP to rise in somewhat of a time-dependent manner although only the 21 day test group had pressures significantly raised above the sham-operated group. Because LVP is a measure of intrahepatic pressure rather than PVP (Lautt et al., 1986; Legare and Lautt, 1987), it is possible to determine whether the increased resistance within the hepatic vascular bed is pre- or postsinusoidal. Changes in LVP paralleled those of PVP with no change in the PVP-LVP gradient for the 10 and 14 day test groups (figure 30). Thus, the changes in PVP could be fully accounted for by the changes in LVP, suggesting that the majority of the change in vascular resistance to portal and hepatic blood flow was taking place at a postsinusoidal site. CBDL increased presinusoidal resistance in the 21 day test group, indicated by a significant increase in PVP over LVP, and the relative contribution of presinusoidal resistance to the trans-hepatic pressure gradient increasing to 32.9%. This is best illustrated by the PVP-LVP gradient depicted in figure 30. Thus, this model of CBDL develops an initial increase in resistance at a postsinusoidal site, followed by an increase in the presinusoidal component. Postsinusoidal resistance still constitutes the major site of resistance to portal and hepatic blood flow. Other investigators have reported postsinusoidal (Lebrec et al., 1976; Franco et al., 1979; Bosch et al., 1983) and presinusoidal (Sherlock, 1959; Trams and Symeonidis, 1957) sites of increased resistance in humans with primary biliary cirrhosis and in experimental animals with biliary obstruction.

The mechanisms by which resistance increases in the liver has been a matter of debate. It was previously believed that the increase in intrahepatic resistance in cirrhosis was due to the increase in fibrosis and nodule formation (Popper and Zak, 1958). This theory has not been supported by the empirical data (Lebrec et al., 1976; Sherlock et al., 1959). PVP has also not correlated with the degree of fibrosis (Krogsgaard et al., 1984). Orrego et al. (1981) and Blendis et al. (1982), support the concept that enlarged hepatocyte surface area is the cause of intrahepatic resistance and PHT. It must be emphasized that, despite the large degree of portal tract fibrosis incurred by the test animals, PVP was only 9.5 $\pm$ 0.3 mmHg in the 21 day test group, a value which is still within the normal range for PVP. Although fibrosis was not quantitated in this study, the PVP data from the current investigation would tend to support the finding by Krogsaard et al. (1984) that hepatic fibrosis does not correlate well with the elevation of PVP. This does not imply that the collagenization and fibrosis had no influence on intrahepatic resistance. Portal and hepatic veins were found to be fibrosed and the postsinusoidal resistance sites were lengthened in the 21 day test animals. It is possible that these events may have contributed to the rise in pre- and postsinusoidal resistance by altering the distensibility of the resistance sites and impairing the ability of the liver to autoregulate PVP and LVP. It should also be noted that in a recent study from our laboratory, hepatic venous compliance was not altered after 2 weeks of complete biliary obstruction (Schafer et al., submitted).

The fluctuation in PVP and LVP between the 10 and 14 day test animals is difficult to explain, but may simply be due to transient changes taking place in the sinusoids or parenchyma, such as inflammatory responses which were noted in the histological analysis

of these animals. This early stage after bile duct-ligation has been referred to as a "volatile" stage since these animals are undergoing many simultaneous changes in their cardiovascular system, making this a difficult period to assess (Bomzon and Blendis, 1990).

The hepatic venous pressure gradient (HVPG), calculated as the difference between PVP and the inferior vena caval pressure (IVCP) has been a poorly reported pressure index in most studies of experimental PHT. This is surprising since the HVPG is as important as the measurement of PVP when determining the severity of PHT. In humans, an HVPG of 10 mmHg is considered necessary for variceal development (Sherlock, 1989) and a gradient of 12 mmHg or greater is considered life-threatening due to the distinct possibility of variceal rupture (Bosch et al., 1989). The peak HVPG measured in this investigation averaged 5.2 ± 0.3 mmHg in the 21 day group, having increased significantly from 1.6 ± 0.3 mmHg in the sham-operated group. Bosch et al. (1983), reported an average HVPG of 11 mmHg in CBDL dogs after 8 weeks of obstruction. It is possible that if the duration of obstruction had been extended in this study, greater elevations in PVP and HVPG may have occurred. Other studies of CBDL of comparable or longer duration in the dog and rat have reported PVP in the range of 13 to 17 mmHg (Ohlsson et al., 1970a; Franco et al., 1979; Bosch et al., 1983), although Mathie et al. (1988) recorded a peak PVP of only 6.4 mmHg after 2 weeks of CBDL in greyhounds. According to Bomzon and Blendis (1990), PVP in the dog may still be within normal ranges after 3 weeks of biliary obstruction, after which it may begin to rise. However, by 3 weeks post-ligation, the clinical condition of the animals in this investigation were becoming quite severe, despite a rigorous maintenance protocol. Indeed, there were also animals in the 14 day test group whose condition had

severely deteriorated. It was mutually agreed upon by all parties involved in this investigation that prolongation of biliary obstruction past 3 weeks would be unethical. Thus, the clear conclusion from the venous pressure measurements is that, despite statistically significant elevations in PVP and the HVPG, PHT had not developed by the third week of complete biliary obstruction.

b) Effect on Basal Systemic Arterial Blood Pressure

It has been reported that systemic arterial pressure may be reduced in animals with PHT (Bomzon and Blendis, 1990) and cholemia (Green et al., 1984), but this is not definitive. The hypothesis most commonly cited to explain the reduced vascular tone and blood pressure is the development of a hyperdynamic circulation caused by an increase in the concentration of a circulating dilator substance that can antagonize endogenous pressor agents or reflexes. Glucagon has been viewed as the most likely candidate substance for this theory (Benoit et al., 1986).

Arterial pressure in all 3 groups of test animals was reduced in this study. Arterial pressure in the sham-operated group was unexpectedly reduced below normal levels (100-120 mmHg) and was not significantly different from the test groups. These data strongly suggest that some aspect of the protocol, unrelated to biliary obstruction, was capable of producing arterial hypotension in the sham-operated group and possibly affected the test groups as well. It is possible that some aspect of the initial (set-up) or acute experimental surgery was involved. Pentobarbital anesthesia is known to have hypotensive effects which might be enhanced in animals with liver damage. However, the amount of anesthetic administered

during the acute experimental surgery was tailored to the needs of the individual animal and, therefore, the depth of anesthesia throughout the experiments was consistent between the sham-operated and test animals. The experimenter was also well experienced with using pentobarbital and neither sham-operated animals nor test animals were overdosed with anesthetic.

Dehydration may have contributed to the hypotension in the test and sham-operated animals. Cholemia is known to induce a diuresis that can lead to volume depletion (Green et al., 1984). Most test animals displayed some degree of dehydration and, although they were treated with subcutaneous fluid supplements, many animals remained dehydrated. This mechanism cannot explain the hypotension occurring in the sham-operated animals, and therefore, this group may have suffered from a reduced fluid intake during the 10 day recovery period.

Effect of CBDL on Vascular Responses to Sympathetic Stimulation

The dose of norepinephrine used in this investigation (0.5 μg/kg/min) is equal to the ED_{42} for the change in portal venous resistance. This estimate was derived from a previous study that used nonlinear regression to calculate the pharmacodynamic estimates of Rmax and the ED_{50} for the change in portal venous resistance in response to norepinephrine (Lautt and Legare, 1991). Nerve frequency-response data for portal venous resistance is not available. However, the 8 Hz frequency stimulation - equivalent to a Hz_{80} in the superior mesenteric artery (Lockhart et al., 1988) - was used to improve the chance of producing a detectable response if CBDL severely inhibited the nerve-induced responses. Thus, by using

the 0.5 μg/kg/ml dose of norepinephrine and the 8 Hz frequency nerve stimulation in all animals, conclusions can be drawn with reasonable certainty, regarding the effect of CBDL in the hepatic vascular bed.

CBDL produced a selective inhibition of the nerve-mediated constrictions in the hepatic venous bed (PVP and LVP responses) and was reflected in the systemic arterial responses (FAP responses; figures 31, 32, 33), despite PHT not being present. These responses were affected most profoundly in the 10 day test group for all pressures recorded, and appeared to undergo a time-dependent or partial compensation. This was most evident for the PVP responses in the 21 day test group. Impairment of the PVP responses initially affected both the pre- and postsinusoidal resistance sites, but a compensatory process appeared to occur at the presinusoidal sites, evidenced by a progressive return of the PVP-LVP gradient responses towards the control responses, while the LVP-IVCP gradient reactivity remained impaired (figures 30, 32A and 33A).

FAP, PVP and LVP responses to norepinephrine, on the other hand, remained intact in all test groups. The PVP and LVP responses to the ED_{42} dose of norepinephrine in the 10 and 14 day test groups were not significantly different from the responses in the sham-operated group. Thus it is possible to conclude that the potency of norepinephrine in these test animals was not affected by CBDL. PVP and LVP responses demonstrated a potentiated response in the 21 day test animals which was consistent with the timing of the compensatory process for the nerve-mediated responses. These results also indicate that the CBDL-induced impairment of the responses in this investigation is related primarily to the hepatic nerves, rather than the hepatic or systemic vasculature.

The reduced reactivity of the systemic arterial circuit to the nerve-mediated responses is most likely related to a blunted constrictor response in the hepatic arterial circuit or in the hepatic capacitance vessels, resulting in less blood flow redistribution or blood volume mobilization into the arterial circuit during nerve stimulation. This is consistent with the findings of Aarseth et al. (1979), who reported a significantly reduced redistribution of splanchnic blood volume after a small (10%) hemorrhage in 7 day bile-duct ligated rats. Other investigators have found impaired blood volume responses in rats (Findberg et al., 1982) and dogs (Williams et al., 1960) subjected to biliary obstruction. A recent investigation from this laboratory found that in 14 day CBDL cats, hepatic blood volume compensation for hemorrhage was reduced by approximately 50% (Schafer et al., submitted). This same study also revealed a selective impairment of nerve-mediated hepatic blood volume responses, with exogenous norepinephrine-induced responses remaining intact, a finding that is consistent with the data from the present investigation.

There are many possible explanations for the diminished nerve-mediated responses. Cholemia has long been know to have detrimental effects on the cardiovascular system (Williams et al., 1960; Aarseth et al., 1979; Findberg et al., 1981, 1982; Green et al., 1986; Blendis and Bomzon, 1990) but the mechanisms have not been well understood. It is known that bilirubin and bile salts are capable of disrupting cellular and subcellular membranes, and have deleterious effects on normal membrane function and structure (Zetterstrom and Ernster, 1956; Yamamoto et al., 1978; Kanai et al., 1991). Various receptor types have also been shown to be affected under conditions of elevated bilirubin. Lee et al. (1990) reported a functional impairment of cardiac chronotropic responses resulting from a reduction in β_1-

adrenergic receptors in bile-duct ligated rats. Likewise the Bmax for hepatic parenchymal α_1-adrenergic receptors has been reported to be significantly reduced 2 days after biliary obstruction in rats (Aggerbeck et al., 1983). However, the fact that norepinephrine-induced responses in the current study were fully intact intimates that the vascular smooth muscle was not influenced significantly by cholemia, per se. This strongly points to other mechanisms to explain the diminished reactivity to nerve stimulation.

Structural changes within the liver, as a basis for the selective impairment of nerve-mediated responses, merits consideration. The rapid enlargement of the portal and hepatic veins and the proliferation of ductular tissue may have expanded the area and mass of these regions, such that the number of receptors relative to the tissue mass was reduced. A similar hypothesis was suggested by Jensen et al. (1987) to account for the reduced effects of isoproterenol and serotonin in enlarged mesenteric and esophageal veins from portal hypertensive rabbits. In other studies, norepinephrine-induced hepatic capacitance responses remained intact while nerve-mediated responses were significantly blunted in normal animals with acutely raised hepatic venous pressure (Lautt et al., 1980; Greenway, 1981, 1987). According to Greenway (1987), distension of the liver (as a result of the elevated venous pressure) may alter the anatomical relationship between the sympathetic nerve varicosities and the smooth muscle and\or the amount of norepinephrine released at the these sites. LVP (intrahepatic pressure) and IVCP, however, were not raised above normal levels, thereby excluding elevated pressure, per se, as a cause of the observed neural lesion. Distension and enlargement of the test livers did occur in the present study, as detected by a congested appearance, rounded lobe edges and a significant increase in liver weight (calculated as

absolute and % of total body weight). There is also neurohistochemical evidence suggesting that sympathetic and cholinergic neurons are absent in the liver 4 days after bile duct-ligation in the guinea pig (Ungvary and Donath, 1975). However, by 14 days these neurons could again be demonstrated in expanded interlobular connective tissue. If this phenomenon can be extrapolated to the cat, it may explain, not only the diminished nerve-mediated responses, but also the regeneration of these responses, particularly for PVP. Whether innervation of the hepatic venous sphincters is permanently affected is not certain, but clearly there was a prolonged functional lesion occurring in the LVP responses.

The cause of the enhanced potency of norepinephrine in the 21 day test animals is not clear. CBDL may have caused a supersensitivity of α-adrenergic receptors, possibly by a decreased hepatic clearance of an unknown substance. Alternatively, up-regulation of these receptors on hepatic vascular smooth muscle, in response to the initial disappearance of the hepatic nerves or expansion of the portal tract tissue, could possibly explain the temporal sequence of events culminating in the potentiated norepinephrine responses. This, however, is highly speculative. Furthermore, elevated levels of circulating norepinephrine is a consistent finding in patients and experimental animals with PHT and cirrhosis. The increased norepinephrine, in theory, would tend to produce a down-regulation of adrenergic receptors. However, whether the levels of this amine were high enough to produce receptor down-regulation after 21 days is not known and is not consistent with the results from this study. The potentiated reactivity of the hepatic vasculature does not necessarily represent the final state of these vessels or that of the systemic arterial vessels. It is very possible that with prolongation of the cholestasis and increases in hepatic injury, further alteration may

occur in the reactivity of these vessels.

A final comment on the development of this model is required. Of the numerous studies using CBDL as a model of cirrhosis, obstructive jaundice or PHT, very little is reported about the clinical condition of the animals and whether any measures were taken to maintain their health. In this investigation, medical attention required by the cats was dealt with promptly and animals were maintained and monitored on a daily basis (exercise and play, grooming, housing, diet). Whether our efforts to maintain the health of the cats was responsible for the absence of PHT is not easily determined. Nevertheless, CBDL still severely affected the clinical condition of several animals in the 14 and 21 day test groups. It is possible that the cat is especially sensitive to this type of pathology, but it is surprising that dogs have been able to withstand the prolonged exposure to biliary obstruction that have been reported (3-6 months). It is very likely that of the few animals that survive the duration, they are moribund. The data from this investigation indicate that a 3 week duration of CBDL in the cat is not an effective model of PHT, and exposing experimental animals to long periods of CBDL and the ensuing discomfort is unnecessary. In my opinion, findings from this study also raise questions about the validity of the results from previous investigations of this nature, due to the possible condition of the animals. It is interesting, however, that despite the absence of PHT, cardiovascular complications often associated with PHT were observed. Moreover, these effects appear to be most profound in the early stages of CBDL (10 days after obstruction), when the physical condition of the animals has not yet deteriorated.

SUMMARY

In summary, a 21 day duration of CBDL did not produce PHT in cats, although basal PVP, LVP and the HVPG were significantly elevated from levels in the sham-operated cats. These pressures may have reached pathological levels by extending the duration of CBDL, but it was the opinion of the all the investigators involved that this would subject the animals to unnecessary suffering. CBDL did produced liver injury and dysfunction in all test animals and severe wasting in a number of cats exposed to 14 and 21 days of biliary obstruction. A selective impairment of the hepatic nerves was observed, although this lesion may have been only transient. It is of interest, however, that the development of extensive fibrosis, morphological disruptions and biochemical dysfunction occurred in the absence of greatly elevated hepatic vascular resistance. Nevertheless, the conclusion from this investigation is that CBDL in the cat is not an effective model of PHT, but it may be useful for the investigation of the neural dysfunction and fibrogenesis associated with short term (10 day) biliary obstruction.

GENERAL SUMMARY

The studies presented in this thesis addressed several aspects of the hepatic and mesenteric vascular bed. Using glucagon as a research tool in the initial investigations, new methodologies were developed for the investigation of the pharmacology of the hepatic vascular bed. Using these new techniques, the pharmacodynamic estimates of Rmax and ED_{50} for glucagon in the hepatic artery were determined for the first time without the confounding influence of the hepatic arterial buffer response. By conducting comparative studies in the mesenteric vascular bed, it was determined that the hepatic artery was approximately 9 times more sensitive to glucagon, whereas the maximum dilation in the mesenteric artery, in response to glucagon, was double that of the hepatic artery.

Having assessed the pharmacology of glucagon in these two vascular beds, functional aspects of this agent in the vascular system could be addressed. In particular, the effect of glucagon on the regulatory systems of the hepatic and mesenteric vascular beds were investigated. Glucagon was without effect on the adenosine-mediated, intrinsic regulatory mechanism of the HA, the hepatic arterial buffer response. Thus, glucagon can be used in future pharmacological studies in this vessel without interfering with this control system. In respect to the extrinsic control system of the hepatic and mesenteric arterial vessels (neural and humoral), glucagon was not found to be an inhibitory modulator of nerve- and norepinephrine-induced peak constrictor responses in either arterial vascular bed, which is in contrast to previous reports. Furthermore, it is highly unlikely that this peptide has any significant vascular effects at physiological or even pathophysiological levels. Interestingly, although glucagon had very little or no effect on the peak constrictor response, it did

significantly inhibit the vascular escape response from peak constriction in the hepatic artery in a dose-dependent manner and at a post-synaptic site. By virtue of the fact that this effect of glucagon was not present in the mesenteric vasculature, it suggests that the actions of glucagon on the hepatic artery may be related to a glucagon-liver interaction whereby the liver releases an inhibitory factor or modifies the blood in a manner capable of inhibiting vascular escape.

In the vascular escape studies, it was also determined that conductance was superior to resistance as an index of vascular tone with which to quantitate the escape phenomenon in arterial vessels, in vivo. This stems from the linear relationship between conductance and blood flow and the ability to carry out mathematical manipulation of the conductance data. The exponential relationship between resistance and blood flow does not permit such manipulation of the data. Attempts to carry out simple arithmetic functions using resistance, such as vascular escape indexes, was shown to introduce errors into the calculated values, which may be a cause of some of the discrepancies found in previous investigations.

In the final research series, the aim of the investigation was to study the portal and hepatic venous system of the liver, rather than the arterial vasculature (ie., the hepatic artery). To this end, the technique of chronic bile duct-ligation (CBDL) was assessed as a model of portal hypertension (PHT) in the cat, and investigated as to the possible mechanism by which this pathology may develop. In short, CBDL did not produce PHT after a 21 day period of total biliary obstruction. Portal and intrahepatic venous pressures (PVP and LVP) were elevated at this point, but not to pathological levels. CBDL did produce major structural disturbances of the hepatic architecture as well as biliary hyperplasia and severe

collagenization of the portal triad regions.

CBDL produced a selective impairment of the nerve-induced constrictor responses of the portal and hepatic venous resistance sites. That is, vascular reactivity of the portal and hepatic venous resistance sites to nerve stimulation was severely depressed although portal venous reactivity (but not hepatic venous) underwent a partial compensatory or regenerative process. Portal and hepatic venous responses to infused norepinephrine were potentiated after 3 weeks of CBDL. Thus, CBDL was not an effective means of producing portal hypertension in the cat. This technique does, however, produce some of the cardiovascular complications associated with chronic liver disease and PHT, despite the absence of greatly elevated hepatic vascular resistance.

REFERENCES

Aarseth, S., Bergan, A., and Aarseth, P. Circulatory homeostasis in rats after bile duct ligation. Scand. J. Clin. Lab. Invest. 39: 93-97, 1979.

Abboud, F.M., Eckberg, D.L., Johannsen, V.J., and Mark, A.L. Carotid and cardiopulmonary baroreceptor control of splanchnic and forearm vascular resistance during venous pooling in man. J. Physiol. Lond. 286: 173-184, 1979.

Abergel, A., Braillon, A., Gaudin, C., Kleber, G., and Lebrec, D. Persistence of a hyperdynamic circulation in cirrhotic rats following removal of the sympathetic nervous system. Gastroenterology 102: 656-660, 1992.

Aggerbeck, M., Ferry, N., Zafrani, E-S., Billon, M-C., Barouki, R., and Hanoune, J. Adrenergic regulation of glycogenolysis in rat liver after cholestasis. Modulation of the balance between $alpha_1$ and $beta_2$ receptors. J. Clin. Invest. 71: 476-486, 1983.

Ahren, B. and Lundquist, I. Secretin potentiates cholinergically induced glucagon secretions in the mouse. Acta Physiol. Scand. 128: 575-578, 1986.

Ahren, B. and Lundquist, I. α-Adrenoceptor blockade by phentolamine inhibits ß-adrenergically and cholinergically induced glucagon secretion in the mouse. Horm. Metab. Res. 19: 600-603, 1987.

Ahren, B., Veith, R.C., and Taborsky, G.J., Jr., Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: effects of basal release of insulin and glucagon. Endocrinology 121: 323-331, 1987.

Alon, U., Berant, M., Mordechovich, D., and Better, O.S. The effect of intrarenal infusion of bile on kidney function in the dog. Clin. Sci. 62: 431-433, 1982.

Andia-Waltenbaugh, A.M., Kimura, S., Wood, J., Divakaran, P., and Friedmann, N. Effects of glucagon, insulin and cyclic-AMP on mitochondrial calcium uptake in the liver. Life Sci. 23: 2437-2444, 1978.

Angehrn, W., Schmid, E., Althaus, F., Niedermann, K., and Rothlin, M. Effect of dopamine on hepatosplanchnic blood flow. J. Cardiovasc. Pharmacol. 2: 257-265, 1980.

Arber, N., Zajicek, G., and Ariel, I. The streaming liver II. Hepatocyte life history. Liver 8: 80-87, 1988.

Arey, L.B. Throttling veins in the livers of certain mammals. Anat. Rec. 81: 21-31, 1941.

Arey, L.B. and Simonds, J.P. The relation of the smooth muscle in the hepatic veins to shock phenomena. Anat. Rec. 18: 219, 1920.

Aronsen, K.F. Late effects of biliary stasis on the effective liver blood flow. Acta Chir Scand. 134: 278-281, 1968.

Aronsen, K.F., Nylander, G., and Ohlsson, E.G. Liver blood flow studies during and after various periods of total biliary obstruction in the dog. Acta Chir. Scand. 135: 55-59, 1969.

Bashour, F.A., Geumei, A., Nafrawi, A.G., and Downey, H.F. Glucagon: Its effects on the hepatic arterial and portal venous beds in the dog. Pflugers Arch. 334: 83-92, 1973.

Bass, L., Keiding, S., Winkler, K., and Tygstrup, N. Enzymatic elimination of substrate flowing through the intact liver. J. Theor. Biol. 61: 393-409, 1976.

Bass, L., Robinson, P., and Bracken, A.J. Hepatic elimination of flowing substrates: the distributed model. J. Theor. Biol. 72: 161-184, 1978.

Bauer, A. Clinical and experimental studies of the alkaline phosphatase content of blood serum in outflow obstruction at various levels of the biliary passage. Acta Chir. Scand. 100: 228-244, 1950.

Bauer, W., Dale, H.H., Poulsson, L.T., and Richards, D.W. The control of circulation through the liver. J. Physiol. Lond. 74: 343-375, 1932.

Benoit, J.N., Barrowman, J.A., Harper, S.L., Kvietys, P.R., and Granger, D.N. Role of humoral factors in the intestinal hyperemia associated with chronic portal hypertension. Am. J. Physiol. 247: G486-G493, 1984.

Benoit, J.N., Womack, W.A., Hernandez, L., and Granger, D.N. "Forward" and "backward" flow mechanisms of portal hypertension. Gastroenterology 89: 1092-1096, 1985.

Benoit, J.N., Zimmerman, B., Premen, A.J., Go, V.L.X.N., and Granger, D.N. Role of glucagon in splanchnic hyperemia of chronic portal hypertension. Am. J. Physiol. 251: G674-G677, 1986.

Bentinck-Smith, J. and French, T.W. In: Kirk, R.W. and Bonagura, J.D. eds. Kirks's current veterinary therapy. Small animal practice. 10th edition. W.W. Saunders Company, Philadelphia, pp. 1251-1256, 1989.

Better, O.S., Aisnebrey, G.A., Berl, T., Anderson, R.J., Handelman, W.A., Linus, S.L., Guggenheim, S.J., Schrier, R.W. Role of antidiuretic hormone in impaired urinary dilution associated with chronic bile duct ligation. Clin. Sci. 58: 493-500, 1980.

Binah, O., Bomzon, A., Blendis, L.M., Mordechovich, D., and Better, O.S. Obstructive jaundice blunts myocardial contractile response to isoprenaline in the dog: a clue to susceptibility of jaundiced patients to shock. Clin. Sci. 69: 647-653, 1985.

Blackard, W.G., Nelson, N.C., and Andres, S.S. Portal and peripheral vein immunoreactive glucagon concentrations after arginine or glucose infusions. Diabetes 23: 199-202, 1974.

Blendis, L.M., and Bomzon, A. The cardiovascular complications of cirrhosis - a history. Ch. 1, In: Cardiovascular Complications of Liver Disease. Edited by A. Bomzon and L.M. Blendis. CRC Press, Boca Raton, pp. 1-8, 1990.

Blendis, L.M., Orrego, H., Crossley, I.R., Blake, J.E., Medline, A., and Israel, Y. The role of hepatocyte enlargement in hepatic pressure in cirrhotic and noncirrhotic alcoholic liver disease. Hepatology 2: 539-546, 1982.

Bloom, S.R. and Edwards, A.V. Characteristics of the neuroendocrine responses to stimulation of the splanchnic nerves in bursts in the conscious calf. J. Physiol. 362: 303-310, 1985.

Bobbioni, E., Marre, M., Helman, A., and Assan, R. The nervous control of rat glucagon secretion in vivo. Horm. Metab. Res. 15: 133-138, 1983.

Bohlen, H.G., Maass-Moreno, R., and Rothe, C.F. Hepatic venular pressures of rats, dogs, and rabbits. Am. J. Physiol. 261: G539-G547, 1991.

Bomzon, A., Vascular reactivity in liver disease. Ch 10, In: Cardiovascular Complications of Liver Disease. Edited by A. Bomzon and L.M. Blendis. CRC Press, Boca Raton, pp. 207-224, 1990.

Bomzon, A. and Blendis, L.M. Animal models of liver disease. Ch. 2, In: Cardiovascular Complications of Liver Disease. Edited by A. Bomzon and L.M. Blendis. CRC Press, Boca Raton, pp. 2-28, 1990.

Bosch, J. Effect of pharmacological agents on portal hypertension: a hemodynamic appraisal. Clin. Gastroenterol. 14: 169-184, 1985.

Bosch, J., Enriquez, R., Groszmann, R.J., and Storer, E.H. Chronic bile duct ligation in the dog: hemodynamic characterization of a portal hypertensive model. Hepatology 3: 1002-1007, 1983.

Bosch, J., Navasa, M., Garcia-Pagan, J.C., DeLacy, A.M., and Rodes, J. Portal hypertension. Med. Clin. North Amer. 73: 931-953, 1989.

Branch, R.A., Nies, A.S., and Shand, D.G. The disposition of propranolol. VIII. General implications of the effects of liver blood flow on elimination from the perfused rat liver. Drug Metab. Dispos. 1: 687-690, 1973.

Brown, J.C. and Otte, S.C. Gastrointestinal hormones and the control of insulin secretion. Diabetes 27: 782-787, 1978.

Burton-Opitz, R. The vascularity of the liver. II. The influence of the portal blood flow upon the flow in the hepatic artery. Q. J. Exp. Physiol. 4: 93-102, 1911.

Cameron, G.R. and Oakley, C.L. Ligation of the common bile duct. J. Pathol. Bact. 35: 789-798, 1932.

Carlsten, A., Edlund, Y., and Thulesius, O. Bilirubin, alkaline phosphatase and transaminases in blood and lymph during biliary obstruction in the cat. Acta Physiol. Scand. 53: 58-67, 1961.

Center, S.A., Baldwin, B.H., King, J.M., and Tennant, B.C. Hematologic and biochemical abnormalities associated with induced extrahepatic bile duct obstruction in the cat. Am. J. Vet. Res. 44: 1822-1829, 1983.

Cerini, R., Koshy, A., Hadengue, A., Lee, S.S., Garnier, P., and Lebrec, D. Effects of glucagon on systemic and splanchnic circulation in conscious rats with biliary cirrhosis. J. Hepatology, 9:69-74, 1989.

Chen, L.Q., Riedel, G.L., and Shepherd, A.P. Norepinephrine release during autoregulatory escape: effects of α_2-receptor blockade. Am. J. Physiol. 260: H400-H408, 1991.

Child, C.G. The hepatic circulation and portal hypertension. W.B. Saunders Company, Philadelphia, 1954.

Claria, J., Jimenez, W., Ros, J., Asbert, M., Castro, A., Arroyo, V., Rivera, F., and Rodes, J. Pathogenesis of arterial hypertension in cirrhotic rats with ascites: role of endogenous nitric oxide. Hepatology 15: 343-349, 1992.

Cohn, R. and Kountz, S. Factors influencing control of arterial circulation in the liver of the dog. Am. J. Physiol. 205: 1260-1264, 1963.

Combettes, L., Berthon, B., Binet, A., and Clarel, M. Glucagon and vasopressin interactions on Ca^{+2} movements in isolated hepatocytes. Biochem. J. 237:675-683, 1986.

Crissinger, K.D., Kvietys, P.R., and Granger, D.N. Autoregulatory escape from norepinephrine infusion: roles of adenosine and histamine. Am. J. Physiol. 254: G560-G565, 1988.

Cummings, S.A., Groszmann, R.J., and Kaumann, A. Hypersensitivity of mesenteric veins to 5-hydroxytryptamine- and to ketanserin-induced reduction of portal pressure in portal hypertensive rats. Br. J. Pharmacol. 89: 501-513, 1986.

Davis, M.A., Williams, P.E. and Cherrington, A.D. Effect of glucagon on hepatic lactate metabolism in the conscious dog. Am. J. Physiol. 248: E463-E470, 1985.

Del Rio Lozano, I. and Andrews, W.H.H. Changes in the hepatic vascular pattern that follow ligation of the common bile duct in rabbits. J. Pathol. Biol. 90: 471-477, 1965.

Deysach, L.J. The nature and location of the "sphincter mechanism" in the liver as determined by drug actions and vascular injections. Am. J. Physiol. 132: 713-724, 1941.

Dockray, G.J. Comparative biochemistry and physiology of gut hormones. Ann. Rev. Physiol. 41: 83-95, 1979.

Dunn, C.W., Horton, J.W., Megison, S.M., and Vuitch, M.F. Contribution of portal systemic shunt to Kupffer cell dysfunction in obstructive jaundice. J. Surg. Res. 50: 234-239, 1991.

Einzig, S., Rao, G.H.R., and White, J.G. Differential sensitivity of regional vascular beds in the dog to low-dose prostacyclin infusion. Can. J. Physiol. Pharmacol. 58: 940-946, 1980.

Elias, H. and Sherrick, J.C. Morphology of the Liver. Academic Press, New York and London, 1969.

Emmanouel, D.S., Jaspan, J.B., Rubenstein, A.H., Huen, A.H-J., Fink, E. and Katz, A.I. Glucagon metabolism in the rat. J. Clin. Invest. 62: 6-13 1978.

Everett, R.M., Duncan, J.R., and Prasse, K.W. Alkaline phosphatase, leucine aminopeptidase, and alanine aminotransferase activities with obstructive and toxic hepatic disease in cats. Am. J. Vet. Res. 38: 963-966, 1977a.

Everett, R.M., Duncan, J.R., and Prasse, K.W. Alkaline phosphatase in tissues and sera of cats. Am. J. Vet. Res. 38: 1533-1538, 1977b.

Ezzat, W.R. and Lautt, W.W. hepatic arterial pressure-flow autoregulation is adenosine mediated. Am. J. Physiol. 252: H836-H845, 1987.

Farah, A.E. Glucagon and the circulation. Pharmacol. Rev. 35: 181-217, 1983.

Felig, P., Gusberg, R., Hendler, R., Gump, F.E., and Kinney, J.H. Concentrations of glucagon and the insulin: glucagon ratio in the portal and peripheral circulation. Proc. Soc. Exp. Biol. Med. 147: 88-90, 1974.

Felig, P., Wahren, J., Hendler, R. Influence of physiologic hyperglucagonemia on basal and insulin inhibited splanchnic glucose output in normal man. J. Clin. Invest. 58: 761-762, 1976.

Findberg, J.P.M., Seidman, R., and Better, O.S. Cardiovascular responsiveness to vasoactive agents in rats with obstructive jaundice. Clin. Exp. Pharmacol. Physiol. 9: 639-643, 1982.

Findberg, J.P.M., Syrop, H.A., and Better, O.S. Blunted pressor response to angiotensin and sympathomimetic amines in bile duct-ligated dogs. Clin. Sci. 61: 535-539, 1981.

Fisher, J.E. and Baldessarini, R.J. False neurotransmitters and hepatic failure. Lancet 2: 75-80, 1971.

Flint, E.R. Obstructions of the common bile duct. Br. J. Med.,253(2): 253-256, 1937.

Flood, C.A., Benedict-Gutman, E., and Gutman, A.B. Serum and urine phosphatase activity in the cat after ligation of the common bile duct. Am. J. Physiol. 120: 696-702, 1937.

Floras, J.S., Legault, L., Morali, G.A., Hara, K., and Blendis, L.M. Increased sympathetic outflow in cirrhosis and ascites direct evidence from intraneural recordings. Ann. Int. Med. 114: 373-380, 1991.

Folkow, B., Lewis, D.H., Lundren, O., Mellander, S., and Wallentin, I. The effect of graded vasoconstrictor fibre stimulation on the intestinal resistance and capacitance vessels. Acta Physiol. Scand. 61: 445-457, 1964a.

Folkow, B., Lewis, D.H., Lundgren, O., Mellander, S., and Wallentin, I. The effect of the sympathetic vasoconstrictor fibres in the distribution of capillary blood flow in the intestine. Acta Physiol. Scand. 61: 458-466, 1964b.

Forssmann, W.G. and Ito, S. Hepatocyte innervation in primates. J. Cell. Biol. 74: 299-313, 1977.

Franco, D., Gigou, M., Szekely, A.M., and Bismuth, H. Portal hypertension after bile duct obstruction. Arch. Surg. 114: 1064-1067, 1979.

Fraser, R., Bowler, L.M., and DeZanger, R.B. Agents related to fibrosis, such as alcohol, CCl_4 acutely affect endothelial fenestrae which may cause fatty liver. In: Connective Tissue of the Normal and Fibrotic Human Liver. Edited by V. Gerlach. Thieme Verlag, Stuttgart, pp.159-160, 1982.

Frenzel, H., Kremer, B., and Hucker, H. The liver sinusoids under various pathological conditions. A TEM and SEM study of rat liver after respiratory hypoxia, telecolbalt-irradiation and endotoxin application. In: Kupffer Cells and Other Liver Sinusoidal Cells. Edited by E. Wisse and D.L. Knook. Elsevier, Amsterdam, pp.213-222, 1977.

Friedmann, N. and Park, C.R. Early effects of 3',5'-adenosine monophosphate on the fluxes of calcium and potassium in the perfused liver of normal and adrenalectomized rats. Proc. Natl. Acad. Sci. 61: 504-508, 1968.

Gagnon, G., Regoli, D., and Rioux, F. A new bioassay for glucagon. Br. J. Pharmacol. 64: 99-108, 1978.

Gagnon, G., Regoli, D., and Rioux, F. Studies on the mechanism of action of glucagon in strips of rabbit renal artery. Br. J. Pharmac. 69: 389-396, 1980.

Galambos, J.T. Esophageal variceal hemorrhage: diagnosis and an overview of treatment. Sem. Liver Dis. 2: 211-226, 1982.

Gaudin, C., Braillon, A., Poo, J.L., Kleber, G., Moreau, R., and Lebrec, D. Hepatology 13: 913-916, 1991.

Gaudin, C., Ruget, G., Braillon, A., Selz, F., Cuche, J.L., and Lebrec, D. Life Sci. 45: 1333-1339, 1989.

Gelman, S., Dillard, E., and Parks, D.A. Glucagon increases hepatic oxygen supply-demand ratio in pigs. Am. J. Physiol. 252: G648-G653, 1987.

Gerich, J.E., Langlois, M., Noacco, C., Schneider, V., and Forsham, P.H. Adrenergic modulation of pancreatic glucagon secretion in man. J. Clin. Invest. 53: 1441-1446, 1974.

Gimson, A.E.S., Westaby, D., Hegarty, J., Watson, A., and Williams, R. A randomized trial of vasopressin and vasopressin plus nitroglycerin in the control of acute variceal hemorrhage. Hepatology 6: 410-413, 1986.

Goodman, P.S. Vitamin A metabolism and the liver. In: The Liver: Biology and Pathobiology, Chapter 26, 2nd Edition. Edited by I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter, and D.A. Shafritz. Raven Press, New York, 1988.

Goresky, C.A. Tracer behaviour in the hepatic microcirculation. In: Hepatic Circulation in Health and Disease, ed. W.W. Lautt, Raven Press, New York, NY, pp. 25-39, 1981.

Granger, D.N., Miller, T., Allen, R., Parker, R.E., Parker, J.C., and Taylor, A.E. Permselectivity of cat liver blood-lymph barrier to endogenous macromolecules. Gastroenterology 97: 103-109, 1979.

Green, J.G. Pressure-flow and volume-flow relationships of the systemic circulation of the dog. Am. J. Physiol. 229: 761-769, 1975.

Green, J., Beyar, R., Bomzon, L., Findberg, J.P.M., and Better, O.S. Jaundice, the circulation and the kidney. Nephron 37: 145-152, 1984.

Green, J., Beyar, R., Siderman, S., Mordechovitz, D., Better, O.S. The "jaundiced heart": A possible explanation for postoperative shock in obstructive jaundice. Surgery, 100: 14-19, 1986.

Greenway, C.V. Discussion. In: Hepatic Circulation in Health and Disease. Edited by W.W. Lautt. Raven Press, New York, p. 224, 1981a.

Greenway, C.V. Hepatic plethysmography. In: Hepatic Circulation in Health and Disease. Edited by W.W. Lautt. Raven Press, New York, pp. 41-54, 1981b.

Greenway, C.V. Role of splanchnic venous system in overall cardiovascular homeostasis. Fed. Proc. 42: 1678-1684, 1983.

Greenway, C.V. Autoregulatory escape in arteriolar resistance vessels. In: Smooth Muscle Contraction. (Ed) N.L. Stephens, Marcel Dekker Inc., New York, p.473-484, 1984a.

Greenway, C.V. Effects of hemorrhage and hepatic nerve stimulation on venous compliance and unstressed volume in cat liver. Can. J. Physiol. Pharmacol. 65: 2168-2174, 1987.

Greenway, C.V. and Lautt, W.W. Effects of hepatic venous pressure on transsinusoidal fluid transfer in the liver of the anesthetized cat. Circ. Res. 26: 697-703, 1970.

Greenway, C.V. and Lautt, W.W. Effects of infusions of catecholamines, angiotensin, vasopressin and histamine on hepatic blood volume in the anesthetized cat. Br. J. Pharmacol. 44: 177-184, 1972.

Greenway, C.V. and Lautt, W.W. Blood volume, the venous system, preload, and cardiac output. Can. J. Physiol. Pharmacol. 64: 383-387, 1986.

Greenway, C.V. and Lautt, W.W. Distensibility of hepatic venous resistance sites and consequences on portal pressure. Am. J. Physiol. 254: H452-H458, 1988.

Greenway, C.V. and Lautt, W.W. Hepatic circulation. In: Handbook of Physiology - The Gastrointestinal System I, Volume 1, Part 2, Chapter 41. Eds. S.G. Schultz, J.D. Wood, and B.B. Rauner, Am. Physiol. Soc., Oxford University Press, New York, NY, pp.1519-1564, 1989.

Greenway, C.V. and Lawson, A.E. ß-Adrenergic receptors in the hepatic arterial bed of the anesthetized cat. Can. J. Physiol. Pharmacol. 47: 415-419, 1969.

Greenway, C.V., Lawson, A.E., and Mellander, S. The effects of stimulation of the hepatic nerves, infusion of noradrenaline, and occlusion of the carotid arteries on liver blood flow in the anesthetized cat. J. Physiol. Lond. 192: 21-41, 1967.

Greenway, C.V. and Oshiro, G. Comparison of the effects of hepatic nerve stimulation on arterial flow, distribution of arterial and portal flows and blood content in the livers of anesthetized cats and dogs. J. Physiol. Lond. 227: 487-501, 1972.

Greenway, C.V. and Oshiro, G. Effects of histamine on hepatic volume (outflow block) in anesthetized dogs. Br. J. Pharmacol. 47: 282-290, 1973.

Greenway, C.V., Seaman, K.L., and Innes, I.R. Norepinephrine on venous compliance and unstressed volume in cat liver. Am. J. Physiol. 248: H468-H496, 1985.

Greenway, C.V., Scott, G.D., and Zink, J. Sites of autoregulatory escape of blood flow in the mesenteric vascular bed. J. Physiol. 259: 1-12, 1976.

Greenway, C.V. and Stark, R.D. Hepatic vascular bed. Physiol. Rev. 51: 23-65, 1971.

Grimaud, D., Livrelli, N., Dolisi, C.L., Philip, F., and Maestracci, P. Effects de la dopamine sur les index hepatique et cardiaque chez l'homme. Nouv Presse Med. 10: 1031-1035, 1981.

Grisham, J.W. A morphological study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver; autoradiography with thymidine-H^3. Canc. Res. 22: 842-849, 1962.

Groszmann, R.J. and Atterbury, C.E. The pathophysiology of portal hypertension: a basis for classification. Sem. Liver Dis. 2: 177-186, 1982.

Groszmann, R.J., Kravetz, D., and Bosch, J., Glickman, M., Bruix, J., Bredfeldt, J., Conn, H.O., Rodes, J., and Storer, E.H. Nitroglycerin improves the hemodynamic response to vasopressin in portal hypertension. Hepatology 2: 757-762, 1982.

Gumucio, J.J. Hepatocyte heterogeneity: the coming of age from the description of a biological curiosity to a partial understanding of its physiological meaning and regulation. Hepatology 9: 154-160, 1989.

Gumucio, J.J. and Chianale, J. Liver cell heterogeneity and liver function. In: The Liver: Biology and Pathobiology, Chapter 53, 2nd Edition. Edited by I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter, and D.A. Shafritz. Raven Press, New York, 1988.

Hadengue, A., Lee, S.S., Moreau, R., Braillon, A., and Lebrec, D. Beneficial hemodynamic effects of ketanserin in patients with cirrhosis: possible role of serotinergic mechanisms in portal hypertension. Hepatology 7: 644-647, 1987.

Hales, M.R., Allan, J.S., and Hall, E.M. Injection-corrosion studies of normal and cirrhotic livers. Am. J. Physiol. 35: 909-927, 1959.

Hall, C., Bergan, A., and Henriksen, J.E. Blood flow in normal and cholestatic dog liver as measured by intraparenchymal injection of xenon^{133}. Eur. Surg. Res. 9: 357-363, 1977.

Hanson, K. and Johnson, P.C. Local control of hepatic arterial and portal venous flow in the dog. Am. J. Physiol. 211: 712-720, 1966.

Harber, M.H. and Rees, K. The production and composition of white bile in the rat. J. Path. Bact. 85: 127-137, 1963

Hase, T. and Brim, J. Observations on the microcirculatory architecture of the rat liver. Anat. Rec. 156: 157-174, 1966.

Haussinger, D. Glutamine metabolism in the liver: overview and current concepts. Metabolism 38(8), suppl 1: 14-17, 1989.

Heimburger, I., Teramoto, S., and Shumaker, H.B. Effect of surgical pituitrin upon portal and hepatic circulation. Surgery 48: 706-715, 1960.

Henderson, J.M., MacKay, G.J., Hooks, M., Chezmar, J.L., Galloway, J.R., Dodson, T.F., and Kutner, M.H. High cardiac output of advanced liver disease persists after orthotropic liver transplantation. Hepatology 15: 258-262, 1992.

Henderson, J.M., Millikan, W.J., Hooks, M., Noe, B., Kutner, M., and Warren, D. Increased galactose clearance after liver transplantation: a measure of increased blood flow through the denervated liver? Hepatology 10(3): 288-291, 1989.

Henriksen, J.H., Christensen, N.J., and Ring-Larsen, H. Continuous infusion of tracer norepinephrine may miscalculate unidirectional nerve uptake of norepinephrine in man. Circ. Res. 65: 388-395, 1989.

Henriksen, J.H. and Lassen, N.A. Pressure profile in liver sinusoids. Liver 8: 88-94, 1988.

Henriksen, J.H., Ring-Larsen, H., and Christensen, N.J. Hepatic intestinal uptake and release of catecholamines in alcoholic cirrhosis. Evidence of enhanced hepatic-intestinal sympathetic nervous activity. Gut 28: 1637-1642, 1987.

Henriksen, J.H., Ring-Larsen, H., and Christensen, N.J. Aspects of sympathetic nervous system regulation in patients with cirrhosis: a 10 year experience. Clin. Physiol. 11: 293-306, 1991.

Henriksen, J.H., Ring-Larsen, H., Kanstrup, I.L., and Christensen, N.J. Splanchnic and renal elimination and release of catecholamines in cirrhosis. Evidence of enhanced sympathetic nervous activity in patients with decompensated cirrhosis. Gut 25: 1034-1043, 1984.

Hirsh, A.T., Levenson, D.J., Cutler, S.S., Dzau, V.J., and Creager, M.A. Regional vascular responses to prolonged lower body negative pressure in normal subjects. Am. J. Physiol. 257: H219-H225, 1989.

Hirsch, L.J., Ayabe, T., and Glick, G. Response of portal vein, hepatic artery and superior mesenteric artery flows to direct dopamine infusion. Fed. Proc. 34: 459, 1975.

Hisida, A., Honda, N., Sudo, M., and Nagase, M. Mechanisms of altered renal perfusion in the early stage of obstructive jaundice. Kidney Int. 17: 223-230, 1980.

Hoffmann, W.E., Renegar, W.E., and Dorner, J.L. Serum half-life of intravenously injected intestinal and hepatic alkaline phosphatase isoenzymes in the cat. Am. J. Vet. Res. 38: 1637-1639, 1977.

Holick, M.F. Vitamin D: photobiology, metabolism, and clinical application. In: The Liver: Biology and Pathobiology, Chapter 27, 2nd Edition. Edited by I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter, and D.A. Shafritz. Raven Press, New York, 1988.

Holst, J.J. A radioreceptor-assay for glucagon: binding of enteroglucagon to liver plasma membranes. Diabetalogica 11: 211-219, 1975.

Holst, J.J., Gronholt, R., Shaffalitzky de Muckadill, O.B., and Fahrenkrug, J. Nervous control of pancreatic endocrine secretion in pigs. V. Influence of the sympathetic nervous system on the insulin and glucagon response to vagal stimulation. Acta Physiol. Scand. 113: 279-283, 1981.

Hunt, D.R. Changes in liver blood flow with development of biliary obstruction in the rat. Aust. N.Z. J. Surg. 49: 733-737, 1979.

Ideo, G., Bellati, G., Fesco, E., and Grimoldi, D. Nadolol can prevent the first gastrointestinal bleeding in cirrhosis: a prospective, randomized study. Hepatology 8: 6-9, 1988.

Ipp, E., Dobbs, R.E., and Unger, R.H. Morphine and ß-endorphin influence the secretions of the endocrine pancreas. Nature 178: 190-191, 1978.

Iversen, J. Adrenergic receptors and the secretion of glucagon and insulin from the isolated, perfused canine pancreas. J. Clin. Invest. 52: 2102-2116, 1973.

Iwai, M., Gardemann, A., Puschel, G., and Jungermann, K. Potential role for prostaglandin $F_{2\alpha}$, D_2, E_2 and thromboxane A_2 in mediating the metabolic rate and hemodynamic actions of sympathetic nerves in perfused rat liver. Eur. J. Biochem. 175: 45-50, 1988.

Iwai, M. and Jungermann, K. Possible involvement of eicosanoids in the actions of sympathetic hepatic nerves on carbohydrate metabolism and hemodynamics in perfused rat liver. FEBS Let. 221: 155-160, 1987.

Jaspan, J.B., Polonsky, K.S., Lewis, M., Pensler, J., Pugh, W., Moossa, A.R., and Rubenstein, A.H. Hepatic metabolism of glucagon in the dog: contribution of the liver to overall metabolism in disposal of glucagon. Am. J. Physiol. 240: E233-E244, 1981.

Jenkins, S., Baxter, J., and Corbett, W., Devitt, P., Ware, J., Shields, R. A prospective randomized controlled clinical trial comparing somatostatin and vasopressin in controlled acute variceal hemorrhage. Br. Med. J. 290: 275-278, 1985.

Jensen, L.S., Juhl, C.O., and Mulvany, M.J. Mechanical, morphological and pharmacological properties of esophageal varices and small mesenteric veins in portal hypertensive rabbits. Acta. Physiol. Scand. 130: 649-656, 1987.

Johnson, J.M., Rowell, L.B., Niederberger, M., and Eisman, M.M. Human splanchnic and forearm vasoconstrictor responses to reductions of right atrial and aortic pressures. Circ. Res. 34: 515-523, 1974.

Jungermann, K. Metabolic zonation of liver parenchyma. Sem. Liv. Dis. 8: 329-341, 1988.

Jungermann, K., Gardemann, A., Beuers, U., Ballé, C., Sannemann, J., Beckh, K., and Hartmann, H. Regulation of liver metabolism by the hepatic nerves. Adv. Enz. Reg. 26: 63-88, 1987.

Kanai, M., Tanaka, M., Nimura, Y., Nagino, M., Katoh, T., and Ozawa, T. Mechanism of adaptive increase of respiratory enzymes in rat liver mitochondria during obstructive jaundice. Biochem. Int. 23: 1165-1173, 1991.

Kaneto, A., Kawagu, S., Sato, H., Kaneko, T., Yanaihara, C., Yanaihara, N., and Kasaka, K. Effect of the vagal and splanchnic nerve stimulation on the release of somatostatin, pancreatic polypeptide, glucagon and insulin. Biomed. Res. 2: 166-178, 1981.

Kaneto, A., Miki, E., and Kosaka, K. Effects of $beta_1$- and $beta_2$-adrenoceptor stimulants infused intrapancreatically on glucagon and insulin secretion. Endocrinology 97: 1166-1173, 1975.

Kaneto, A. and Kosaka, K. Stimulation of glucagon and insulin secretion by acetylcholine infused intrapancreatically. Endocrinology 95: 676-681, 1974.

Karam, J.H., Pancreatic hormones and antidiabetic drugs. Ch. 40, In: Basic and Clinical pharmacology, 3rd edition, B.G. Katzung, Ed., Appleton and Lange, Los Altos, pp. 484-496, 1987.

Katz, N., Jungermann, K. Autoregulatory shift from fructolysis to lactate gluconeogenesis in rat hepatocyte suspensions: The problem of metabolic zonation of the liver parenchyma. Hoppe-Seyler's Z. Physiol. Chem., 357: 359-375, 1976.

Katz, N., Teutsch, H.F., Jungermann, K., and Sasse, D. Heterogenous reciprocal localization of fructose-1, 6-Bis-phosphatase and of glucokinase in microdissected periportal and perivenous rat liver tissue. FEBS Letters, 83: 272-276, 1977.

Kawasaki, T., Carmichael, F.J., Saldavia, V., Roldan, L., and Orrego, H. Relationship between portal venous and hepatic arterial blood flows: spectrum of response. Am. J. Physiol. 259: G1010-G1018, 1990.

Kelly, D.F., Baggott, D.G., and Gaskell, C.J. Jaundice in the cat associated with inflammation of the biliary tract and pancreas. J. Small Anim. Pract. 16: 163-172, 1975.

Kiel, J.W., Pitts, V., Benoit, J.N., Granger, D.N., and Shepherd, A.P. Reduced vascular sensitivity to norepinephrine in portal hypertensive rats. Am. J. Physiol. 248: G192-G195, 1985.

Kitano, S., Koyanagi, N., Sugimachi, K., Kobayashi, M., and Inokuchi, K. Mucosal blood flow and modified vascular responses to norepinephrine in the stomach of rats with liver cirrhosis. Eur. Surg. Res. 14: 221-230, 1982.

Knudtzon, F. Adrenergic effects on plasma levels of glucagon, insulin, glucose and free fatty acids in rabbits. Horm. Metab. Res. 16: 415-422, 1984.

Koch-Weser, D., Meyer, K.A., Yesinick, C., and Popper, H. Influence of the site of experimental biliary obstruction upon functional and morphological hepatic injury. Lab. Invest. 1: 324-331, 1952.

Kock, N.G., Roding, B., Hahnloser, P., Tibblin, S. and Schenk, W.G., Jr. The effect of glucagon on hepatic blood flow. Arch. Surg. 100: 147-149, 1970a.

Kock, N.G., Tibblin, S., and Schenk, W.G., Jr. Hemodynamic responses to glucagon: an experimental study of central, visceral and peripheral effects. Ann. Surg. 171: 373-379, 1970b.

Kock, N.G., Tibblin, S., and Schenk, W.G., Jr. Modification by glucagon of the splanchnic vascular responses to activation of the sympathicoadrenal system. J. Surg. Res. 1: 12-17, 1971.

Kong, C., Lay, C., and Tsai, Y. The hemodynamic effect of verapamil on portal hypertension in patients with post-necrotic cirrhosis. Hepatology 6: 423-426, 1986.

Koshy, A., Moreau, R., Cerini, R., Roulot, D., Bacq, Y., Hadengue, A., and Lebrec, D. Effects of oxygen inhalation on tissue oxygenation in patients with cirrhosis. Evidence for an impaired arterial baroreflex control. J. Hepatol. 9: 240-245, 1989.

Kountouras, J., Belling, B.H., and Scheuer, P.J. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. Br. J. Exp. Pathol. 65: 305-311, 1984.

Krarup, N. Effects of histamine, vasopressin, and angiotensin II on hepatosplanchnic hemodynamics, liver function, and hepatic metabolism in cats. Acta Physiol. Scand. 95: 311-317, 1975.

Krarup, N. and Larsen, J.A. The effect of glucagon on hepatosplanchnic hemodynamics, functional capacity, and metabolism of the liver in cats. Acta Physiol. Scand. 91: 42-52, 1974.

Kravetz, D., Bosch, J., Teres, J., Bruix, J., Rimola, A. and Rodes, J. Comparison of intravenous somatostatin and vasopressin infusion in treatment of acute variceal hemorrhage. Hepatology 4: 442-446, 1984.

Kroeger, R.J. and Groszmann, R.J. Increased portal venous resistance hinders portal pressure reduction during the administration of ß-adrenergic blocking agents in a portal hypertensive model. Hepatology 5: 97-101, 1985.

Krogsgaard, K., Gluud, C., Henriksen, S.H., and Christoffersen, P. Correlation between liver morphology and portal pressure in alcoholic liver disease. Hepatology 4: 699-703, 1984.

Kullmann, R., Breull, W.R., Wassermann, K., and Konopatzki, A. Blood flow redistribution by dopamine in the feline gastrointestinal tract. N.S. Arch. Pharmacol. 323: 145-148, 1983.

Lagerkranser, M., Irestedt, L., Sollevi, A., and Andreen, M. Central and splanchnic hemodynamics in the dog during controlled hypotension with adenosine. Anesthesiology 60: 547-552, 1984.

Laragh, J.G., Cannon, P.J., Bentzel, C.J., Sicenski, A.M., and Meltzer, J.I. Angiotensin II, norepinephrine, and renal transport of electrolytes and water in normal man and in cirrhotics with ascites. J. Clin. Invest. 42: 1179-1192, 1963.

Larner, J. Insulin and oral hypoglycemic drugs: glucagon. Ch. 64, In: Goodman and Gillman's the pharmacological basis of therapeutics, 7th edition, A. Goodman-Gillman, L.S. Goodman, T.W. Rall, F. Murad Eds. MacMIllan Publishing Company, New York, pp. 1490-1516, 1985.

Latzina, A., Brown, H., Brown, U.E., and McDermott, W.V. Hepatic blood flow alteration following the common bile duct ligation. Surg. Forum 19: 340-344, 1968.

Lautt, W.W. Method for measuring hepatic uptake of oxygen or other blood-borne substances in situ. J. Appl. Physiol. 40: 269-274, 1976.

Lautt, W.W. Control of hepatic and intestinal blood flow: effect of isovolumetric hemodilution on blood flow and oxygen uptake in the intact liver and intestine. J. Physiol. 265: 313-326, 1977a.

Lautt, W.W. Effect of stimulation of hepatic nerve on hepatic O_2 uptake and blood flow. Am. J. Physiol. 232: H652-H656, 1977b.

Lautt, W.W. Hepatic presinusoidal sphincters affected by altered arterial pressure and flow, venous pressure and nerve stimulation. Microvasc. Res. 15: 309-317, 1978.

Lautt, W.W. Hepatic nerves: a review of their functions and effects. Can. J. Physiol. Pharmacol. 58: 105-123, 1980a.

Lautt, W.W. Control of hepatic arterial blood flow: independence from liver metabolic activity. Am. J. Physiol. 239: H559-H564, 1980b.

Lautt, W.W. Evaluation of surgical denervation of the liver in cats. Can. J. Physiol. Pharmacol. 59: 1013-1016, 1981a.

Lautt, W.W. Role and control of the hepatic artery. In: Hepatic Circulation in Health and Disease. Ed. W.W. Lautt, Raven Press, New York, NY, pp.203-226, 1981b.

Lautt, W.W. Afferent and efferent neural roles in liver function. Prog. Neurobiol. 21: 323-348, 1983a.

Lautt, W.W. Relationship between hepatic blood flow and overall metabolism: the hepatic arterial buffer response. Fed. Proc. 42: 1662-1666, 1983b.

Lautt, W.W. Mechanism and role of intrinsic regulation of hepatic arterial blood flow: hepatic arterial buffer response. Am. J. Physiol. 249: G549-G556, 1985.

Lautt, W.W. Resistance or conductance for expression of arterial vascular tone. Microvasc. Res. 37: 230-236, 1989.

Lautt, W.W. Noncompetative antagonism of adenosine by caffeine on the hepatic and superior mesenteric arteries of anesthetized cats. J. Pharm. Exp. Therap. 254: 400-406, 1990.

Lautt, W.W., Brown, L.C., and Durham, J.S. Active and passive control of hepatic blood volume responses to hemorrhage at normal and raised venous pressure in cats. Can. J. Physiol. Pharmacol. 58: 1049-1057, 1980.

Lautt, W.W. and Carroll, A.M. Evaluation of topical phenol as a means of producing autonomic denervation of the liver. Can. J. Physiol. Pharmacol. 62: 849-853, 1984.

Lautt, W.W., d'Almeida, M.S., McQuaker, J., and D'Aleo, L. Impact of the hepatic arterial buffer response on splanchnic vascular responses to intravenous adenosine, isoproterenol and glucagon. Can. J. Physiol. Pharmacol. 66: 807-813, 1988a.

Lautt, W.W. and Daniels, T.R. Differential effect of taurocholate acid on hepatic arterial resistance vessels and bile flow. Am. J. Physiol. 244: G366-G369, 1983.

Lautt, W.W. and Greenway, C.V. Conceptual review of the hepatic vascular bed. Hepatology 7: 952-963, 1987.

Lautt, W.W., Greenway, C.V., and Legare, D.J. Effect of hepatic nerves, norepinephrine, angiotensin, and elevated central venous pressure on postsinusoidal resistance sites and intrahepatic pressure in cats. Microvasc. Res. 33: 50-61, 1987a.

Lautt, W.W., Greenway, C.V., and Legare, D.J. Index of contractility: quantitative analysis of hepatic venous distensibility. Am. J. Physiol. 260: G325-G332, 1991a.

Lautt, W.W., Greenway, C.V., Legare, D.J., and Weisman, H. Localization of intrahepatic portal vascular resistance. Am. J. Physiol. 251: G375-G381, 1986.

Lautt, W.W. and Legare, D.J. The use of 8-phenyltheophylline as a competitive antagonist of adenosine and an inhibitor of the intrinsic regulatory mechanism of the hepatic artery. Can. J. Physiol. Pharmacol. 63: 717-722, 1985.

Lautt, W.W. and Legare, D.J. Adenosine modulation of hepatic arterial but not portal venous constriction by sympathetic nerves, norepinephrine, angiotensin and vasopressin in the cat. Can. J. Physiol. Pharmacol. 64: 449-454, 1986.

Lautt, W.W. and Legare, D.J. Effect of histamine, norepinephrine, and nerves on vascular pressures in the dog liver. Am. J. Physiol. 252: G472-G478, 1987.

Lautt, W.W. and Legare, D.J. The importance of selecting the correct index of vascular tone for in vivo pharmacological studies. Proc. West. Pharmacol. Soc. 34: 229-232, 1991.

Lautt, W.W. and Legare, D.J. Passive autoregulation of portal venous pressure: distensible hepatic resistance. Am. J. Physiol. (in press), 1992.

Lautt, W.W., Legare, D.J., and d'Almeida, M.S. Adenosine as putative regulator of hepatic arterial flow (the buffer response). Am. J. Physiol. 248: H331-H338, 1985.

Lautt, W.W., Legare, D.J., and Daniels, T.R. The comparative effect of administration of substances via the hepatic artery or portal vein on hepatic arterial resistance, liver blood volume and hepatic extraction in cats. Hepatology 4: 927-932, 1984.

Lautt, W.W., Legare, D.J., and Greenway, C.V. Effect of hepatic venous sphincter contribution on transmission of central venous pressure to lobar and portal pressure. Can. J. Physiol. Pharmacol. 65: 2735-2243, 1987b.

Lautt, W.W., Legare, D.J., and Lockhart, L.K. Vascular escape from vasoconstriction and post-stimulatory hyperemia in the superior mesenteric artery of the cat. Can. J. Physiol. Pharmacol. 66: 1174-1180, 1988b.

Lautt, W.W., Martins, E.S., and Legare, D.J. Insulin and glucagon response during hemorrhage induced hyperglycemia. Can. J. Physiol. Pharmacol. 60: 1624-1629, 1982.

Lautt, W.W., and McQuaker, J.E. Methodological approach to pharmacodynamic calculations of vascular responses in vivo. Proc. West. Pharmacol. Soc. 32: 227-230, 1989.

Lautt, W.W., Schafer, J., and Legare, D.J. Effect of adenosine and glucagon on hepatic blood volume responses to sympathetic nerves. Can. J. Physiol. Pharmacol. 69: 43-48, 1991b.

Lautt, W.W. and Wong, C. Hepatic glucose balance in response to direct stimulation of sympathetic nerves in the intact liver of cats. Can. J. Physiol. Pharmacol. 56: 1022-1028, 1978a.

Lautt, W.W. and Wong, C. Hepatic parasympathetic neural effect on glucose balance in the intact liver. Can. J. Physiol. Pharmacol. 56: 679-682, 1978b.

Lebrec, D., Nouel, O., Corbic, M., and Benhamou, J-P. Propranolol - a medical treatment for portal hypertension? Lancet 2: 1280-1281, 1980.

Lebrec, D., Sicot, C., Degott, C., and Benhamou, J-P. Portal hypertension and primary biliary cirrhosis. Digestion, 14: 220-226, 1976.

Lee, J.A., Ahmed, Q., Hines, J.E., Burt, A.D. Disappearance of hepatic parenchymal nerves in human liver cirrhosis. Gut, 33: 87-91, 1992a.

Lee, S.S., Johansen, K., and Lebrec, D. Circulatory changes induced by portal venous diversion and mesenteric hypertension in rats. Hepatology 15: 117-121, 1992b.

Lee, S.S., Marty, J., Mantz, J., Samain, E., Braillon, A., and Lebrec, D. Desensitization of myocardial ß-adrenergic receptors in cirrhotic rats. Hepatology 12: 481-485, 1990.

Leevy, C.M., Tenhove, W., and Opper, A. Influence of ethanol and microsomal drugs on hepatic hemodynamics. Ann. N.Y. Acad. Sci. 170: 315-331, 1970.

Legare, D.J. and Lautt, W.W. Hepatic venous resistance site in the dog: localization and validation of intrahepatic pressure measurements. Can. J. Physiol. Pharmacol. 65: 352-359, 1987.

Lindberg, B. and Darle, N. Effect of glucagon on hepatic circulation in the pig. Arch. Surg. 111: 1379-1383, 1976.

Lindsey, C.A., Faloona, G.R., and Unger, R.H. Plasma glucagon levels during rapid exsanguination with and without adrenergic blockade. Diabetes 24: 313-316, 1975.

Lockhart, L.K., Legare, D.J., and Lautt, W.W. Kinetics of adenosine antagonism of sympathetic nerve-induced vasoconstriction. Proc. West Pharmacol. Soc. 31: 105-107, 1988.

Lutz, J. and Henrich, H. Comparison of the vascular escape phenomenon in the intestinal and renal circulation under neural and humoral induction. Pflugers Arch. 339: 37-48, 1973.

Maass-Moreno, R. and Rothe, C.F. Contribution of the large hepatic veins to postsinusoidal vascular resistance. Am. J. Physiol. 262: G14-G22, 1992.

Macmathuna, P., O'Reilly, T., Kelliher, D., Barry, M., Feely, J., and Keeling, P.W.N. The effect of calcium channel blockade with nifedipine on splanchnic and systemic hemodynamics in cirrhosis. Aliment. Pharmacol. Therap. 1: 639-642, 1987.

Madden, J.J., Ludewig, R.M., and Wangensteen, S.L. Effects of glucagon on the splanchnic and the systemic circulation. Am. J. Surg. 122: 85-90, 1971.

Magari, S. Hepatic lymphatic system: structure and function. J. Gastroent. Hepatol. 5: 82-93, 1990.

Mak, K.M. and Lieber, C.S. Alterations in endothelial fenestrations in liver sinusoids of baboons fed alcohol: a scanning electron microscopic study. Hepatology 4: 386-391, 1984.

Marco, J., Calle, C., Roman, P., Diaz-Fierros, M., Villanueva, M., Valverde, I. Hyperglucagonism induced by glucocorticoid treatment in man. N. Eng. L. Med. 288: 128-131, 1973.

Marliss, E.B., Girardier, L., Seydoux, J., Wollheim, C.B, Kanazawa, Y., Orci, L., Renold, A.E., and Porte, D., Jr. Glucagon release induced by pancreatic nerve stimulation in the dog. J. Clin. Invest. 52: 1246-1259, 1973.

Marteau, P., Ballet, F., Chazouilleres, O., Chretien, Y., Rey, C., Petit, D., and Poupon, R. Effect of vasodilators on hepatic microcirculation in cirrhosis: a study in the isolated perfused rat liver. Hepatology 9: 820-823, 1989.

Mastai, R., Rochaleau, B., and Huet, P.M. Serotonin blockade in conscious, unrestrained cirrhotic dogs with portal hypertension. Hepatology 9: 265-268, 1989.

Mathie, R.T. and Alexander, B. The role of adenosine in the hyperemic response of the hepatic artery to portal vein occlusion (the "buffer response"). Br. J. Pharmacol. 100: 626-630, 1990.

Mathie, R.T. and Blumgart, L.H. The hepatic hemodynamic response to acute portal venous blood flow reductions in the dog. Pflugers Arch. 399: 223-227, 1983.

Mathie, R.T., Lam, P.H.M., Harper, A.M., and Blumgart, L.H. Hepatic arterial blood flow response to portal vein occlusion in the dog. Pflugers Arch. 386: 77-83, 1980.

Mathie, R.T., Nagarney, D.M., Lewis, M.H., and Blumgart, L.H. Hepatic hemodynamics after chronic obstruction of the biliary tract in the dog. Surg. Gynecol. Obstet. 166: 125-130, 1988.

Matthews, D.R. and Clark, A. Neural control of the endocrine pancreas. Proc. Nutr. Soc. 46: 89-95, 1987.

McIntyre, N. The limitations of conventional liver function tests. Sem. Liv. Dis. 3: 265-274, 1983.

Merkel, C., Gatta, H., Bolognesi, M., Padreni, R., Finucci, G.F., Angeli, P. and Ruol, A. The calcium-channel blocker, verapamil, does not improve portal pressure in patients with alcoholic cirrhosis. Br. J. Clin. Pharm. 26: 273-277, 1988.

Mesh, C.L., Joh, T., Korthuis, R.J., Granger, D.N., and Benoit, J.N. Intestinal vascular sensitivity to vasopressin in portal hypertensive rats. Gastroenterology 100: 916-921, 1991.

Metz, W. and Forssmann, W.G. Innervation of the liver in guinea pig and rat. Anat. Embryol. 160: 239-252, 1980.

Miller, D.L. Quantitative morphological assessment of the sinusoids of the hepatic acinus. In: Hepatic Circulation in Health and Disease, ed. W.W. Lautt, Raven Press, New York, pp. 111-135, 1981.

Mitzner, W. Hepatic outflow resistance, sinusoid pressure and the vascular waterfall. Am. J. Physiol. 227: 513-519, 1974.

Moreau, R. and Lebrec, D. Nitrovasodilators and portal hypertension. J. Hepatol. 10: 263-267, 1990.

Moreau, R., Lee, S.S., Hadengue, A., Braillon, A., and Lebrec, D. Hemodynamic effects of a clonidine-induced decrease in sympathetic tone in patients with cirrhosis. Hepatology 7: 145-154, 1987.

Moreau, R., Lee, S.S., Hadengue, A., Ozier, Y., Sicot, C., and Lebrec, D. Relationship between O_2 transport and O_2 uptake in patients with cirrhosis: effects of vasoactive drugs. Hepatology 9: 427-432, 1989.

Moreno, A.H., Burchell, A.R., Rousselot, L.M., Panke, W.F., Slafsky, S.F. and Burke, J.H. Portal blood flow in cirrhosis of the liver. J. Clin. Invest. 46: 436-445, 1967.

Motta, P., Muto, M., and Fujita, T. The Liver: An Atlas of Scanning Electron Microscopy. 1st Edition, Igaku-Shoin Ltd., Tokyo, 1978.

Muggaberg, J. and Brockman, R.P. Effect of adrenergic drugs on glucose and plasma glucagon and insulin responses to xylozine in sheep. Res. Vet. Sci. 33: 118-120, 1982.

Mundschau, G.A., Zimmerman, S.W., Gildersleeve, J.W., and Murphy, Q.R. Hepatic and mesenteric resistances after sinoaortic denervation and hemorrhage. Am. J. Physiol. 211: 77-82, 1966.

Murphy, G.J., Hruby, V.J., Trivedi, D., Wahelam, J.D., and Houslay, M.D. The rapid desensitization of glucagon stimulated adenylate cyclase is a cyclic AMP-independent process that can be mimicked by hormones which stimulate inositol phospholipid metabolism. Biochem. J. 243: 39-46, 1987.

Murray, B.M. and Paller, M.S. Decreased pressor reactivity to angiotensin II in cirrhotic rats. Evidence for a post-receptor defect in angiotensin action. Circ. Res. 57: 424-431, 1985.

Narimiya, M., Yamada, H., Matsuba, I., Ikeda, Y., Tanese, T., and Abe, M. Adrenergic modulation of insulin and glucagon secretion from the isolated perfused rat pancreas. Endocrinol. Japan. 28: 281-286, 1981.

Navasa, M., Bosch, J., Reichen, J., Bru, C., Mastai, R., Zysset, T., Silva, G., Chesla, J., and Rodes, J. Effects of verapamil on hepatic and systemic hemodynamics and liver function in patients with cirrhosis and portal hypertension. Hepatology 8: 850-854, 1988.

Netter, F.H. The CIBA collection of medical illustrations, Vol. 3, Digestive system, part III. Liver, biliary tract and pancreas. plate 6, E. Oppenheimer, Ed. CIBA Pharmaceutical Company, New York, P. 8, 1972.

Nicholls, K.M., Shapero, M.D., Vanputten, V.J., Kluge, R., Chung, H.M., Bichet, D.G., and Shrier, R.W. Elevated plasma norepinephrine concentrations in decompensated cirrhosis. Association with increased secretion roles, normal clearance rates, and suppressibility by control blood volume expansion. Circ. Res. 56: 457-461, 1985.

Nobin, A., Falck, B., Ingemansson, S., Jarhult, J., and Rosengnen, E. Organization and function of the sympathetic innervation of human liver. Acta Physiol. Scand. Suppl.452: 103-106, 1977.

Nopanitaya, W., Lamb, J.L., Grisham, J.W., and Carson, J.L. Effect of hepatic venous outflow obstruction on pores and fenestrations in sinusoidal endothelium. Br. J. Exp. Pathol. 57: 604-609, 1976.

Oberg, B. and Rossel, S. Sympathetic control of consecutive vascular sections in canine subcutaneous adipose tissue. Acta Physiol. Scand. 71: 42-56, 1967.

O'Brien, T.R. and Mitchum, G.D. Case report. J.A.V.M.A. 156: 1015-1017, 1970.

Oda, S., Ohtomo, Y., Ohneda, A., Sasaki, Y., and Tsuda, T. Adrenergic modulation of pancreatic glucagon and insulin secretion in goats. Comp. Biochem. Physiol. 84: 723-728, 1986.

Ohlsson, E.G., Rutherford, R.B., Boitnott, J.K., Haalebos, M.M.P., and Zuidema, G.D. Changes in portal circulation after biliary obstruction in dogs. Am. J. Surg. 120: 16-22, 1970a.

Ohlsson, E.G., Rutherford, R.B., Haalebos, M.M.P., Wagner, H.N., and Zuidema, G.D. The effect of biliary obstruction on hepatosplanchnic blood flow in dogs. J. Surg. Res. 10: 201-208, 1970b.

O'Leary, D.S. Regional vascular resistance vs. conductance: which index for baroreflex responses? Am. J. Physiol. 260: H632-H637, 1991.

Orrego, H., Blendis, C.M., Crossley, I.R., Medline, A., MacDonald, A., Ritchie, S., and Israel, Y. Correlation of intrahepatic pressure with collagen in the Disse space and hepatomegaly in humans and in the rats. Gastroenterology 80: 546-556, 1981.

Orrego, H., Medline, A., and Blendis, L.M., Rankin, J.G. and Kreadon, D.A. Collagenization of the Disse space in alcoholic liver disease. Gut 20: 673-679, 1979.

Ozier, Y., Braillon, A., Gaudin, C., Roulot, D., Hadengue, A., and Lebrec, D. Hepatic denervation alters hemodynamic responses to hemorrhage in conscious rats. Hepatology 10(4): 473-476, 1989.

Pang, K.S. and Rowland, M. Hepatic clearance of drugs. I. Theoretical considerations of a "well stirred" model and a "parallel tube" model. Influence of hepatic blood flow, plasma and blood cell binding and the hepatocellular enzymatic activity on hepatic drug clearance. J. Pharmacokin. Biopharm. 5: 625-653, 1977.

Pascal, J.P. and Cales, P. Propranolol in the prevention of first upper gastrointestinal tract hemorrhage in patients with cirrhosis of the liver and esophageal varices. N. Eng. J. Med. 317: 856-861, 1987.

Peschl, L. Klinische und experimentelle untersuchungen uber die wirkung von dopamin auf die hamodynamik und funktion von niere und leber. Wien. Klin. Wschr. 90(Suppl.86): 3-33, 1978.

Phillips, M.J., Poucell, S., Patterson, J., and Valencia, P. The Liver: An Atlas and Text of Ultrastructural Pathology. Raven Press, New York, 1987.

Popper, H. Pathological aspects of cirrhosis. Am. J. Pathol. 87: 228-258, 1977.

Popper, H. and Zak, F.G. Pathological aspects of cirrhosis. Am. J. Med. 24: 593-619, 1958.

Premen, A.J. Splanchnic and renal hemodynamic responses to intraportal infusion of glucagon. Am. J. Physiol. 253: F1103-F1112, 1987.

Rappaport, A.M., Borowy, Z.J., Lougheed, W.M., and Lotto, W.N. Subdivision of hexagonal liver lobules into a structural and functional unit. Anat. Rec. 119: 11-33, 1954.

Rector, W.G. Drug therapy for portal hypertension. Ann. Int. Med. 105: 96-107, 1986.

Rector, W.G., Hossock, K.F., and Ready, J.B. Nitroglycerin for portal hypertension: a controlled comparison of the hemodynamic effects of graded doses. J. Hepatol. 10: 375-380, 1990.

Reichen, J., Hirlinger, A., Ha, H.R., Saegesser, H. Chronic verapamil administration lowers portal pressure and improves hepatic function in rats with liver cirrhosis. J. Hepatol. 3: 49-58, 1986.

Reichen, J. Liver function and pharmacological considerations in pathogenesis and treatment of portal hypertension. Hepatology 11: 1066-1078, 1990.

Reichen, J. and Le, M. Verapamil favourably influences hepatic microvascular exchange and function in rats with cirrhosis of the liver. J. Clin. Invest. 78: 448-455, 1986.

Reilly, F.D., McCuskey, P.A., and McCuskey, R.S. Intrahepatic distribution of nerves in the rat. Anat. Rec. 191: 55-68, 1978.

Remak, G., Hottenstein, O.D., and Jacobson, E.D. Sensory nerves mediate neurogenic escape in rat gut. Am. J. Physiol. 258: H778-H786, 1990.

Reynolds, T.B., Hidemura, R., and Michel, H. and Peters, R. Portal hypertension without cirrhosis in alcoholic liver disease. Ann. Intern. Med. 70: 497-506, 1969.

Ribes, G., Trimble, E.R., Blayac, J.B., Wollheim, C.B., and Loubatieres-Mariani, M.M. In vivo stimulation of pancreatic hormone secretion by norepinephrine infusion in the dog. Am. J. Physiol. 246: E339-E343, 1984.

Richardson, P.D.I. and Withrington, P.G. The vasodilator actions of isoprenaline, histamine, prostaglandin E_2, glucagon and secretin on the hepatic arterial vascular bed of the dog. Br. J. Pharmac. 57: 581-588, 1976a.

Richardson, P.D.I. and Withrington, P.G. The inhibition by glucagon of the vasoconstrictor actions of noradrenaline, angiotensin, and vasopressin on the hepatic arterial vascular bed of the dog. Br. J. Pharmac. 57: 93-102, 1976b.

Richardson, P.D.I. and Withrington, P.G. Glucagon inhibition of hepatic arterial responses to hepatic nerve stimulation. Am. J. Physiol. 233: H647-H654, 1977.

Richardson, P.D.I. and Withrington, P.G. The effects of intraportal infusions of glucagon on the hepatic arterial and portal venous vascular beds of the dog: inhibition of hepatic arterial vasoconstrictor responses to noradrenaline. Pflugers Arch. 378: 135-140, 1978a.

Richardson, P.D.I. and Withrington, P.G. Responses of the simultaneously-perfused hepatic arterial and portal venous vascular beds of the dog to histamine and 5-hydroxytryptamine. Br. J. Pharmac. 64: 581-588, 1978b.

Richardson, P.D.I. and Withrington, P.G. The effects of intra-arterial and intra-portal injections of vasopressin on the simultaneously perfused hepatic arterial and portal venous vascular beds of the dog. Circ. Res. 43: 496-503, 1978c.

Richardson, P.D. and Withrington, P.G. Liver blood flow II. Effects of drugs and hormones on liver blood flow. Gastroenterology 81: 356-375, 1981.

Rikkers, L.F. Is the distal splenorenal shunt better? Hepatology 8: 1705-1707, 1988.

Rikkers, L.F. New concepts of pathophysiology and treatment of portal hypertension. Surgery 107: 481-488, 1990.

Rojdmark, S., Bloom, G., Chou, M.C.Y., Jaspan, J.A., and Field, J.B. Hepatic insulin and glucagon extraction after their augmented secretion in dogs. Am. J. Physiol. 235(1): E88-E96, 1978.

Ross, G. Effects of epinephrine and norepinephrine on the mesenteric circulation of the cat. Am. J. Physiol. 212: 1037-1042, 1967.

Ross, G. Regional circulatory effects of pancreatic glucagon. Br. J. Pharmac. 38: 735-742, 1970.

Ross, G. Escape of mesenteric vessels from adrenergic and nonadrenergic vasoconstriction. AM. J. Physiol. 221(5): 1217-1222, 1971.

Ross, G. and Kurrasch, M. Adrenergic responses of the hepatic circulation. Am. J. Physiol. 216: 1380-1385, 1969.

Rothe, C.F. Reflex control of veins and vascular capacitance. Physiol. Rev. 63: 1281-1342, 1983.

Roulot, D., Braillon, A., Gaudin, C., Ozier, Y., Girod, C., and Lebrec, D. Mechanism of clonidine-induced decreases in portal pressure in normal and cirrhotic conscious rats. Hepatology 10: 477-481, 1989.

Rowland, M., Benet, L.Z., and Graham, G.G. Clearance concepts in pharmacokinetics. J. Pharmacokin. Biopharm. 1: 123-136, 1973.

Sakai, H. Changes of the insulin and glucagon receptors in bile duct ligated rats. Nippon. Geka. Gakkai. Zasshi. 93: 36-42, 1992.

Sakoda, K. and Atik, M. Influence of common bile duct ligation on hepatic blood flow. Am. Surg. 36: 731-736, 1970.

Samols, E. and Weir, G.C. Adrenergic modulation of pancreatic A, B and D cells. α-Adrenergic suppression and ß-adrenergic stimulation of somatostatin secretion, α-adrenergic stimulation of glucagon secretion in the perfused dog pancreas. J. Clin. Invest. 63: 230-238, 1979.

Sancetta, S.M. Dynamic and neurogenic factors determining the hepatic arterial flow after portal occlusion. Circ. Res. 1: 414-418, 1953.

Sasaki, H., Rubalcava, B., Baetens, D., Blasquez, E., Srikant, C.B., Orci, L. and Unger, R.H. Identification of glucagon in the gastro-intestinal tract. J. Clin. Invest. 56: 135-145, 1975.

Schafer, J., d'Almeida, M.S., Weisman, H., and Lautt, W.W. Reduced hepatic blood volume responses to hemorrhage and nerve stimulation but normal compliance and norepinephrine effects in cats with chronic bile duct ligation. Hepatology (submitted), 1992.

Schuit, F.C. and Pipeleers, D.G. Differences in adrenergic recognition by pancreatic A and B cells. Science 232: 875-877, 1986.

Shanbour, L.L. and Hinshaw, L.B. Effects of dopamine on the liver before and following administration of endotoxin. Can. J. Physiol. Pharmacol. 47: 923-928.1986, 1969.

Shasha, S.M., Better, O.S., Chaimovitz, C., Doman, J., and Kishor, Y. Hemodynamic studies in dogs with chronic bile duct ligation. Clin. Sci. Mole. Med. 50: 533-537, 1976.

Shepherd, J.T. and Vanhoutte, P.M. Local modulation of adrenergic neurotransmission in blood vessels. J. Cardiovasc. Pharmacol. 7(S3): S167-S178, 1985.

Sherlock, S. Primary biliary cirrhosis (chronic intrahepatic obstructive jaundice). Gastroenterology, 37: 574-586, 1959.

Sherlock, S. Diseases of the Liver and Biliary System. 8th edition, Blackwell Scientific Publications, Oxford, 1989.

Sherwin, R.S., Fisher, M., Bessoff, J., Snyder, N., Hendler, R., Conn, H.O., and Felig, P. Hyperglucagonemia in cirrhosis: altered secretion and sensitivity to glucagon. Gastroenterology 74: 1224-1228, 1978.

Shimazu, T. Regulation of glycogen metabolism in liver by the autonomic nervous system. V. Activation of glycogen synthetase by vagal stimulation. Biochm. Biophys. Acta. 252: 28-38, 1971.

Shimazu, T., and Fujimoto, T. Regulation of glycogen metabolism in liver by the autonomic nervous system. IV. Neural control of glycogen biosynthesis. Biochm. Biophys. Acta. 252: 18-27, 1971.

Shoemaker, W.C., Van Itallie, T.B., and Walker, W.F. Measurement of hepatic glucose output and hepatic blood flow in response to glucagon. Am. J. Physiol. 196(2):315-318, 1959.

Shulman, G.I., Liljenquist, J.E., Williams, P.E., Lacy, W.W., Cherrington, A.D. Glucose disposal during insulinopenia in somatostatin-treated dogs: the roles of glucose and glucagon. J. Clin. Invest. 62: 487-491, 1978.

Siderys, H., Tyson, K.T., Henrendeen, T.L., Glover, J.L. Experimental augmentation of portal blood flow. Ann. Surg. 160: 910-918, 1964.

Sikuler, E. and Groszmann, R.J. Interaction of flow and resistance in maintenance of portal hypertension in rat model. Am. J. Physiol. 250: G205-G212, 1986a.

Sikuler, E. and Groszmann, R.J. Hemodynamic studies in long- and short-term portal hypertensive rats: the relation to systemic glucagon levels. Hepatology 6: 414-418, 1986b.

Sikuler, E., Kravetz, D., and Groszmann, R.J. Evolution of portal hypertension and mechanisms involved in its maintenance in a rat model. Am. J. Physiol. 248: G618-G625, 1985.

Silva, G., Gomis, R., Bosch, J., Casamitjana, R., Mastai, R., Navasa, M., Rivera, F. and Rodes, J. Hyperglucagonism and glucagon resistance in cirrhosis. Paradoxical effect of propranolol on plasm glucagon levels. J. Hepatol. 6: 325-331, 1988.

Sirica, A.E., Elmore, L.W., and Sano, N. Characterization of rat hyperplastic bile ductular epithelial cells in culture and in vivo. Dig. Dis. Sci. 36: 494-501, 1991.

Sistare, F.D., Picking, R.A., Haynes, Jr., R.C. Sensitivity of the response of cytosolic calcium in Quin-2-loaded rat hepatocytes to glucagon, adenine nucleosides, and adenine nucleotides. J. Biol. Chem. 260: 12744-12747, 1985.

Sitzmann, J.V., Buckley, G., Mitchell, M.C., and Campbell, K. Role of prostacyclin in the splanchnic hyperemia contributing to portal hypertension. Ann. Surg. 209: 322-327, 1989.

Skoglund, G., Lundquist, I., and Ahren, B. α_1- and α_2-adrenoceptor activation increases plasma glucagon levels in the mouse. Eur. J. Pharmacol. 143: 83-88, 1987.

Smitherman, T.C. Osborn, R.C., and Atkins, J.M. Cardiac dose-response relationship for intravenously infused glucagon in normal intact dogs and men. Am. Heart J. 96: 363-371, 1978.

Staddon, J.N. and Hansford, R.G. Evidence indicating that the glucagon-induced increases in cytoplasmic-free Ca^{+2} concentration in hepatocytes is mediated by an increase in cyclic AMP concentration. Eur. J. Biochem. 179: 47-52, 1989.

Sutherland, E.W., Robison, G.A. and Butcher, R.W. Some aspects of the biological role of adenosine 3', 5'-monophosphate (cyclic AMP). Circulation 37: 279-306, 1968.

Tai, T-Y., Pek, S. Direct stimulation by growth hormone of glucagon and insulin release from isolated rat pancreas. Endocrinology 9: 669-677, 1976.

Ternberg, J. and Butcher, H.R. Blood-flow relation between hepatic artery and portal vein. Science 150: 1030-1031, 1965.

Tibblin, S., Kock, N.G., and Schenk, W.G. Splanchnic hemodynamic responses to glucagon. Arch. Surg. 100: 84-89, 1970.

Tibblin, S., Kock, N.G., Worthington, G. and Schenk, W.G., Jr. Response of mesenteric blood flow to glucagon. Arch. Surg. 102: 65-70, 1971.

Trams, E.G. and Symeonidis, A. Morphologic and functional changes in the livers of rats after ligation of the or excision of the common bile duct. Am. J. Pathol. 33: 13-27, 1957.

Tsai, Y.T., Lay, C.S., Lai, K.H., Ng, W.W., Yeh, Y.S., Wang, J.Y., Chiang, T.T., Lee, S.D., Chiang, B.N., and Lo, K.J. Controlled trial of vasopressin plus, vasopressin plus nitroglycerin vs. vasopressin alone in the treatment of bleeding esophageal varices. Hepatology 6: 406-409, 1986.

Tsuneki, K. and Ichihara, K. Electron microscope study of vertebrate liver innervation. Arch. Histol. Jpn. 44: 1-13, 1981.

Unger, R.H., Ohneda, A., Aguilar-Parada, E., Eisentraut, A.M. The role of aminogenic glucagon secretion in blood-glucose homeostasis. J. Clin. Invest. 48: 810-822, 1969.

Unger, R.H. and Orci, L. Physiology and pathophysiology of glucagon. Physiol. Rev. 56: 778-826, 1976.

Unger, R.H. and Orci, L. Glucagon and the A cell. N.E. J. Med. 304: 1518-1524, 1981.

Ungvary, G., and Donath, T. Neurohistochemical changes in the liver of guinea pigs following ligation of the common bile duct. Exp. Mol. Pathol. 22: 29-34, 1975.

Vallance, P. and Moncada, S. Hyperdynamic circulation in cirrhosis: a role for nitric oxide? Lancet 337: 776-778, 1991.

Vorobioff, S., Bredfeldt, J.E., and Groszmann, R.J. Hyperdynamic circulation in portal hypertensive rat model: a primary factor for maintenance of chronic portal hypertension. Am. J. Physiol. 244: G52-G57, 1983.

Wahren, J. and Ericksson, L.S. The influence of a long-acting somatostatin analogue in splanchnic hemodynamics and metabolism in healthy subjects and patients with liver cirrhosis. Scand. J. Gastroenterol. 21(S119): 103-108, 1986.

Wakelam, M.J.O., Murphy, G.J., Hruby, V.J. and Houslay, M.D. Activation of two signal-transduction systems in hepatocytes by glucagon. Nature, 323: 68-71, 1986.

Walker, W.F., MacDonald, J.S., and Pickard, C. Hepatic vein sphincter mechanism in the dog. Br. J. Surg. 48: 218-220, 1960.

Wanless, I.R., Medline, A., Phillips, M.J. Pathology of the hepatic vasculature including hepatic vascular tumors. In: Hepatic circulation in health and disease. W.W. Lautt, Ed. Raven Press, New York, pp. 257-281, 1981.

Warren, W.D., Zeppa, R., Fomon, J.J. Selective trans-splenic decompression of gastroesophageal varices by distal splenorenal shunt. Ann. Surg. 166: 437-455, 1967.

Whipple, A.D. The problem of portal hypertension in relation to the hepatosplenopathies. Ann. Surg. 122: 449-475, 1945.

Wilkinson, G.R. and Shand, D.G. A physiological approach to hepatic drug clearance. Clin. Pharmacol. Ther. 18: 377-390, 1975.

Willet, I., Esler, M., Burke, F., Leonard, P., and Dudley, F. Total and renal sympathetic nervous system activity in alcoholic cirrhosis. J. Hepatol. 1: 639-648, 1985.

Willet, I.R., Jennings, G., Esler, M., Dudley, F.J. Sympathetic tone modulates portal venous pressure in alcoholic cirrhosis. Lancet 2: 939-943, 1986.

Williams, R.D., Elliott, D.W., and Zollinger, R.M. The effect of hypotension in obstructive jaundice. Arch. Surg. 81: 334-340, 1960.

Wisse, E., De Zanger, R.B., Charles, K., Van Der Smissen, P., and McCuskey, R.S. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Hepatology 5: 683-692, 1985.

Witte, C.L., Witte, M.H., Bair, G., Mobley, W.P., Morton, D. Experimental study of hyperdynamic vs. stagnant mesenteric blood flow in portal hypertension. Ann. Surg. 179: 304-310, 1974.

Yamaguchi, N. Sympathoadrenal system in neuroendocrine control of glucose: mechanisms involved in the liver, pancreas, and adrenal gland under hemorrhagic and hypoglycemic stress. Can. J. Physiol. Pharmacol. 70: 167-206, 1992.

Yamamoto, M., Sato, M., Ido, T., Ukigusa, M., and Ozawa, K. Obstructive jaundice and hemorrhagic shock. Circ. Shock 5: 235-249, 1978.

Yamamoto, K., Sherman, I., Phillips, M.J., and Fisher, M.M. Three dimensional observations of the hepatic arterial terminations in the rat, hamster and human liver by scanning electron microscopy of microvascular casts. Hepatology 5: 452-456, 1985.

Yeung, R.T.T. and Wang, C.C.L. A study of carbohydrate metabolism in postnecrotic cirrhosis of liver. Gut 15: 907-912, 1974.

Zetterstrom, R. and Ernster, L. Bilirubin, an uncoupler of oxidative phosphorylation in isolated mitochondria. Nature, 178: 1335-1337, 1956.

Zito, R.A., Diez, A.R., and Groszmann, R.J. Comparative effect of nitroglycerin and nitroprusside on vasopressin-induced cardiac dysfunction in the dog. J. Cardiovasc. Pharmacol. 5: 586-591, 1983.

Zollinger, R.M. and Williams, R.D. Surgical aspects of jaundice. Surgery 39: 1016-1030, 1956.