

# A COMPARATIVE STUDY

### OF THE ARTERIAL VASCULATURE OF THE STOMACH

WITH SPECIAL REFERENCE TO THE SUBMUCOUS PLEXUS

A thesis submitted in fulfilment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in the

UNIVERSITY OF LONDON

by

COLIN ARTHUR WYATT, M.A. (Cantab.)

Department of Anatomy

September 1989

The Royal Free Hospital School of Medicine

University of London

London

ProQuest Number: U051174

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest U051174

Published by ProQuest LLC (2017). Copyright of the Dissertation is held by the Author.

All rights reserved. This work is protected against unauthorized copying under Title 17, United States Code Microform Edition © ProQuest LLC.

> ProQuest LLC. 789 East Eisenhower Parkway P.O. Box 1346 Ann Arbor, MI 48106 – 1346

#### ABSTRACT

A vascular factor may be implicated in the initiation of peptic ulceration. However, a review of the literature showed that information on the gastric and duodenal arterial vascular patterns, though plentiful in man, was scarce in animals. Hence, this study was undertaken to remedy the situation and to seek for particular features which might be implicated in the aetiology of ulceration.

The literature describing the arterial vasculature of the stomach and first part of the duodenum in the dog, monkey, cat, pig, rat, guinea pig, rabbit, ferret and dolphin was reviewed. The methods used to investigate the gastric vessels were also assessed.

The gastroduodenal arterial vasculature of dogs, monkeys, cats, pigs, rats, guinea pigs, rabbits, ferrets and a dolphin was perfused with a contrast medium, the muscularis externa was removed and the mucosa and submucosa were cleared. The topology and dimensions of the vessels were determined, with particular reference to the submucous plexus. In the dolphin and ferret, the extramural arterial vasculature was dissected and examined.

This work establishes the vascular architecture of the submucous plexus and the origin of the mucosal arteries in the species named, with the exception of the dolphin, where only the extramural arteries were described. Three notable features emerge.

- A gradation was found in the relative density of the submucous plexus between the various animals, from the richest in cats to the poorest in guinea pigs and rabbits.
- 2. In most species, a plexus was found in both the submucosa and the mucosa. There was a spectrum, between the dog, which had only a submucous plexus, through the cat, where

both were equally dense, to the rabbit, where most anastomoses were located in the mucosa.

3. In rabbits and guinea pigs, patches of mucosa were found which were supplied by end arteries. These arose outside the gut wall, not from the submucous or mucosal plexus.

This work defines certain vascular characteristics of potential animal models for the investigation of the role of end arteries in peptic ulceration. Since end arteries have been reported in man, and their role in the initiation of peptic ulceration has been hypothesized, it provides a specific animal model whereby the vulnerability of mucosa supplied by such an end artery system can tested in vivo.

#### **ACKNOWLEDGEMENTS**

I should first and foremost like to express my thanks to Dr. Christopher Piasecki for his guidance and support throughout the duration of this study and his patient forebearance and encouragement during the preparation of this manuscript.

I must also convey my appreciation of the support and facilities provided by many of the staff of the Royal Free Hospital School of Medicine. It is not possible to mention everyone who helped me by name, but particular mention must be made of several. My thanks go to Professor Ruth Bowden and latterly Professor John Harries for the use of the facilities of their department. Invaluable technical assistance was given by Mr. L.V. Grayson and his staff, particularly Mr. J. Norton. I am indebted to Mr. A. Rahim for instruction in, and assistance with, histology. Much of excellent photographic the work was undertaken enthusiastically by Mr. B. Pike and his staff. I am also grateful for much additional assistance from Miss. P. Fear and her library staff, and the staff of the animal house. In addition, I must record my gratitude to the Royal Free Hospital School of Medicine for the award of a Junior Research Fellowship held during the course of this work.

I am grateful to the National Institute of Biological Standards and Control for the provision of primate material, and to Professor K. Hobbs for pig stomachs. The dolphin was made available through the good offices of H.M. Inspector of Wrecks and the Natural History Museum, London.

Finally, it is with the very deepest gratitude that I acknowledge the tolerance, support and sacrifice of my wife and family, without which this thesis could never have been produced.

For Eileen who made this work possible

and

•

Geoffrey, Olivia, Edmund and Antonia

who made it worthwhile.

# TABLE OF CONTENTS

TITLE	1
ABSTRACT	2
ACKNOWLEDGEMENTS	4
DEDICATION	5
TABLE OF CONTENTS	6
<u>PART</u> <u>I</u> - INTRODUCTION	16
<u>PART II</u> - REVIEW OF THE LITERATURE	18
<u>CHAPTER</u> <u>1</u> - NOMENCLATURE	18
<u>Chapter</u> <u>2</u> - Materials and Methods	22
1. Direct Methods	22
a. Post Mortem	22
i. Increased Resolution	22
ii. Preservation of Surrounding	
Tissue	23
b. In Vivo	25
2. Indirect Methods	26
a. Appearance	27
b. Blood Flow	27
3. Conclusions	28
<u>CHAPTER</u> <u>3</u> - THE CAT	29
1. Non-vascular Gastroduodenal Anatomy	29
a. External Form	29
b. Mucosal Folds	30
c. Gastro-Oesophageal Junction	30
d. Epithelial Lining	31
e. Musculature	33
f. Conclusions	34
2. Extramural Vasculature	35
a. Description	36
b. Conclusions	39

3.	Intramural Vasculature	39
	a. Description	39
	b. Conclusions	41
<u>CHAPTER</u>	$\underline{4}$ - THE PRIMATE	42
1.	Non-vascular Gastroduodenal Anatomy	42
	a. External Form	42
	b. Gastro-Oesophageal Junction	43
	c. Epithelial Lining	43
	d. Musculature	43
2.	Extramural Vasculature	44
	a. Description	44
	b. Conclusions	46
3.	Intramural Vasculature	47
<u>CHAPTER</u>	<u>5</u> - THE RABBIT	49
1.	Non-vascular Gastroduodenal Anatomy	49
	a. External Form	49
	b. Mucosal Folds	49
	c. Gastro-Oesophageal Junction	49
	d. Epithelial Lining	50
	e. Musculature	51
	f. Conclusions	52
2.	Extramural Vasculature	52
	a. Description	53
	b. Conclusions	54
3.	Intramural Vasculature	55
	a. Description	55
	b. Conclusions	56
<u>CHAPTER</u>	<u>6</u> - THE PIG	57
1.	Non-vascular Gastroduodenal Anatomy	57
	a. External Form	57
	b. Mucosal Folds	58
	c. Gastro-Oesophageal Junction	58
	d. Epithelial Lining	59
	e. Musculature	61
	f. Conclusions	62

2	2.	Extramural Vasculature	64
		a. Description	64
		b. Conclusions	<b>6</b> 6
3	3.	Intramural Vasculature	67
		a. Description	67
		b. Conclusions	73
<u>CHAPTE</u>	ER	<u>7</u> - THE GUINEA PIG	76
נ	1.	Non-vascular Gastroduodenal Anatomy	76
		a. External Form	76
		b. Mucosal Folds	77
		c. Gastro-Oesophageal Junction	77
		d. Epithelial Lining	77
		e. Musculature	79
		f. Conclusions	79
2	2.	Extramural Vasculature	80
		a. Description	81
		b. Conclusions	83
	3.	Intramural Vasculature	83
CHAPTI	<u>ER</u>	<u>B</u> - THE FERRET	84
-	1.	Non-Vascular Gastroduodenal Anatomy	84
		a. External Form	84
		b. Mucosal Folds	85
		c. Gastro-Desophageal Junction	86
			80
		f Conclusions	87
	<b>^</b>		88
•	۷.	Extramural Vasculature	88
	2		88
•	5.		09
CHAPTI	ER	9 - THE RAT	90
<u></u>	1.	Non-vascular Gastroduodenal Anatomy	90
-		a. External Form	90
		b. Mucosal Folds	92
		c. Gastro-Oesophageal Junction	94
		d. Epithelial Lining	94
		- <b>-</b>	

e.	Musculature
f.	Conclusions
2. Extr	amural Vasculature 98
a.	Description
b.	Arterioarterial Anastomoses102
c.	Conclusions107
3. Intr	amural Vasculature108
a.	Description108
b.	Duodenum121
c.	Physiological Studies122
d.	Conclusions123
<u>CHAPTER</u> 10 -	<b>THE DOG127</b>
1. Non-	vascular Gastroduodenal Anatomy127
a.	External Form127
b.	Mucosal Folds128
c.	Gastro-Oesophageal Junction128
d.	Epithelial Lining129
e.	Musculature130
f.	Conclusions131
2. Extr	amural Vasculature133
a.	Description133
b.	Conclusions137
3. Intr	amural Vasculature137
a.	Description138
b.	Conclusions142
<u>PART III</u> - ORIGIN	AL OBSERVATIONS143
<u>CHAPTER</u> 11 -	Materials and Methods143
1. Spec	imens143
2. Sacr	ifice143
3. Inje	ction Studies145
a.	Method145
b.	Media146
	i. Gelatine146
	ii. Latex146
	iii. Micropaque147

	c.	Dissection147
	d.	Dehydration148
	e.	Clearing148
	f.	Observations149
	g.	Photography149
	h.	Mapping150
4.	Hist	ology150
5.	Netw	ork Analysis151
	a.	Definitions151
	b.	Measurement152
	c.	Statistical Analysis153
6.	Disc	ussion153

<u>CHAPTER 12</u> - THE CAT163
1. Non-Vascular Gastroduodenal Anatomy163
a. General Appearance
b. Omental Attachments
c. Muscularis Externa
d. Miscellaneous164
2. Extramural Vasculature
3. Intramural Vasculature
a. Supply to Muscularis Externa166
b. Spatial Variations in Supply to M.E168
c. Submucous Plexus
d. Spatial Variations in SMP169
e. Connective Tissue Plexus
f. Spatial Variations in CTP
g. Mucosal Plexus
h. Spatial Variations in Mucosal Plexus.172
i. Network Analysis175
j. Mucosal Arteries
k. Spatial Variations in M.A's178
1. Arteriovenous Anastomoses
m. Duodenum178
4. Discussion180
CHAPTER 13 - THE PRIMATE184

1.	Non-Vascular Gastroduodenal Anatomy184
	a. General Appearance
	b. Omental Attachments184
	c. Muscularis Externa185
	d. Miscellaneous185
2.	Extramural Vasculature186
3.	Intramural Vasculature188
	a. Supply to Muscularis Externa188
	b. Spatial Variations in Supply to M.E188
	c. Submucous Plexus188
	d. Spatial Variations in SMP
	e. Connective Tissue Plexus
	f. Spatial Variations in CTP
	g. Mucosal Plexus193
	h. Spatial Variations in Mucosal Plexus.195
	i. Mucosal Arteries198
	j. Spatial Variations in M.A's198
	k. Arteriovenous Anastomoses198
	1. Duodenum
4.	Discussion
4.	1. Duodenum
4. <u>CHAPTER</u>	1. Dubdenum
4. <u>CHAPTER</u> 1.	1. Duodendam
4. <u>Chapter</u> 1.	1. Duodenda
4. <u>CHAPTER</u> 1.	1. Duodenda.   Discussion.   200   14 - THE RABBIT.   205   Non-Vascular Gastroduodenal Anatomy.   205   a. General Appearance.   205   b. Omental Attachments.   205   c. Muscularis Externa.
4. <u>CHAPTER</u> 1.	1. Duodendam. 200   Discussion. 200   14 - THE RABBIT. 205   Non-Vascular Gastroduodenal Anatomy. 205   a. General Appearance. 205   b. Omental Attachments. 205   c. Muscularis Externa. 205   d. Miscellaneous. 206
4. <u>CHAPTER</u> 1. 2.	1. Duodenum
4. <u>CHAPTER</u> 1. 2.	1. Duodenum.Discussion.20014 - THE RABBIT.205Non-Vascular Gastroduodenal Anatomy.205a. General Appearance.205b. Omental Attachments.205c. Muscularis Externa.205d. Miscellaneous.206Extramural Vasculature.208Intramural Vasculature.210
4. <u>CHAPTER</u> 1. 2. 3.	1. Duodenum.Discussion.20014 - THE RABBIT.205Non-Vascular Gastroduodenal Anatomy.205a. General Appearance.205b. Omental Attachments.205c. Muscularis Externa.205d. Miscellaneous.206Extramural Vasculature.208Intramural Vasculature.210a. Supply to Muscularis Externa.210
4. <u>CHAPTER</u> 1. 2. 3.	1. Duodenum.Discussion.20014 - THE RABBIT.205Non-Vascular Gastroduodenal Anatomy.205a. General Appearance.205b. Omental Attachments.205c. Muscularis Externa.205d. Miscellaneous.206Extramural Vasculature.208Intramural Vasculature.210a. Supply to Muscularis Externa.210b. Spatial Variations in Supply to M.E. 211
4. <u>CHAPTER</u> 1. 2. 3.	<pre>1. Duodenda</pre>
4. <u>CHAPTER</u> 1. 2. 3.	<pre>1. Duodendm</pre>
4. <u>CHAPTER</u> 1. 2. 3.	1. Dubuenum200Discussion20014 - THE RABBIT205Non-Vascular Gastroduodenal Anatomy205a. General Appearance205b. Omental Attachments205c. Muscularis Externa205d. Miscellaneous206Extramural Vasculature208Intramural Vasculature210a. Supply to Muscularis Externa210b. Spatial Variations in Supply to M.E. 211211d. Spatial Variations in SMP212e. Connective Tissue Plexus212
4. <u>CHAPTER</u> 1. 2. 3.	1. Buddendm
4. <u>CHAPTER</u> 1. 2. 3.	1. DubuenumDiscussion
4. <u>CHAPTER</u> 1. 2. 3.	1. Duodenum200Discussion

.

•

	j.	Arteriovenous	Anastomoses229	Э
	k.	Duodenum		9
4.	Disc	ussion		D

<u>CHAPTER</u>	<b><u>15</u></b> - THE PIG236
1.	Non-Vascular Gastroduodenal Anatomy236
	a. General Appearance
	b. Omental Attachments236
	c. Muscularis Externa
	d. Miscellaneous239
2.	Extramural Vasculature240
3.	Intramural Vasculature243
	a. Supply to Muscularis Externa243
	b. Spatial Variations in Supply to M.E244
	c. Submucous Plexus244
	d. Spatial Variations in SMP247
	e. Connective Tissue Plexus254
	f. Spatial Variations in CTP254
	g. Mucosal Arteries254
	h. Spatial Variations in M.A's255
	i. Arteriovenous Anastomoses259
4.	Discussion
<u>CHAPTER</u>	<b><u>16</u></b> - THE GUINEA PIG263
1.	Non-Vascular Gastroduodenal Anatomy263
	a. General Appearance263
	b. Omental Attachments263
	c. Muscularis Externa263
_	d. Miscellaneous264
2.	Extramural Vasculature264
3.	Intramural Vasculature266
	a. Supply to Muscularis Externa266
	b. Spatial Variations in Supply to M.E269
	c. Submucous Plexus
	d. Spatial Variations in SMP272
	e. Connective Tissue Plexus277
	f. Mucosal Plexus277

	g. Spatial Variations in Mucosal Plexus.278
	h. Mucosal Arteries
	i. Spatial Variations in M.A's282
	j. Arteriovenous Anastomoses
	k. Duodenum286
4.	Discussion
<u>CHAPTER</u>	<b><u>17</u></b> - THE FERRET
1.	Non-Vascular Gastroduodenal Anatomy292
	a. General Appearance
	b. Omental Attachments
	c. Muscularis Externa
	d. Miscellaneous294
2.	Extramural Vasculature
	a. Conclusions
3.	Intramural Vasculature
	a. Supply to Muscularis Externa
	b. Submucous Plexus
	c. Spatial Variations in SMP
	d. Connective Tissue Plexus
	e. Mucosal Arteries
	f. Spatial Variations in M.A's312
	g. Arteriovenous Anastomoses
	h. Duodenum
4.	Discussion

.

<u>CHAPTER</u>	<u>18</u> - THE RAT
1.	Non-Vascular Gastroduodenal Anatomy316
	a. General Appearance
	b. Omental Attachments
	c. Muscularis Externa
	d. Miscellaneous
2.	Extramural Vasculature
3.	Intramural Vasculature
	a. Supply to Muscularis Externa322
	b. Spatial Variations in Supply to M.E322
	c. Submucous Plexus

	d. Spatial Variations in SMP
	e. Connective Tissue Plexus
	f. Mucosal Plexus
	g. Mucosal Arteries
	h. Spatial Variations in M.A's326
	i. Arteriovenous Anastomoses
	k. Duodenum328
1.	Discussion
7 D	19 - THE DOG
1	Non-Vaccular Castroduodenal Anatomy 331
L •	Non-Vasculal Gascioulodenal Anacomy
	a. General Appearance
	b. Omental Attachments
	c. Muscularis Externa
	d. Miscellaneous332
2.	Extramural Vasculature
3.	Intramural Vasculature
	a. Supply to Muscularis Externa335
	b. Spatial Variations in Supply to M.E336
	c. Submucous Plexus
	d. Spatial Variations in SMP
	e. Connective Tissue Plexus
	f. Mucosal Plexus
	g. Mucosal Arteries
	h. Spatial Variations in M.A's
	i. Arteriovenous Anastomoses
	j. Duodenum
4.	Discussion
	4.

<u>CHAPTER</u>	<b><u>20</u></b> - THE DOLPHIN
1.	Introduction
2.	Gastroduodenal Anatomy - Review
3.	Original Observations
	a. Gross Anatomy
	b. Extramural Vasculature
	c. Intramural Vasculature

4. Discussion	.360
---------------	------

# 

PART	<u>IV</u> - Appe	ENDIC	<b>ES</b>
	Appendix	1 -	Spalteholz Technique for Tissue
			Clearing
	Appendix	2 -	Modified Spalteholz Technique378
	Appendix	3 -	Sorenson's Phosphate Buffer379
	Appendix	4 -	Technique to Counterstain Cleared
			Tissue
	Appendix	5 -	Haematoxylin and Eosin Method381
	Appendix	6 -	Van Gieson Method
	Appendix	7 -	Estimates of Areas Sampled by Oi383
	Appendix	8 -	Abbreviations in Diagrams & Text384
	Appendix	9 -	Protocol for Kolmogorov-Smirnov Two
			Sample Test
	Appendix	10 -	-Piasecki, C. & Wyatt, C.A. (1980).
			Comparative Study of the Intramural
			Blood Supply to the Gastric Mucosa
			with Special Reference to End Artery
			Systems.
			J. Anat. <u>130(2)</u> , 650
	Appendix	11 -	-Piasecki, C. & Wyatt, C. (1986).
			Patterns of Blood Supply to the
			Gastric Mucosa. A Comparative Study
			Revealing an End Artery Model.
			J. Anat. <u>149</u> , 21-39Inside Back Cover

# PART I INTRODUCTION

The treatment of peptic ulceration has undergone a revolution during the last decade, as effective pharmacological therapy has obviated much of the need for surgical intervention. However, both gastric and duodenal ulceration remains a common and disabling disorder, with a significant mortality. In 1986, 4528 deaths were attributed in England and Wales to these causes (ICD 531 + 532 + 533).

This disease, or group of diseases, has been investigated for over 80 years, and although great strides have been made in treatment, there is still no unequivocal comprehensive theory to account for its aetiology. More recently, the multifactorial nature of the condition has been accepted, encompassing earlier hypotheses, such as bile reflux, genetics, mucosal ischaemia, environmental factors and neurogenic influences.

Two particular aspects of ulceration which still require explanation are the tendency of ulcers to occur singly and their predominant localisation to the lower lesser curvature in the case of gastric ulcers, and to the anterior and posterior aspects of the first 2cm of the duodenum in the case of duodenal ulcers. Various hypotheses have been proposed to explain these phenomena, such as the occurence of localised compression (Ivy, Grossman & Bachrach 1950), islets of ectopic mucosa (Ivy et al. 1950; Spira 1956), a "pyloric jet" of gastric contents into the duodenum (Kirk 1968) and the interaction of mucosal boundaries and intramural muscle bundles (Oi et al. 1969).

The significance of a vascular factor is suggested by three pieces of evidence. Tension on the stomach downwards and to the left results reversibly in an area of pallor on the antral wall of the first 2.5cm of the duodenum - "Mayo's anaemic spot" (Mayo 1908; Kirk 1968). Post-mortem anatomical examination in man, largely using injection techniques, has

shown that the lesser curvature is less well vascularized than the remainder of the stomach (Disse 1903; Reeves 1920; Jatrou 1920; Hoffman & Nather 1921; Berlet 1923; Barlow, Bentley & Walder 1951; Piasecki 1974). Finally, vessels have been demonstrated in man which penetrate the gastric muscularis and supply patches of mucosa, but which do not communicate with the intramural arterial plexus - "Mucosal arteries of extramural origin" (Piasecki 1974). They occur singly or in small numbers in a distribution which parallels that of gastric ulcer localisation.

Experimental investigation of peptic ulceration has used a range of species, but comparative regional differences in gastroduodenal vascular anatomy have hardly been studied. This study was therefore undertaken to compile a detailed description of the intramural arterial vasculature of the stomach and first part of the duodenum in a range of laboratory species (dog; primate; cat; pig; rat; guinea pig; rabbit; ferret). In addition, the dolphin was examined as the occurence of spontaneous peptic ulceration is well documented in them, in contrast to other species (Ridgeway 1965; Ridgeway & Johnson 1965; Testi et al. 1971). The extramural arterial vasculature was analysed in greater detail in those species in which it had not been described in the literature (ferret and dolphin). In particular, the occurence and characteristics of intramural end arteries were investigated in all species. The overall objective was to provide a firm foundation for the selection and interpretation of animal models in which further investigation of vascular factors in the aetiology of peptic ulceration could be more rationally investigated.

# <u>PART II</u> <u>REVIEW OF THE LITERATURE</u>

# <u>CHAPTER</u> <u>1</u> <u>NOMENCLATURE</u>

A system of scientific classification must not only be self consistent and reflect the inherent structure and divisions of a phenomenon, but must also be universally accepted. The early anatomists, such as Vesalius (1555), devised their own nomenclature for the structures they observed. Although there was some awareness of the classifications used by contemporaries and predecessors in the field, the individualist tradition prevented the formation of a consensus. Thus, von Haller (1745) suggested that the Left Gastric Artery ("Coronaria superior arteria") should be named the " Arteria gastrohepatica sinistra", due to the common occurrence of a large branch of the artery passing to the liver. (this has been revived by Michels (1955)). However, the majority of subsequent authors ignored von Haller's suggestion. This approach resulted in the existence of a number of synonyms for each structure. Bell (1826) speaks of the " Arteria coeliaca (or coeliac artery or coeliac trunk or axis arteriae coeliacae)" and of the "arteria coronaria ventriculis ( or coronary artery of the stomach or gastric artery)".

The first attempt to remedy this was made in 1895, with the publication of the Basle Nomina Anatomica (B.N.A.) This reduced (Jamieson 1916). approximately 30,000 anatomical terms in use to about 5000. It was based upon the body in the anatomical position, but was not widely adopted. In 1933, the Birmingham Revision (B.R.) of the BNA was issued, which was also based upon the anatomical position (for examples see Cunningham 1951). This was followed by the Jena Nomina Anatomica (J.N.A. - 1936) in which structural descriptions were related directly to position in the body and not to arbitrary spatial co-ordinates. In 1955, the Paris Nomina Anatomica (P.N.A.), which is the basis of

current terminology, was published. This returned to the anatomical position standard (Nomina Anatomica 1963).

The Basle Nomina Anatomica (1916) was unsuitable for direct application to veterinary anatomy as it was based upon the anatomical position. An adaptation was published in 1923, the Nomina Anatomica Veterinaria (N.A.V.), but this was not generally accepted. In response to the Paris Nomina Anatomica, the International Association of Veterinary Anatomists was formed, and this produced a revised Nomina Anatomica Veterinaria (1963; 1973)

The evolution of anatomical terms from Gray (1858) to P.N.A. with reference to the coeliac trunk and its branches in Man has been reviewed by Piasecki (1973). Due to the non applicability of the anatomical position to animals and actual differences in structure, the P.N.A. and N.A.V.  $(\tau_{a}(\tau_{a}))$  terminologies are not identical. For example, terms such as "superior" (P.N.A.) are replaced by position invariant terms such as "cranialis" (N.A.V.). There are also differences between the orders in the nomenclature. For example, in swine (Sus), the left gastric artery is listed under the splenic artery. Wherever possible the terms used by the authors themselves will be used in this work, rather than attempting to reduce them to a common terminology, with the consequent risk of misinterpretation.

## Table. 1

## A COMPARISON OF HUMAN (PARIS NOMINA ANATOMICA)

# <u>AND</u>

## VETERINARY (NOMINA ANATOMICA VETERINARIA)

#### NOMENCLATURE

#### <u>Notes</u>

1. Corresponding vessels are not always identically named.

2. Branching order is indicated by indentation order.

3. Branching order is not constant. Corresponding displaced vessels are joined.

4. The absence of a corresponding vessel at a branch point is indicated.

5. The NAV has separate nomenclatures for Carnivora, Sus, Ruminantia and Equus. Only the first two are included.

(Table overleaf)

PARIS NOMINA ANATOMICA	NOMINA ANATOM	ICA VETERINARIA
MAN	CARNIVORA	SUS (PIG)
Truncus cellacus	A. Cellaca	A. celiaca
(A. phrenica superior - irom aorta)	A. phrenica caudalis (felidae)	A. phrenica caudalis
A. gastrica sinistra	A. gastrica sinistra	
Rr. esophage1	Rr. esophagei	
A. hepatica communis	A. hepatica	A. hepatica
A. gastrica dextra		
A. hepatica propria		
		Dr nancreatici
K. GeXter	K. Gexter Lateralis	K. Gexter lateralls
	A. IOD1 CAUDAT1	A. lobi caudati
		(A. gastroduodenalis
		( A. pancreaticoduodenalis
		( cranialis
		( A.gastroeninloica dextra
	R. dexter medialis	R. devter medialis
A. cvatica		A cvetica
R. einister	R. sinister	R. sinister
		Dr cinictri lateralec
	Rr sinistri modialos	Dr. cinictri madialee
	Dr. cinictri lateraled	
	A restrict doutre	A gentrice doutre
	A. yastrodindenslie	A. YASULICA WEALEA
A. gastrouuouenalis	A. yaseroutoutiaria	
A. pancreaticoguogenalis superior	A. Pailoteactcourouenaits	
Kr. pancreatici		
K. duodenales		
A. gastroepipioica dextra	A. gastroepipioica dextra)	
Rr. epiploici		
A. lienales	A. lienalis	A. lienalis
		(A. gastrica sinistra
		(Rr. esophagei
*****		A. diverticuli
Rr. pancreatici	Rr. pancreatici	R. pancreaticus
		R. gastrolienalis
A. gastroepiploica sinistra		
Rr. epiploici		
Aa. gastrici breves	Aa. gastrici breves	
Kr. lienales		
	A. gastroepiploica sinistra	A.gastroepiploica sinistra
	NOTES	
	1. Corresponding vessels are not	the second structure of the second se
	2. Branching Order is Indicated	by indentation order.
	vessels are joined by a broke	anti corresponding dispided
	4. The absence of a correspondir	ig vessel at a branch point
	is indicated.	•
	5. The NAV has separate nomencla Ruminata and Eduna, Only the	atures for Carnivora, Sus, first two are indicated.
	Table 1 A Comparison of Human (	(Paris Nomina Anatomica) and
	<u>Veterinary (Nomina Anat</u>	<u>comica Veterinaria)</u>
_	Nomenclature.	

.

.

# <u>CHAPTER 2</u> <u>MATERIALS AND METHODS</u>

Many techniques are available to examine the blood vessels at all levels of diameter. These fall into two main categories, direct and indirect.

#### DIRECT

These methods render the vessels clearly visible so that their characteristics may be measured and analysed. They comprise the most widely used techniques and can be subdivided into two further categories, post mortem and in vivo.

#### Post Mortem

The simplest way to visualise the blood vessels directly is to remove the surrounding tissue by dissection. This technique has been used by many authors to investigate the gastroduodenal circulation (e.g. Vesalius 1555; von Haller 1745; El-Eishi, 1973). However, when used alone, it has two principal limitations.

1. The maximum resolution which can be obtained is low.

2. It involves the destruction of surrounding tissue and, in the case of the deep vascular system of an organ, also of the more superficial vascular bed. This makes it difficult to relate the vessels to the tissues through which they pass and which they supply.

#### Increased Resolution

The resolution obtainable from dissection may be increased by prior injection of an optically opaque material into the vascular bed. This augments the contrast between the blood vessel and the surrounding tissue. Such a substance may be a dye which stains the vascular wall, such as haemotoxylin or methylene blue, or a particulate material, such as india ink or carmine. The latter is often suspended in a medium which will subsequently solidify, such as gelatine, latex rubber or acrylic resin. (Mall 1896; Djorup 1922; Greene 1935; Michels 1955; Schnitzlein 1957; Berg 1961; Bielak 1967;Obel 1967, 1968a; Perneczky 1969; Wiland 1970; Gannon 1982).

The maximum resolution is still limited by the process of manual dissection. This may be obviated by corrosion casting, whereby the vessels are injected with a monomer, usually coloured acrylic resin, and, after its polymerization, the tissue is removed by acidic or enzymic maceration, leaving the resistant vascular cast. This process accurately replicates details down to the capillary level. (Busch 1973; Gannon 1982).

## Preservation of Surrounding Tissue

The spatial relationship of the vascular system to the surrounding tissue is destroyed by all dissection techniques, but other processes permit the vessels to be visualised without this. These are primarily clearing, radiography and histology.

A result analogous to that of dissection may be obtained by making the tissue transparent - clearing. This is done by dehydration, followed by the replacement of the water by a liquid with a high refractive index. The most commonly used clearing agents are glycerine (Thompsett 1970) and benzene / benzyl benzoate / methyl salicylate (Spalteholtz 1914). The previously injected blood vessels can be seen in the transilluminated specimen. Either the arterial system alone (single) or both the arterial and venous systems (double - Hase & Moss 1973) may be injected, using the particulate materials discussed above. (de Busscher 1948; Leone 1948; Barlow 1951; Jacobsen & Noer 1952; Arabehety 1959; Boulter & Parks 1963; Bellamy 1973; Busch 1973; Hase & Moss 1973; Kobayashi 1973; Piasečki 1973. 1974, 1975).

An alternative approach is to examine the specimen radiographically, after the injection into the vessels of a radio-opaque material such as barium sulphate solution. (Mignot 1956; Minne 1952; Piasecki 1975). Three dimensional information may be preserved by taking angularly displaced radiographs, which are viewed stereoscopically, or by computer based tomography, perhaps with digital image reconstruction in three dimensions.

Conventional histological techniques display the arrangement and characteristics of blood vessels in the plane of the section, and the three dimensional architecture may be reconstructed from serial sections (Ware 1975). The identification of vessels is facilitated by their prior injection with a contrast medium (de Busscher 1948; Schnitzlein 1957; Boulter & Parks 1963; Sherman 1963; Bellamy 1973).

All of the above techniques rely upon the filling of a vessel by injectate to enable it to be seen. However, not all vessels may be patent at the time of injection or, if they are, they may not fill with injectate. Although it is normally possible to detect the presence of unfilled vessels, if the injectate is miscible with blood (Piasecki 1973), their nature or course cannot be determined. Hence, observations which demonstrate the presence of a vascular phenomenon must carry greater weight than those which show its absence. In contrast, the pressure applied to the injectate may force open vessels which were not patent at the time. This can be avoided by the use of a low pressure injection in vivo, or by limiting the applied force to the normal systemic blood pressure in that species.

The observed anatomy is, therefore, dependent on the filling pattern which is, in turn, a function of ante mortem physiological parameters and of the mode of death. Ante mortem injections will be modified by such factors as surgical shock (Basu Mallik 1955), drugs and anaesthetics (Baumel 1970; Altura & Altura 1975), all of which will affect the vasculature. Post mortem injections will be modified by the mode of death and by the length of time elapsing between killing and injecting the animal. Immediately after death, there is generalized vasoconstriction due to spasm of the vascular smooth muscle, which takes up to 24 hours to subside (Baumel 1970). This may alter the filling pattern.

The conditions under which the injection takes place are, therefore, important factors in determining the anatomy which is observed. Thus, patterns displayed by different techniques are not necessarily comparable. The same is true of the bores of the vessels, which vary as a function of the methods used (Hase & Moss 1973).

# <u>In Vivo</u>

Many of the above problems can be overcome by observing the gastroduodenal circulation in the living animal. This may be done in an anaesthetised animal by exposing and transilluminating the area of interest (Basu Mallik 1955; Baez 1959; Guth 1970, 1972a;1975). Although this technique overcomes the problem of post mortem artefacts, the results are not necessarily identical to those that would be obtained from the hypothetical 'intact' animal. Any invasive process must produce physiological effects which may modify the vasculature. For example, anaesthetics and drugs are given (Altura & Altura 1975), the animal is in surgical shock (Basu Mallik 1955) and superficial tissue and vessels must often be removed to obtain adequate resolution in deeper planes. Despite this, the resolution which is obtained is less than that resulting from injection techniques. This factor can be improved to a limited extent by the introduction of a contrast or fluorescent medium into the circulation. In the latter case (fluorescence angiography), a material such as fluorescin, coupled to a large molecule to retard its loss from the blood vessels, is introduced into the circulation and irradiated by ultra violet radiation, whereupon it emits light in the visible region of the spectrum, thereby increasing the contrast between the vessels and the surrounding tissues. Three dimensional reconstruction using holographic techniques has been achieved, but only of mesenteric vessels (Donaho 1972).

The vessels in the conscious, intact animal are currently most commonly studied after the injection of a radio-opaque medium, such as a suspension of barium sulphate, into the systemic circulation (Adams 1965; Mincev

1971; Enge 1972). Unfortunately, this method has a limited resolution capability, since focussing and magnification of the image is not possible, and it can be difficult to distinguish between overlapping and intercommunicating vessels. More recently, techniques such as computerised axial tomography, nuclear magnetic resonance and positron emission tomography have been used to examine larger vessels, although still only in two dimensions. Preservation of the three dimensional information can be achieved by taking two laterally displaced radiographs and viewing them stereoscopically. Computerised reconstruction of the full three dimensional network is possible, as a research rather than a clinical tool (IBM Scientific Centre - unpublished data). In addition, dynamic information can be retained by enhanced, if fluoroscopy, necessary, by image intensification. An alternative method if visualising the vasculature of the conscious animal is to remove the tissue overlying the vessels and to replace these layers with a transparent material, such as glass, mica or 'Perspex'. After healing, the underlying vessels may be observed by transillumination. Such techniques have only been used on thin structures, such as the rabbit's ear (Nims 1973).

## INDIRECT METHODS

Use is made of the relationship between changes of physiological parameters in response to experimental manoeuvres and the underlying vascular anatomy. The physiological parameters themselves may be indirectly determined from physical measurements e.g. blood flow from radioactive isotope distribution. Since the relationship between these quantities is often imperfectly understood, or based upon a number of assumptions which are made to reduce the complexity of the mathematical model, the amount of anatomical information obtainable is usually limited and often of uncertain validity.

#### <u>Appearance</u>

The physiological parameter may be the appearance of the tissue. For example, changes in the colour of gastric venous blood after handling the stomach may be interpreted in terms of arteriovenous shunts (Barclay & Bentley 1949a). The extent of the interarterial anastomoses and the areas supplied by individual vessels may be investigated by selective arterial ligature and observation of the consequent changes in the mucosa and blood flow patterns (Bernheim 1932; Blalock 1939; Babkin 1943; Layne 1943; Berg 1947; Baronofsky 1948; Holm 1949a, 1949b; Guth 1972, 1975; Varhaug 1979). An analogous technique was used by Seirafi (1962) who 'devascularized' the stomach of the dog by blocking its arterioles and capillaries with microspheres.

## Blood Flow

This is normally investigated by the use of exogenous tracers. These can be non-radioactive or radioactive isotopes, either in solution or bound to microscopic spheres, which can also be used in the unlabelled form. The results from the use of these techniques can be interpreted to give information of such factors as the volume of a region, the destinations of certain vessels and the presence and size of arteriovenous channels.

Examples of the use of non-radioactive tracers are Schnitzlein's work to assess the density of vessels in regions of the rat's stomach by measuring its optical density after the vascular injection of india ink (1957). Another is the use of aminopyrine clearance (Jacobsen 1966a, 1966b) from the mucosal capillaries to the gastric lumen to estimate mucosal blood flow.

A number of radioactive isotopes have been used to estimate blood flow e.g.  $^{99}$ Tc,  $^{47}$ K,  $^{86}$ RbCl and ( $^{17}$ C) analine (Delaney & Grim 1964; Taylor 1974, 1975a, 1975b, 1975c; Zinner 1975; Whittle 1976). These may be differentiated by their decay characteristics, so that more than one isotope may be used simultaneously. A proportion of the isotope is taken up by the tissue through which the blood passes, so that the final radioactivity of that tissue is a function of its blood flow, amongst other factors.

Microscopic spheres are available in a number of materials, such as glass, plastic, agar and latex. If such particles are injected into the circulation of an organ and are slightly larger than the anticipated maximum capillary size, they are trapped in the capillary bed in numbers which are a function of the blood flow. Counting unlabelled spheres in tissue sections or the radioactive assay of tissue samples containing isotopically labelled spheres, determines the number of particles that have been retained. The presence and extent of arteriovenous shunting may be indicated by the presence of microspheres in the venous effluent that have been injected into the arterial inflow. (Walder 1951; Sherman & Newman 1954; Peters 1958; Delaney 1972; Sales 1975; Zinner 1976).

The results from all the above techniques are open to interpretation, since the techniques are based upon theoretical relationships between the observed parameter and the required information, which necessarily simplify a complex system. Nevertheless, the validity of the radioactive tracer technique as a determinant of blood flow has been demonstrated by Taylor(1975), who showed that its results were in agreement with those derived from a number of other techniques. This has also been demonstrated for radioactive microspheres (Buckberg 1971; Archibald 1975), despite the suggestion that the tracer may elute from the microspheres and appear in the venous effluent (Buchin & Edlich 1969) and Grayson's reservations on a technique which depends upon the blockage of small blood vessels (1974). CONCLUSIONS

A number of techniques are available to study the gastroduodenal vasculature, all of which are limited in certain respects. There is no non-invasive method which will map this area in high resolution in the intact conscious animal, but the use of a number of techniques, with varying characteristics, can enable a more comprehensive description to be synthesized.

# <u>CHAPTER 3</u> <u>THE CAT</u>

#### NON-VASCULAR GASTRODUODENAL ANATOMY

There are a number of texts which describe the gross anatomy of the cat, such as Stromsten (1952), Taylor & Weber (1951), Bourdelle & Bressou (1953) and Crouch (1969). These authors concur in their descriptions of the foregut.

## External Form

The oesophagus passes through the diaphragmatic crura and, after a short course, enters the stomach. There is no enlargement of the oesophagus at the cardia and indeed Botha (1958a) described a slight constriction there, although this was denied by El-Ramli (1964) and Fatani (1971). The latter presented photographs which appeared to support his contention.

The 'J-shaped' stomach has a volume of 300 - 350 ml, with 80% of its bulk lying to the left of the midline. The oesophagus enters at almost the most rostral point of the stomach and the fundus is small. The right lateral aspect of the oesophagus is colinear with the lesser curvature whilst the incisura cardiaca, between its left lateral aspect and the greater curvature, is acute as is the incisura angularis.

The duodenum is approximately 15cm long with a dilatation in its first centimetre and is, according to Carleton (1935), unusually mobile so that it may form a single or a double loop. The arrangement more usually described (Taylor & Weber 1951; Crouch 1969) comprises an initial cranial flexure, immediately distal to its origin at the pylorus, followed by a descending portion, 8cm long, which passes slightly dextrad. This is succeeded by the caudal flexure, at approximately the level of the fifth lumbar vertebra, which leads into the ascending portion. The duodenum terminates at the duodenojejunal flexure. The common bile and pancreatic duct enters the descending portion of the viscus at the Ampulla of Vater (or major

papilla) which Taylor & Weber and Carleton place 2.0 - 2.5cm distal to the pylorus although Villemin (1922) located it 4.0cm from the pylorus. The bile and pancreatic ducts enter the wall of the duodenum separately and join intramurally. Taylor & Weber also described the entry of an accessory pancreatic duct at the minor papilla, 2.0cm distal to the Ampulla.

### <u>Mucosal</u> Folds

The folds the distal oesophagus have been variously described as "coarse longitudinal and fine transverse" (Fatani 1971), "vague and irregular" (Botha 1958b) and "scale 1969). like" (Crouch In the stomach Bourdelle & Bressou(1953) and Crouch described basically longitudinal, though tortuous, folds which began abruptly in the pylorus and faded out in the fundus. According to Crouch, the tortuosity is at a minimum on either side of the lesser curvature, forming a 'gastric groove' from the cardia to the antrum. By contrast, the duodenal folds are irregular and are invaginated by particularly long and slender villi.

## The Gastro-oesophageal Junction

Authors disagree over the existence of an intrinsic anatomical sphincter at the gastro-oesophageal junction. Its presence was described by Taylor & Weber and El-Ramli, whereas Crouch and Botha (1958a) reported its absence. Fatani (1971) inferred from this that Botha and El-Ramli were in conflict, but the difference appears to be semantic rather than factual. Thus, El-Ramli states elsewhere that "no thickening was found at the lower end of the oesophagus sphincter and no special was demonstrable histologically...the muscular walls were continuous with the muscle coats of the stomach without any pronounced change in thickness". In this, he was in agreement with both Fatani and Botha, as he himself noted. The confusion arises from his use of the word 'sphincter' in two senses; as an anatomical mechanism with a sphincteric function or a localised thickening of the tunica muscularis.

The presence of a sphincteric mechanism was indicated by Clarke & Vane (1961) who found that a gastro-oesophageal pressure gradient of 12-16 cm  $H_2O$  (1.18 - 1.57 KPa) could be supported in the chloralose anaesthetised cat before the stomach contents emptied into the oesophagus. A similar differential was reported by Botha (1962).

The nature of this mechanism was first suggested by Botha (1958b) who observed a ring of mucosal folds around the oesophageal orifice in the living animal and in "recently killed" specimens (1958a). These observations were supplemented by El-Ramli whose histological examinations showed mucosal folds, raised by a thickening of the submucosa and muscularis mucosae, at the cardia.

It therefore seems that the cat does possess a physiological mechanism which limits the reflux of gastric contents into the oesophagus, but that this is not due to a localised sphincteric muscular thickening. Its precise nature is uncertain, but mobile mucosal folds around the aperture, raised by thickened muscularis mucosae, may have an occlusive function.

In contrast, there is general agreement on the existence of an intrinsic anatomic sphincter at the gastroduodenal junction formed by a thickening of the gastric circular muscle.

## Epithelial Lining

The oesophagus is lined by stratified squamous epithelium (Fatani 1971) which contains no glands (Trautman & Fiebiger 1957; El-Ramli 1964). There is an abrupt transition between the oesophageal and gastric secretory mucosae, but the precise level is disputed. Most authors are content to locate it "at the gastro-oesophageal junction " or " at the cardia " but do not define their terminology. Botha (1962) pointed out the difficulty of precisely placing the gastro-oesophageal junction and the confusion in the literature on this subject. He defined it as coincident with the cardia and located along a line from the incisura cardiaca, which is marked by the junction of the oblique

sling fibres and the greater curvature, to the point at which the right lateral aspect of the oesophagus merged with the lesser curvature. Using this terminology, he found the mucosal interface to be 3 to 5cm proximal to the gastrooesophageal junction. Since the information presented by the other authors is inadequate, it is not possible to ascertain whether this discrepancy is a semantic or factual one.

The entire stomach is lined by a gastric secretory mucosa, comprising three types of glands, cardiac, fundic and pyloric. The cardiac glands are found in a restricted 'collar" around the entry of the oesophagus. They secrete mucus, although Trautman & Fiebiger found that they did not stain for mucin, in contrast to the results obtained by Bensley (1902). They were described as small and singly branched by Taylor & Weber but as highly branched and coiled by Trautman & Fiebiger, No other authors described their histological characteristics.

The fundic glands are found in the fundus and corpus of the stomach. They are highly branched structures, emptying into deep pits and secreting hydrochloric acid (from parietal cells) and pepsin (from chief cells). The bases of the glands do not reach the muscularis mucosae, from which they are separated by the tunica subglandularis, which is differentiated into an inner stratum granulosum and an outer stratum compactum. In the Carnivora, Trautman & Fiebiger distinguished two fundic zones, a small proximal 'light' area and a larger distal 'dark' area. However, Ellenberger & Baum (1943) found his pattern only in dogs, and described a homogeneous fundic area in the cat.

The pyloric glands are singly branched structures which lack parietal cells and which produce mucus. Intermediate gland types are found in the interfaces between the mucosal regions.

The distribution of the parietal cells in the stomachs of five cats was studied histologically by Oi et al.(1958) who sampled each stomach at 58 sites. They found a maximal parietal cell concentration in the central corpus, decreasing caudally and rostrally, combined with decreasing concentration gradients from the greater curvature to the lesser curvature and from the necks to the bases of the glands. They did not mention the cardiac gland area of low parietal cell density, perhaps because of the coarse spatial resolution of their samples and hence of their results. The thickness of the mucosa and gland length distributions followed a similar pattern. Oi's results are consistent with the bipartite fundic gland area described by Trautman & Fiebiger. However, the total area of Oi's samples constituted only a very small proportion of the entire mucosal surface (0.01%). In addition, no samples were taken from the antrum.

The duodenum is lined by Brunner's glands which are found in connective tissue, deep to the muscularis mucosae. Villemin (1922) was the first to point out that these glands extended no further than the major papilla in several species. In the cat, he sited this point 4.0cm from the pylorus. Carleton (1935) confirmed this concept in the cat, but she located the major papilla 2.0cm from the pylorus, a distance which was confirmed by Trautman & Fiebiger.

#### Musculature

It is agreed that the tunica muscularis of the distal portion of the oesophagus is composed of smooth muscle whereas the cranial portion is striated. Taylor & Weber placed the transition between the end of the cranial and the beginning of the caudal third, but Trautman & Fiebiger (1957) found it more distally. The tunica is composed of an outer thin longitudinal layer, an inner circular layer and, according to Trautman & Fiebiger, a thin innermost oblique layer. Fatani claimed that Bourdelle & Bressou (1953) had reported that all the oesophagus comprised striated muscle but a search of their text has not revealed this assertion.

The musculature of the stomach is entirely smooth and comprises three layers. Firstly, an incomplete outer longitudinal layer, running along the greater and lesser curvatures, which is united by intermediate fibres in the antrum. Secondly, a middle circular layer, which thickens to

the right and gives rise to the pyloric sphincter. Finally, an incomplete inner oblique layer, which originates at the incisura cardiaca, runs dorsal and ventral to the oesophageal aperture and along the dorsolateral and ventrolateral aspects of the stomach. This is equivalent to the 'sling fibres' of Botha and the 'cardial muscle loops' of Trautman & Fiebiger.

In the duodenum, Trautman & Fiebiger described an outer, thin, longitudinal layer, an inner circular layer and a thin innermost oblique layer. No further details were given.

## <u>Conclusions</u>

There is general agreement on most aspects of the anatomy of the foregut. The oesophagus, which has no distal dilatation, enters the 'J-shaped' stomach at almost its most rostral point, so the fundus is small. The duodenum is 'Cshaped' and is entered by a common bile and pancreatic duct, the parent trunks of which unite intramurally. There is no sphincteric muscular thickening at the gastro-oesophageal junction, but peri-oesophageal mucosal folds, raised by a thickening of the muscularis mucosae at the cardia, may have an occlusive function. The pylorus and the corpus are lined by tortuous mucosal folds, with a degree of longitudinal orientation, which is most marked near the lesser curvature. The oesophageal mucosa is aglandular whereas that of the stomach is entirely secretory, the transition being 'in the region of the cardia'. The gastric epithelium is divisible into mucus secreting cardiac and pyloric glandular areas, and mucus, acid and pepsinogen secreting fundic glandular areas. The duodenum is lined by Brunner's glands as far as the major papilla. The musculature of the proximal oesophagus is striated and the distal is smooth. Both of these are divided into three layers, including an inner oblique, as is the gastric muscle.

There are a few significant gaps in knowledge concerning this species. There is uncertainty over the disputed transition point between oesophageal and gastric

mucosae, the possible occurence of two types of fundic gland and the position of the fundus - antrum interface. Other minor points concern the location of the transition between oesophageal striated and gastric smooth muscle, the nature of the functional sphincter at the gastro-oesophageal junction, the presence of an accessory bile duct and the position of the common bile duct.

#### EXTRAMURAL VASCULATURE

There are few reports in the literature of detailed observations upon the cat's abdominal vascular anatomy, despite the species' widespread use in biomedical experimentation. Most of the information that is available is found in veterinary textbooks and dissection manuals which generally have a number of limitations. Firstly, there is rarely a description of the evidence on which the conclusions given are based, of the experimental methods used or of the number of specimens studied. This makes an assessment of the work's validity impossible. Secondly, only the larger vessels are normally described. Finally, there is rarely any attempt to assess the relative frequencies of variant patterns. Such texts have been written by Reighard & Jennings (1929), Taylor & Weber (1951), Stromsten (1952), Harrison (1952), Bourdelle & Bressou (1953), Crouch (1969), Gilbert (1971) and McClure (1973).

Reighard & Jennings, while criticizing the prior literature for lack of detail, themselves only described the larger vessels. They gave no account of their experimental methods nor any indication that they found any variation in the vascular patterns. Bourdelle & Bressou, in a volume subtitled "Carnivores: Chien et Chat", described only one pattern, implying that the details given applied equally to both species. In addition, most of the illustrations depicted the dog. McClure et al. stated that their dissection manual was "largely based upon dissection by (one of the authors)" but the number of animals studied and any additional sources of information were not described. The most detailed of these works, by Crouch, was based upon
dissection although the number of animals was unspecified. Whilst he acknowledged the existence of variations in the cat's anatomy, suggesting that the text was the result of the study of a significant number of animals, there are often annotations that the specimen drawn was atypical in some respect. This implies that each regional illustration was a depiction of a particular specimen. Despite these objections, the description given by Crouch appears to be the most complete that is currently available.

## **Description**

The coeliac artery is very short, trifurcating to form the common hepatic, left gastric and lienal arteries, ordered from rostral to caudal. There may also be small phrenic, pancreatic or gastric branches, although no assessment of their frequency was attempted.

The common hepatic artery runs to the porta hepatis, where it gives off the left, middle and right proper hepatic arteries from one or more nodes. The left proper hepatic artery gives rise to the cystic artery. The subsequent course of the common hepatic artery is unclear. Crouch's text states that it gives off a small right gastric artery and a large gastroduodenal artery after giving rise to the proper hepatic arteries. However, his diagram shows the right gastric artery arising from the gastroduodenal artery which arises from the common hepatic artery before the origins of the proper hepatic arteries. The evidence does not permit the resolution of this discrepancy. The right gastric artery runs along the lesser curvature supplying the pylorus, lesser omentum and lesser curvature. The gastroduodenal artery, after giving rise to small pyloric and pancreatic branches, bifurcates to form the right gastroepiploic and cranial pancreaticoduodenal arteries. The former runs in the greater omentum, parallel to the greater curvature, and its branches supply the anterior and posterior aspects of the greater curvature. There are also omental branches. The cranial pancreaticoduodenal artery runs between the ventral pancreas and the duodenum, sending

branches to both.

The left gastric artery, the middle branch of the coeliac triad, passes in the lesser omentum to the fundus and cardia of the stomach. It supplies its anterior and posterior surfaces and gives rise to branches which run along and supply the lesser curvature. It is also the origin of small vessels to the lesser omentum and to the abdominal and thoracic aorta.

The lienal artery travels towards the spleen. After giving rise to a pancreatic branch, it splits into a right and left branch, which travel to the respective halves of the splenic hilum. Before reaching the spleen, the left lienal gives off the short gastric arteries to the greater curvature, whilst the right lienal gives rise to pancreatic and omental branches and to the left gastroepiploic artery. The latter usually comprises two vascular bundles running almost perpendicularly to the greater curvature.

Crouch describes a number of arterioarterial anastomoses:

- \* Between the right and left gastric arteries on the lesser curvature.
- \* Between the gastric branches of the right gastroepiploic and the branches from the right and left gastric arteries on the anterior and posterior wall of the stomach.
- \* Between the right and left gastroepiploics in the greater omentum adjacent to the greater curvature.
- \* Between the cranial and caudal pancreaticoduodenal arteries on the duodenum.
- \* Between the cranial and caudal pancreaticoduodenal arteries within the ventral pancreas.
- \* Between a small vessel from one of the duodenal branches of the cranial pancreaticoduodenal artery and the first jejunal artery on the duodenojejunal junction.

There is a discrepancy in Crouch's account at this point.In the text, he describes the caudal pancreaticoduodenal artery as the second branch of the cranial mesenteric artery, distal to the middle colic artery. However, in the accompanying diagram, the caudal pancreaticoduodenal artery is the first branch, proximal to the middle colic artery. The following additional anastomoses were described by Gomercic & Babic (1972a; 1975).

- \* Between oesophageal branches of the left gastric artery and of the aorta, on the thoracic aorta.
- \* Between a pancreatic branch of the lienal artery and a pancreatic branch of the cranial pancreaticoduodenal artery within the pancreas.
- \* Between the short gastric arteries and branches from the left gastric artery within the stomach wall.

Variations in the coeliac trunk and its branches were described and quantified by Berg (1961) in a dissection study of 100 cats after vascular injection. The overall pattern that he described parallels that given by Crouch. A coeliac artery was formed from the aorta in 98% of the animals studied. This varied in length between 13.9 + 3.5 mm to 19.5 + 2.4 mm. Only 30 of these 98 cats showed the tripodal branching pattern of formation of the left gastric, splenic and hepatic arteries described by Crouch. In 41 cases, a gastrolienal trunk arose distal to the hepatic artery, whilst in 18 there was a gastrohepatic trunk with a separate splenic artery. The gastrolienal trunk was consistently longer than the gastrohepatic (range 2.8-4.2 mm versus 1.6-2.3 mm respectively). In 9 animals, four coeliac branches arose linearly, in the order hepatic, left gastric left gastric II and splenic arteries. Finally, a I, celiacomesenteric trunk was found in 2 of the animals. In one of these, there was sequential branching, the coeliac giving off the hepatic, left gastric, accessory gastric and splenic arteries. In the other, a much shorter coeliac artery gave rise to the hepatic artery and then a qastrolienal trunk.

The frequencies of the variant patterns of the duodenal vasculature were similarly quantified by Gomercic & Babic (1972a; 1975), who dissected 25 cats after fixation and vascular injection. They found that the cranial pancreaticoduodenal artery did not supply the entire descending duodenum in any of their specimens. In 80% it supplied the cranial half and in 20%, the cranial third. The remainder of the duodenum was supplied by the caudal pancreaticoduodenal artery. These two vessels were seen to anastomose in 88% of the specimens. In only 48% of cases was the caudal pancreaticoduodenal artery the first branch of the cranial mesenteric artery. In 28%, it was distal to the middle colic artery and, in 4%, distal to the middle colic and ileocaecocolic arteries. In 20%, it arose at the same level as the middle colic artery.

#### <u>Conclusions</u>

The principal source of information on the cat's gastroduodenal vasculature is the work by Crouch. This has been used to compile the summary diagram, ammended where it conflicts with the experimental reports of Gomercic & Babic and Berg. The main discrepancies are:-

- The trichotomy of the coeliac artery described by Crouch was found in only 30% of Berg's specimens, the most common pattern (41%) being as shown. Other minor variants were the occurence of a gastrohepatic trunk, of two independent left gastric arteries, or of a coeliacomesenteric trunk.

- Gomercic & Babic found that in 80% of cases the superior pancreaticoduodenal artery supplied only the cranial half of the descending limb of the pancreas. In the remainder, it supplied only the cranial third. The rest of the duodenum was supplied by the inferior pancreaticoduodenal artery. Crouch defined a larger duodenal area of supply for this vessel.

- The points of origin of the right gastric and gastroduodenal arteries were unclear.

## INTRAMURAL VASCULATURE

## **Description**

The only study on the micrccirculatory anatomy of the cat stomach which gives any experimental detail is by Leone (1949), who studied specimens from man, rabbit, cat and dog. Unfortunately, he derived only a single homogeneous

description from his observations, making no distinction between the species.

The only other information on the stomach was published by Trautman & Fiebiger in their veterinary histology text (1957). However this does not give any experimental details and rarely quotes the sources of the information. The author describes the stomachs and duodena of all the 'domestic' animals, including the cat, in a single account, giving no species specific information.

A physiological investigation by Svanes et al. (1975) on gastric blood flow gave some anatomical information. Radiolabelled microspheres  $(15 \pm 5 \text{ um})$  were injected into the right carotid artery of 3 starved cats and the radioactivity of 25um sections was used as an index of gastric blood flow. Svanes found that the distribution of radioactivity parallel to the mucosal surface was uniform. The mucosa had the highest count rate, the muscularis less and the submucosa least. Histological examination showed that arterioles arose from the submucous plexus and penetrated the muscularis mucosae perpendicularly. They branched in the lamina propria to form a network running parallel to the mucosa.

Svanes' study was designed from a physiological viewpoint and the anatomical correlates are few. The use of the final tissue radioactivity as a measure of blood flow is complicated by other factors, particularly vessel bore. For example, the submucous plexus vessels would , in general, be larger than the microspheres which would not therefore be trapped. Under such conditions, the activity flow relationship breaks down. In addition, the small number of experimental animals restricts the degree of reliability which can be placed upon the results, and the details of histological processing are not given.

A series of physiological studies by Varhaug et al. (1977; 1979a; 1979b; 1980; 1984) examined the effects of ligating or cutting the vessels close to the lesser or greater curvatures. They found that this reduced mucosal

blood flow, measured by microspheres, and resulted in ulceration on the curvatures. This suggests a relatively poor intramural anastomotic network in these regions.

The only other studies are on the jejunum and/or ileum are are therefore outside the scope of this review. Spanner (1932) and Noer (1943) used injection techniques. Lundgren (1967) utilised radioactive tracers in a primarily physiological study. His description treats the small intestine as homogeneous, although most of his results are based upon jejunal samples.

### <u>Conclusions</u>

There is no species specific account of the feline gastric microcirculation available in the literature which is based upon described observational evidence. There is also little information available on the duodenal angioarchitecture. No assessment of the validity of Trautman iebiger's work can be made as there are no experimental details or cited authorities. Svanes' account provides the only firm information although this is limited in scope and derived from too few animals. He described mucosal arterioles from the submucous plexus forming a plexus at the base of the mucosa which supplied the glands. The degree of vascularization was uniform in the glandular stomach but greater in the mucosa than in the muscularis.

# <u>CHAPTER 4</u> <u>THE PRIMATE</u>

# NON-VASCULAR GASTRODUODENAL ANATOMY

## External Form

The gross anatomy is well described in two comprehensive and well referenced textbooks by Lineback (1961) and Osman Hill (1974). Salomon (1893) and Villemin (1922) described the gastrointestinal histology, whilst the duodenum was examined by Carleton (1935) and Sehgal et al. (1966). Botha (1958a; 1962) included the primate in his series of examinations of the gastrointestinal junction. In most cases, the accounts were based upon the rhesus monkey (Maccacus mulatta).

The oesophagus enters the stomach midway along the lesser curvature, with a large fundus to the left and a piriform pylorus to the right. An unusual feature, noted by both Lineback and Osman Hill, is that the long axis of the stomach is spiral, so that the pylorus curves dorsally . When the stomach is full, the greater curvature is horizontal. The duodenum has a slight initial dilatation and then narrows, running rostrally in a 'U' shaped loop. The bile and pancreatic ducts enter jointly at the Ampulla of Vater which, according to Carleton (1935), was 2.5cm from the gastroduodenal junction in the one animal she examined. Villemin (1922) reported that there was no duodenojejunal valve in the nine primates that he studied.

The omentum on the lesser curvature runs from the fundus to the bile duct, with a broad gastrohepatic ligament forming the lateral boundary of the Foramen of Winslow (Lineback). On the left, it forms the gastrosplenic and gastrophrenic ligaments. Within it run the bile duct, hepatic portal vein and hepatic artery. The large greater omentum runs all along the greater curvature and has a wide free edge.

#### Gastro-oesophageal Junction

The gastro-oesophageal junction was described in detail by Botha (1958a; 1958b; 1962). Although he was unable to find histological evidence of a sphincter, he noted that there was a distinct constriction at the junction which looked like a ring of muscle. Internally, there was no well defined mucosl rosette as in some other species, the only constant feature being a crescentic fold on the greater curvature side of the cardia.

## Epithelial Lining

The histology of the gastrointestinal epithelium was described by Osman Hill, extensively citing the work of Salomon (1893) and Burkl (1958). The epithelium showed the cell types normally found in the mammalian stomach. He noted that there was a sudden transition from oesophageal to gastric mucosa at the cardiac orifice, but that that the interface was irregular - "ora serrata". This was confirmed by Lineback. Botha (1958a) however located the transition 3 - 6mm above the cardia and noted that there was a raised 'cushion' of mucosa immediately distal to this point. According to Lineback, the mucosa was smooth in the fundus, but extensively ridged in the pylorus.

Several worker described the microscopic appearance of the duodenal mucosa. Lineback and Sehgal et al. (1966) observed that the villi were flange rather than finger shaped, orientated, according to Lineback, transverse to the long axis. Sehgal et al. also noted the presence of complex branched villi. The epithelium showed a sharp transition at the gastroduodenal junction, with the appearance of Brunner's Glands. Carleton (1935) found that that they ended abruptly at the entry of the bile duct, but Villemin (1922) reported that isolated groups were found as far as the jejunum.

# <u>Musculature</u>

Lineback and Osman Hill described the muscularis as having the normal mammalian pattern. Lineback also noted that there was a well marked pyloric sphincter, whilst Botha (1958a) asserted that there was no visible gastrooesophageal sphincter.

#### EXTRAMURAL VASCULATURE

Some early work was done by Theile (1852) and Rojecki (1889) but experimental details are few. The first detailed report appears to be that of Grzybowski (1926) who carried out 90 dissections in a study of the coeliac artery. The anatomical account based upon experimental first observations in the rhesus monkey appears to be by Lineback (1961). This was based upon comparatively few dissections and so does not attempt to assess the frequency of variants. The coeliac artery was examined in 100 primates by Bielak (1967) and its branches were examined by Osemlak, Obel et al. (1967; 1968a; 1968b). An exhaustive review and critical analysis of the literature has been published by Osman Hill (1974).

### **Description**

Lineback described the coeliac artery splitting into three unequally sized branches, the left gastric artery being the largest. This is at variance with Grzybowski, who described an equal tripodal branching. According to Lineback, the three branches were the left gastric, splenic and right gastric arteries. The left gastric gave branches to the fundus, greater curvature and spleen. It ran to the pylorus, where it passed into the omentum and reached the right colon. The splenic artery ran to the spleen, giving a small branches to the greater omentum. The right gastric ran along the greater curvature, forming the hepatic and gastroduodenal arteries. The hepatic artery gave rise to the cystic artery and supplied the liver, whilst the gastroduodenal supplied the pylorus and duodenum. There is a wide discrepancy between Lineback's description and the basic mammalian pattern. He provides limited information so the validity of his results cannot be assessed.

The most detailed work on the rhesus coeliac anatomy has been reported in a series of publications (Obel et al. 1967; Osemlak 1968a; 1968b) and by Bielak (1967). Bielak carried out 100 dissections of the coeliac artery, after vascular injection with latex rubber. He found that the it was present in 90% of the specimens, in most cases (86%) arising from the aorta. In the remainder, it arose from the superior mesenteric artery. Where the coeliac was not found, it was replaced by a hepatosplenic trunk (4%), a coeliacomesenteric trunk (4%) or separate hepatomesenteric and gastrosplenic trunks (2%). In nearly all cases, these arose from the aorta.

Several distal branching patterns were seen. Where the coeliac was present, it most frequently branched tripodally to form left gastric, splenic and common hepatic arteries (68%). However, in 22%, a fourth branch was seen, usually a right diaphragmatic branch (16%), but sometimes an accessory hepatic or pancreaticoduodenal artery. Where the coeliac was replaced by a hepatosplenic trunk, it divided into common hepatic and splenic arteries, the former giving rise to the left gastric. A coeliacomesenteric trunk divided either into coeliac and superior mesenteric arteries or immediately into splenic, common hepatic, left gastric and superior mesenteric arteries.

The same techniques were used by Obel, Osemlac et al. to study the common hepatic, splenic and left gastric arteries. They found that the left gastric artery was the thinnest of the coeliac branches (1968a). In most specimens (81%) it divided into an anterior and a posterior branch, each of which supplied the respective aspect of the stomach by numerous short gastric arteries. The posterior branch was paired in 13% and in 4%, arboreal branching was seen. As a minor variant, a left and right branch was formed, each of which had short anterior and posterior branches. Most left gastric arteries also gave rise to one or two cardiac branches (86%) and up to three oesophageal branches. However, anastomosis on the lesser curvature between left and right gastric arteries was rarely seen (3%).

Obel and Osemlac found that the splenic artery was the largest of the coeliac artery branches and ran linearly along the upper border of the pancreas to the splenic hilum (1968b). It divided into 2 or 3 secondary vessels in its distal (71%) or middle third (23%). From the splenic artery arose splenic branches, short gastric, left gastroepiploic, left epiploic and lesser gastric arteries and pancreatic branches. Three to seven splenic branches supplied the spleen by its hilum. The fundus was supplied by two to five short gastric arteries and the lesser gastric artery. The left gastroepiploic artery ran along the greater curvature and supplied the anterior and posterior gastric surfaces and the greater omentum. It anastomosed with the right gastroepiploic artery on the greater curvature in 81%. The left epiploic artery was present in most animals and ran to the greater omentum. Finally, a single or double greater pancreatic artery was present in 85% of animals, often supplemented by a number of small pancreatic branches.

Obel & Osemlac found the common hepatic artery to be present in 78% of the animals, giving rise to the gastroduodenal and proper hepatic arteries. In the remainder, the latter arose directly from the coeliac or superior mesenteric arteries. The proper hepatic artery gave rise to the right gastric, cystic and diaphragmatic arteries and, in 70%, to right and left hepatic branches. The gastroduodenal gave rise to the right gastro-omental and superior pancreaticoduodenal arteries.

#### <u>Conclusions</u>

The descriptions of Bielak and Obel, Osemlak et al. allow a consensus to be obtained, although Grzybowski's anomalous results or terminology can not be resolved. The most common pattern has a coeliac artery arising from the aorta, then branching tripodally to form the left gastric, splenic and common hepatic arteries, although in nearly a quarter of the animals, a fourth branch, usually a right diaphragmatic artery, was present. Opinion seems to be divided on whether the left gastric was thinner than,

thicker than, or equal to the remainder. It usually gave rise to anterior and posterior branches, supplying the same aspects of the stomach, plus cardiac and oesophageal branches. There was rarely a left - right gastric artery anastomsis. The splenic artery supplied the fundus, spleen and pancreas, and its left gastroepiploic branch usually anastomosed with the right gastroepiploic on the greater curvature. The common hepatic artery supplied the liver, distal stomach and duodenum.

There was interesting support for some of Grzybowski's observations by the subsequent authors. He found a number of trends through the phlyogenetic groups which were consistent with the later reports. For example, he reported a single gastrosplenic trunk in lower primate orders, through tripodal coeliac branching (rhesus) to left gastric artery dominance in man. He suggested that the higher the species, the more distal the division of the splenic and left gastric arteries into their secondary branches. Thus, the splenic artery in the primate branches mainly in its distal third, whilst this occurs in the splenic hilum in man. Finally, he suggested that the frequency of cardiac branches of the left gastric is proportional to phylogeny, with an 86% occurence in the primate and 100% in man.

#### INTRAMURAL VASCULATURE

There appears to have been no anatomical study of the gastric microcirculation in the non-human primate. Noer (1949) examined the intramural vessels in the rhesus monkey and the chimpanzee, but limited his attention to the jejunum and ileum. A similar work was published by Reynolds et al. (1967). As neither considered the duodenum, they are outside the scope of this review.

A physiological study of the effects of various drugs on the gastric blood flow was published by Zinner in 1976. As a part of this study, he removed all the vascular inputs to the liver, other than the hepatic portal vein, in seven baboons. Radioactively labelled microspheres (15  $\pm$  5 um) were injected in a retrograde direction into the distally tied common hepatic artery. He was unable to find any significant radioactivity in the lungs and liver and concluded there was no gastric arteriovenous shunting. The use of microspheres as an index of arteriovenous shunting has been questioned by several workers (eg Delaney 1943), although the inherent errors tend to produce spurious positive, rather than negative, results.

# CHAPTER 5 THE RABBIT

#### NON-VASCULAR GASTRODUODENAL ANATOMY

#### External Form

The gross anatomy of the rabbit stomach has been described in a number of texts (eg. Bensley 1948; Craigie 1951). The oesophagus enters the cardia of the stomach slightly ventrally and at an angle of 90°. There is a large sac-like expansion, the fundus, which extends to the left of, and rostrally from, this region, whilst to the right of the cardia, but mainly to the left of the animal's median plane, is the body of the stomach which merges into the pyloric antrum. The cardia and the pylorus are joined by a short lesser curvature, which faces rostrodorsally, the greater curvature having a caudoventral orientation. The junction between the stomach and the duodenum is marked by the pylorus and its anatomical sphincter which is formed by a thickening of the circular muscle layer. The duodenum itself comprises four parts, distinguished by their orientation, and becomes retroperitoneal at its junction with the jejunum. The stomach and duodenum are attached to the liver by the hepatogastric and hepatoduodenal ligaments respectively and the stomach to the spleen by the gastrosplenic ligament.

# <u>Mucosal Folds</u>

The literature contains no information on mucosal folding in this species, other than the specialised area found around the oesophageal opening.

## The Gastro-oesophageal Junction

According to Botha, the terminal 5-10 mm of the oesophagus formed a triangular expansion, the vestibule (1958a; 1958b; 1958c; 1962). At the base of this was a very marked sphincteric thickening, which comprised both striated fibres, derived from the circular muscle of the oesophagus,

ł •

and smooth fibres from the gastric circular muscle layer. This was confirmed by El-Ramli (1964) and Fatani (1971). Botha also described a mucosal fold at the cardia which extended into the gastric cavity and formed a complete 'rosette' around the oesophageal aperture. Observation in vivo (1958b) showed that this was a highly mobile structure, capable of occluding the opening or of allowing the passage of swallowed material. Histological examination showed it to contain a greatly thickened muscularis mucosae, continuous with that of the oesophagus and stomach. The presence of these folds was denied by Fatani, who described the thickening of the muscularis mucosae at this point as 'moderate'. However, these assertions were based upon the observation of only one animal. El-Ramli mentioned neither topic, although he did discuss them in other species. Unfortunately, he was unclear on the period between the death and observation or fixation of his specimens, which he described as 'recently killed', since Botha found that the folds were only visible ante mortem or within a few hours of death.

# Epithelial Lining

Several workers have shown that the stomach is lined by a secretory gastric mucosa which is sharply demarcated from the keratinized oesophageal epithelium at or near the cardia (Oi 1958; Botha 1958a, 1958b, 1958c; El-Ramli 1964; Fatani 1971). Oi sampled the gastric mucosa of seven rabbits at 23 sites in each stomach and examined them histologically. He found that the mucosa was thickest in the body of the stomach, becoming thinner both caudally and rostrally. The variation in gland length was similar. By contrast, the density of the parietal cells was at a maximum in the fundus and decreased caudally, none being found in the antrum. The cells were concentrated towards the lesser curvature and preferentially located at the necks of the glands. The validity of this work is limited since Oi sampled only a very small proportion of the entire mucosa (an estimated 0.004%). Only one sample was taken from the antrum.

Botha (1958a, 1958c), El Ramli(1964) and Fatani (1971) all stated that there was a sharp transition from the keratinized oesophageal epithelium to the gastric secretory mucosa at the gastro-oesophageal opening. Botha maintained that the interface was 2 - 5 mm proximal to the anatomic junction, which he defined as coincident with the cardiac angle, indicated by the junction of the oblique sling fibres and the greater curvature. However, El-Ramli stated that the transition occured 'at the gastro-oesophageal junction', but he failed to define this. Nonetheless, his photograph of the region is consistent with Botha's opinion. Conversely, Fatani stated that the transition occured 'at the cardia', the location of which he had previously specified in terms of Botha's definition. However, this was a judgement based upon the observation of only one animal. Botha described a transitional region between the oesophageal and true gastric epithelia, in which there were no parietal cells and few tubular glands, those that were present appearing poorly developed. The cardiac glands were alveolar with wide ducts, and appeared to be mucus secreting, in confirmation of Bensley's observation (1902). This absence of parietal cells was not observed by Oi (1958), perhaps because of the coarse distribution of his sampling sites.

## Musculature

The musculature of the oesophagus was described by Botha (1958c) as comprising three striated layers, inner and outer longitudinal and a middle circular. All increased in thickness just above the vestibule, particularly the circular, which was the main component of the sphincter. However, the striated layers rapidly tailed away at the distal end of the oesophagus so that they had disappeared at the cardia. Inner circular and outer longitudinal layers appeared just above the cardia and contributed to the sphincter before forming the gastric muscle, which was only half the thickness of the oesophageal.

## <u>Conclusions</u>

The oesophagus enters at the mid point of the stomach, forming an incisura cardiaca of  $90^{\circ}$ , and there is a large fundus to the left of, and rostral to, the cardia. There are no clear external divisions between the different regions of the stomach. The oesophagus displays a pronounced dilatation immediately before the cardia, distal to which is a large sphincter, composed of oesophageal striated and gastric smooth muscle fibres. The stomach itself is entirely lined by secretory mucosa as opposed to the stratified squamous epithelium of the oesophagus. In contrast to many species, the oesophagus has three striated muscle layers, inner and outer longitudinal and middle circular.

However, several features are in need of clarification or elucidation. Nothing seems to be known of the patterns of mucosal folding in the stomach, with the exception of the specialised sphincteric folds around the cardia, the existence of which is disputed, although the weight of experimental evidence suggests their presence. There has been no comprehensive study of the distribution of types of epithelium in the stomach, with particular regard to the acid and non-acid secreting regions, since Oi's study is of questionable accuracy and Botha's description of the cardiac glands is brief. In addition, the transition point between the oesophageal and gastric mucosae is disputed, although it is ' in the region of the cardia'. Finally, there is no description in the literature of the orientation of the muscle layers in the stomach.

#### EXTRAMURAL VASCULATURE

There is a sparse literature upon the gross anatomy of the coeliac artery and its branches in the rabbit, with only one original report on the subject (Adams 1965), the remaining information being found in standard dissection manuals (Gerhardt 1909; Whitehouse 1937; Bensley 1948; Craigie 1951). In none of these texts do the authors state their data sources.

### **Description**

All of the above descriptions are similar, varying in degree of detail. The account given by Craigie may be taken as typical. The first main branch of the A. celiaca, apart from the small Aa. phrenicae inferiores, is the A. lienalis. In its course, this gives off Rami pancreatici and two or more Aa. gastricae breves to the left part of the greater curvature. The final one of these forms the A. gastroepiploica dextra. Rami lienales are given off from the A. lienalis in the splenic hilum. The artery ends by supplying the greater omentum as the omental artery.

The A. celiaca then bifurcates to form the A. gastrica sinistra and the A. hepatica. The former usually comprises a group of vessels. These radiate to the dorsal and ventral gastric surfaces adjacent to the lesser curvature. On the ventral surface, there two branches, one to the left and one to the right of the cardia. The left branch gives rise to the Rami oesophagi, whilst the right bears a small pyloric branch. This runs along the lesser curvature to anastomose with the A. gastric dextra.

The A. hepatica is usually regarded as the continuation of the A. celiaca. This appears to contradict Craigie's previous statement that the A. celiaca "bifurcates", implying a division into two equal parts. The A. hepatica gives off a small Ramus pancreaticus before forming the A. gastroduodenalis. This latter vessel soon divides into two, forming the A. pancreaticoduodenalis superior, supplying the first part of the duodenum, and the A. gastroepiploica dextra, which runs along and supplies the greater curvature and anastomoses with the A. gastroepiploica sinistra. Finally, before entering the liver, the A. hepatica gives rise to the A. gastric dextra, which supplies the pylorus and which subsequently anastomoses on the lesser curvature with the pyloric branch of the A. gastrica sinistra.

The account clearly parallels the "classical' account of the coeliac anatomy in man. To what extent it is based upon direct experimental observation in the rabbit is unknown. In the absence of original descriptions, it is

difficult to assess the account's validity. However, this similarity between the two species is consistent with the results of Noer (1943) and Jacobsen & Noer (1952) who showed that the intramural vasculature in the small intestine of the rabbit is very like that of man. Similarly, a subsequent original investigation of the coeliac anatomy in the rabbit (Adams 1965) supports Craigie's account.

Adams examined ten living rabbits radiographically with image intensification, using an intravascular contrast medium. The coeliac artery had three terminal branches. The first was the splenic artery, which gave off lienal, pancreatic and short gastric branches and which ended as the left gastroepiploic artery. The left gastric artery, which was the largest coeliac branch, was "often multiple". The other member of the terminal trichotomy of the coeliac artery, the common hepatic artery, gave rise to the gastroduodenal artery, before forming the proper hepatic artery, which supplied the liver and, via the cystic artery, the gall bladder. The gastroduodenal artery almost immediately divided to form the superior pancreaticoduodenal artery and the right gastroepiploic artery. Other branches, described by Craigie, were not mentioned.

# <u>Conclusions</u>

Craigie's account, although giving no experimental data, is consistent with Adam's observationally based work. The latter describes fewer branches, which may be a result of the inherently lower resolution of radiography in comparison to dissection. There are a few differences, the most significant being whether the coeliac artery trifurcates or gives off a splenic artery followed by a bifurcation of the gastrohepatic trunk, but it is not possible to differentiate between these on the basis of the limited evidence.

#### INTRAMURAL VASCULATURE

## Description

There is no comprehensive account available of the gastric microcirculation of the rabbit. The first information appears to have been published by de Busscher (1948) as an incidental item in his paper on gastric arteriovenous anastomoses in man. He reported that he was able to find AVA's in the muscularis mucosae of the rabbit stomach. These were simple structures with an epithelioid arterial portion and an endothelioid venous portion. They were a frequent feature but unfortunately he gave no technical details or further information.

Leone (1949) included the rabbit in his vascular injection study, However, it is not possible to differentiate the results from this species in his single description which combined observations from rabbit, man, cat and dog.

The most comprehensive account was given by Bulkley (1970) as a part of a study of the effect of haemorrhagic shock on the gastric microvasculature. He injected the stomachs of 12 rabbits post mortem with silicone rubber, then cleared, sectioned and examined them microscopically. He similarly treated a further 9 animals after one to four hours' restraint. He gave a combined description of the corpus and fundus mucosa which were 'indistinguishable'. At the base of the mucosa were separate arterial and venous plexuses. The arterial plexus gave rise to vertically running arterioles which anastomosed with one another. They terminated in a plexus below the mucosal surface from which arose a capillary network. Bulkley stated that the superficial arteriovenous plexus was drained by 'sparse' venules, which were tributaries of the 'venous plexus of the SMN', but did not define this acronym.

Several workers have examined the intramural circulation of the small intestine, but all restricted their observations to the jejunum and ileum (Testut 1912; Spanner 1932; Noer 1943; Jacobsen & Noer 1952). They are therefore

beyond the scope of this review as there is no evidence to support extrapolation from these regions to the duodenum.

# <u>Conclusions</u>

The paucity and quality of experimental evidence and supporting data make it impossible to give an account of the rabbit gastroduodenal microcirculation.

# <u>CHAPTER 6</u> <u>THE PIG</u>

# NON-VASCULAR GASTRODUODENAL ANATOMY

One of the most authoritative texts on porcine anatomy was first published in 1900 by Ellenberger& Baum (1943). Two subsequent general works were by Sisson, first published in 1910 (Sisson 1953) and by Bourdelle & Bressou (1920). Specific aspects of the anatomy of the foregut have been considered by several authors. Greenwood (1884) studied the gastric glands, whilst the cardiac glands were examined by Bensley (1902) and the duodenal by Kuczynski (1890), Villemin (1922) and Carleton (1935). The histology of the foregut was considered by Sloss (1954) and Trautman & Fiebiger (1957), whilst Weissflog (1903) examined the tunica muscularis. Botha (1958a; 1958b; 1962) described the anatomy of the gastro-oesophageal junction and Kowaczyk (1970) considered the pig from a veterinary care viewpoint.

#### External Form

The oesophagus was described briefly by Sisson. It was short and nearly straight, but with a wider potential lumen at the beginning and end (approximately 7cm diameter) than in the middle (approximately 4.2cm). It entered the stomach obliquely, forming a very acute incisura cardiaca (between the oesophagus and the greater curvature). This was confirmed by Botha (1958a; 1962) who also noted that there was no oesophageal vestibule, but that there was a very well marked, though gradually developing, muscular thickening 'almost caudal to the cardia'.

The most detailed description of the stomach was given by Bourdelle & Bressou. The long axis of the full stomach is transverse, in contrast to the acute incisura cardiaca described by Sisson and Botha. The organ has a volume of six to eight litres and comprises a large central corpus which is slightly flattened dorsally and ventrally with an extremity to the right and to the left. The left expansion, the fundus, ends in a conical sac, the diverticulum ventriculi, which is partly separated anteriorly and posteriorly from the rest of the stomach. The right expansion, the pyloric antrum, is directed cranially, forming an acute incisura angularis, and is constricted at the pylorus.

Bourdelle & Bressou, Carleton and Sisson were in agreement that the duodenum formed a 'C' shaped loop, open to the left and caudally, and was 60 to 80cm long, comprising 3 to 4% of the total length of the small intestine. Villemin described an initial dilatation, but this was not seen by Carleton, in her examination of three animals. All the authors agreed that the bile and pancreatic ducts entered separately, the former 2-5 cm from the pylorus and the latter 12-20 cm from the pylorus. There was no accessory pancreatic duct.

## <u>Mucosal</u> Folds

The mucosal lining of the oesophagus is folded longitudinally (Bourdelle & Bressou 1920; Trautman & Fiebiger 1957). Bourdelle & Bressou also described the cardiac gland area as extremely folded and the gastric gland area as less, but more regularly, so. Conversely, Sisson described irregular folding in the pyloric mucosa and Sloss maintained that the proximal third of the stomach was smooth. In addition, there are specialised folds to the left of the oesophageal aperture and, according to Sisson and Bourdelle & Bressou, around 75% of the perimeter of the opening to the diverticulum. There is also a protruberance, approximately 3cm long and 1cm high, at the pyloric orifice, the torus pyloricus. This comprises mainly fat under the covering mucosa, with a few muscle fibres and can occlude the pylorus.

## Gastro-oesophageal Junction

This region has been studied intensively by Botha (1958a; 1958b; 1962). The oesophagus joined the stomach obliquely, a point confirmed by Sisson but not by Bourdelle & Bressou. The oesophageal circular muscle gradually thickened distally forming a very well developed anatomical sphincter which often bulged into the gastric lumen. The aperture was slit like and was not surrounded by a mucosal rosette. However, a 'curtain like' mucosal fold was always found to the left of the orifice, up to 1.5cm in length. Observations in vivo (Botha 1958b; 1962) showed that this was mobile and could be closely apposed to the lesser curvature. This fold was also seen by Sisson and by Bourdelle & Bressou, the former author reporting that it contained a prolongation of the internal oblique muscle layer.

# Epithelial Lining

There is a consensus that the stomach is divided into a glandular and a non-glandular part, the abrupt transition occuring 2-5 cm (Botha 1962) or 3-7 cm (Bourdelle & Bressou 1920) below the cardia. The glandular area is sub-divided into three equally sized regions, the cardiac, fundic and pyloric gland areas.

The aglandular region forms a collar, 2-7 cm wide, around the oesophageal aperture, extending to the left as far as the entrance to the diverticulum, and comprises a stratified squamous keratinized epithelium, 60-90 cells thick, continuous with that of the oesophagus. Sloss reported the presence of mucous cells in this region.

The left third of the stomach, excluding the aglandular area but including the diverticulum, is lined by cardiac glands. Bensley (1902) reported that these glands were more extensive in the pig than in any other placentate mammal, a comment which Botha (1958a) mistakenly attributed to Greenwood (1884). According to Trautman & Fiebiger, this region was sub-divided into two parts. On the other hand, Botha claimed that the cardiac glands were not obvious and were confined to a narrow, poorly defined band. This is contrary to all other descriptions and is supported by no explanatory details or further evidence. The histology of the cardiac glands has been fully described by several authors, notably Greenwood (1884), Bensley (1902), Sloss

(1954) and Trautman & Fiebiger (1957). In summary, this region is invaginated by pits, each of which receives one or two tubulo-acinar cardiac glands, which are tortuous but which only have one or two branches. The pits are inverted cone shaped and the glands have a basal expansion. There is disagreement on the cell types which are found in the glands. Greenwood and Bensley found a single kind of secretory cell which stained for mucin. Greenwood found these to be absent in the basal third of the gland and Bensley noted that the mucin content of the cells in this region was slightly less than in the rest of the gland, particularly in immature animals. Conversely, Trautman & Fiebiger and Sloss found, in addition to the above mucin cell distribution, a second type of cell, located in the body and base of the gland. Trautman & Fiebiger found that these stained similarly to the fundic parietal cells (i.e Eosin positive) whereas Sloss found that they stained as the fundic chief cell (i.e. Eosin negative).

Fundic glands are found in the middle third of the stomach, although they do not reach the lesser curvature. The glands are lined with columnar cells and the crypts with cuboidal. Three types of secretory cell are found, mucous, chief (pepsinogen) and parietal (hydrochloric acid). The chief cells are found mainly in the distal part of the gland and the parietal cells in the proximal. The latter cells in the middle of the gland are often found in recesses, away from the lumen. Bensley reported that mucous cells were located throughout the gland, whereas Greenwood and Sloss found them only in the gland bases. Also according to Bensley, many glands contained only mucous and chief secretory cells, so that there was a relative deficit of parietal cells, an observation which had already been made by Greenwood, who also noted that mucous cells were found in greater numbers in the more distal parts of the stomach.

The pyloric gland area comprises the distal third of the stomach, including the greater curvature. Most authors described only mucous secretory cells in these glands, which stained similarly to the cardiac gland neck cells. However,

Trautman & Fiebiger reported the presence of a few isolated parietal cells. The transitions between the three glandular areas are gradual and intermediate gland types are seen.

Both Brunner's and Intestinal glands are seen in the duodenum. The latter increase in number distally, but there is disagreement on the extent of the former. Sisson and Trautman & Fiebiger described them extending 3-5 m from the pylorus and Kuczynski 1-4 m. Conversely, Carleton described them extending 57 to 70cm and Sloss 53cm from the pylorus and Villemin as far as the pancreatic duct (i.e. 12 to 20cm). Of the authors describing the lesser extent, Sloss (15 animals) and Carleton (3 animals) based their results on described observations, whereas Trautman & Fiebiger's and Sisson's descriptions were unsubstantiated by cited experimental evidence.

## Musculature

The tunica muscularis in the oesophagus comprises two layers, which initially spiral in opposite senses then form an outer longitudinal and an inner oblique layer. It is proximally smooth and distally striated, although the point of transition is disputed. Most workers described the change occuring just cranial to the cardia, but Sloss reported the presence of both smooth and striated fibres in the middle of the oesophagus. She also observed a few striated fibres remaining in the outer layers at the cardia. The inner circular layer thickens gradually towards the cardia to form a well marked smooth muscle sphincter. The muscularis mucosae was, according to Sloss, divided into two layers, an inner circular and an outer longitudinal, both of which appeared at the level of the cricoid cartilage.

The wall of the stomach contains several muscular layers. The outer is incomplete, comprising oblique fibres from the oesophagus projecting onto adjacent areas of the stomach and longitudinal fibres on the extreme right and left aspects of the greater curvature and on the diverticulum. The deeper circular layer is complete, running from greater to lesser curvature and is thickened at the

pylorus. There is also an incomplete inner oblique layer which forms well developed sling fibres around the oesophagus. The wall of the lesser curvature is up to 1cm thick. The circular layer contributes a few fibres to the core of the torus pyloricus and the inner oblique to the core of the mucosal fold at the left of the oesophageal aperture. It also forms an incomplete ring around the diverticular entrance. Sloss differed slightly from the above account, describing inner circular and middle oblique layers, which both contributed to the pyloric sphincter, and an outer longitudinal layer. She also described the fibres in the torus as having no definite arrangement. The muscularis mucosae (also according to Sloss) was found over the entire stomach and comprised inner circular and outer longitudinal layers, plus bundles of varying orientation.

There is little information available on the duodenal musculature. Trautman & Fiebiger and Sloss were in agreement on the presence of inner circular and outer longitudinal layers of smooth muscle, the former being the thicker, particularly around the entrance of the bile and pancreatic ducts. Inner circular and outer longitudinal layers of the muscularis mucosae were also seen by Sloss although, in this case, the latter was the thicker.

## <u>Conclusions</u>

The external form of the porcine foregut has been fully described and follows the general mammalian pattern. The stomach is large (6 - 8 litres) and is poorly demarcated into fundus, corpus and antrum. The principal atypical feature is the diverticulum ventriculi, a small outgrowth from the fundus, comprising a pouch which is partly separated from the remainder of the gastric lumen. In the duodenum, the bile and pancreatic ducts enter separately, the former proximal to the latter, and there are no accessory ducts.

The location, degree and orientation of mucosal folding is contentious but, as this is subject to several variables, such as the volume of the gastric contents and the interval between death and fixation or observation, few conclusions can be drawn. Constant folds are found at the cardia, the entrance to the diverticulum and the pylorus (torus pyloricus), the first and last being mobile and probably having a sphincteric role.

The angle of the incisura cardiaca is variously described, probably due to unspecified variations in the volume of the gastric contents. In the empty stomach, it is relatively acute. There is a well defined smooth muscle sphincter at the cardia, but no mucosal rosette, although there is a 'curtain like' mucosal fold to the left of the oesophageal aperture.

The gastric epithelium is partly oesophageal in character, a ring of aglandular mucosa 2-7 cm in width being seen around the cardia. The remainder of the stomach is, according to most authors, equally divided into cardiac, fundic and pyloric glandular areas. Only Botha disputed this, describing a narrow ring of cardiac glands around the aglandular area but, in the absence of any confirmatory evidence, his contention is outweighed by the unanimity of the other authors.

The aglandular area probably contains mucin secreting cells, described only by Sloss, since other workers' techniques might not have detected them. Such cells are also found in the cardiac glands, although there is some doubt as to their precise intraglandular location. Most workers described only these cells but some authors also observed parietal-like and chief-like cells. This, and similar disagreements in the fundic and pyloric gland areas, might be explained by the existence of intermediate gland types, particularly in the transitional zones, since no authors stated the number or location of their samples. The fundic gland area contains mucous, parietal and chief cells, although there is dispute over their precise location within the gland and within the region. The pyloric gland area contains mucous cells with, perhaps, a few parietal cells.

The duodenum contains intestinal and Brunner's glands, although the distribution of the latter is contentious,

authors falling into two groups. The experimental evidence is more fully documented by those authors describing the lesser extent, but none gave the size of his animal(s), except Sloss (56.8kg). Additionally, no author described his criterion for the end of the gland distribution, so that isolated glands might or might not have been included. On balance, the evidence appears to favour the lesser extent, but further work is needed.

The oesophageal muscle occupies two layers, initially helical, subsequently longitudinal (outer) and circular (inner). They are proximally striated and distally smooth, the transition occuring in the region of the cardia, although it may be slightly extended orally or aborally. The stomach has the usual three smooth muscle layers, longitudinal, circular and oblique, from superficial to deep. The longitudinal bundles are mainly along the curvatures, the circular over the whole stomach, thickened at the pylorus, and the oblique form sling fibres around the oesophagus. The wall of the lesser curvature is particularly thick. The torus pyloricus and the oesophageal fold contain muscle fibres and are mobile. A layer of muscularis mucosae is found over the entire foregut, caudal to the level of the cricoid cartilage.

### EXTRAMURAL VASCULATURE

Most of the references are textbooks which have limited detail and lack experimental evidence, eg Bourdelle & Bressou (1920) and Sisson (1953). The literature has been augmented by the experimental observations of Busch (1973; 1975) and Trixl (1973). Despite the relative paucity of accounts, there is a significant disparity between them. The account of Sisson may be taken as representative of his and Bourdelle's descriptions. (Fig.l).

# Description

Sisson described the coeliac artery dividing into two branches of equal diameter, the gastrohepatic and splenic arteries. The splenic artery supplied the stomach by posterior gastric branches (not always found), the A. diverticuli to the diverticulum and the A. gastricae breves which supplied the left part of the greater curvature. There were also splenic and pancreatic branches. The parent vessel then continued as the left gastroepiploic artery. Sisson noted the existence of some variants. The posterior gastric branch sometimes arose from the point of bifurcation of the coeliac artery. There were also oesophageal branches which might arise from the posterior gastric branch or from the splenic artery itself.

The gastrohepatic trunk divided into its two components almost immediately. The supply to the stomach comprised an anterior gastric artery which, in turn, gave off an oesophageal artery, and branches to the lesser curvature. The hepatic component split to form the gastroduodenal artery and the A. hepatica propria. The latter gave off the cystic artery to the gall bladder. The former divided to form the cranial pancreaticoduodenal and right gastroepiploic arteries. There were also pyloric and pancreatic branches from the gastroduodenal artery.

Busch's work was primarily directed towards a study of the gastric intramural circulation in the pig, but he gave some information on the gross vasculature. He studied 24 animals by corrosion casting and 22 by tissue clearing (Spalteholz). Unlike Sisson, Busch reported a trifurcation of the coeliac artery, to form the left gastric, hepatic and splenic arteries. The left gastric artery passed to the posterior stomach wall where it supplied the corpus and fundus via many small branches. The hepatic artery, after a short course, also trifurcated to give rise to the right gastric and gastroduodenal arteries and to the rami hepatici. The right gastric artery ran to the lesser curvature of the stomach, giving off a branch to the liver. On the lesser curvature itself, many smaller vessels were given off to supply both it and the anterior gastric surface. These branches anastomosed within the stomach wall with branches from the greater curvature. No anastomosis with the left gastric artery was seen. Distal to the gastric

branches were the origins of an oesophageal branch and pyloric branches.

The gastroduodenal artery gave rise to a small pyloric branch then split to form the cranial pancreaticoduodenal gastroepiploic arteries. cranial The and right pancreaticoduodenal supplied the descending duodenum. The right gastroepiploic artery ran along the greater curvature of the stomach, supplying both of the gastric surfaces. Its anterior branches anastomosed with the branches of the right gastric artery, its posterior ones with those of the left gastric artery. The right gastroepiploic artery itself anastomosed on the greater curvature with the left gastroepiploic artery.

The splenic artery coursed to the left, immediately giving rise to a small diverticular artery, which supplied the diverticulum. The splenic artery then continued as the gastrolienal artery, giving off branches to the spleen, gastric fundus and diverticulum. It terminated as the left gastroepiploic artery which travelled along the greater curvature, supplying it and both surfaces of the stomach. There were intramural anastomoses between its branches and those of the left gastric artery (posterior) and of the right gastric artery (anterior).

Trixl's observations on 15 pigs differed in certain respects from those of both the preceding authors. He reported a coeliac artery bifurcation but into hepatic and gastrosplenic arteries. The hepatic distribution was the same as described by Busch, with the exception that Trixl observed "Rami gastricae" arising from the gastroduodenal artery. From their diagrams and photographs, these appear to be identical to Busch's "Rami pylorici". After splitting into the left gastric and splenic arteries, the distribution of the gastrosplenic artery also appeared to parallel that given by Busch.

## <u>Conclusions</u>

It is difficult to reconcile the three patterns of branching of the coeliac artery which are described by





Sisson, Busch and Trixl and further observations are required. Sisson's description appears to differ from Busch's and Trixl's, but the differences may be largely due to nomenclature. Sisson's anterior gastric artery, a branch of the gastrohepatic trunk, appears to be topologically equivalent to Busch's right gastric artery. Similarly, Sisson's posterior gastric branch, which sometimes originates at the coeliac bifurcation, is topologically equivalent to Busch's left gastric artery. If these two identities are accepted, the three descriptions are largely consistent.

## INTRAMURAL VASCULATURE

The first publication giving any significant detail on the microcirculation of the pig stomach appears to be by Trautman & Fiebiger (1957). This is a general veterinary histology text, encompassing a number of species and does not give any experimental details. Subsequent experimental studies on the stomach were published by Busch (1973; personal communication 1975), Trixl (1973) and Saito (1974). These were all injection based studies. A physiological investigation giving anatomical information was conducted by Sales (1975). In addition, the duodenal microvasculature was investigated by Bellamy (1973).

## Description

A general description of the porcine gastric microvasculature was given by Trautman & Fiebiger (1957). The branches from the extramural arteries pierce the serosa and muscularis and enter the submucosa where they give rise to a plexus. This gives off small branches to the muscularis externa and branches to the mucosa. From the former arises a capillary net with oblong meshes, which is supplemented by supply vessels from the subserous plexus and from the arteries penetrating the muscularis, en route to the submucosa. Arteriovenous anastomoses are found in the submucosa arise at regular intervals and pierce the muscularis mucosae, to which they supply nutrient vessels. In the mucosa, they form the capillary plexus which surrounds the glands from base to opening. Collecting veins drain this plexus and are themselves tributaries of the submucous and subserosal venous plexuses. From them arise the extramural veins.

The value of a report of this nature is severely limited by the absence of methodological information, experimental results and cited authorities. Since species differences are not mentioned, it is not possible to differentiate a lack of data from the contention that the microcirculation of all the domestic animals are identical and may be combined into a single model.

The first work giving experimental details appears to be that of Busch (1973; personal communication 1975). He studied 22 pigs , each of which was subject to one of two injection procedures. Post mortem injections of siloprene rubber or of dye in gelatine were made into the coeliac artery. Corrosion casts of the siloprene preparations were made, coated with metal and examined under the scanning electron microscope. The gelatine injected specimens were fixed and either sectioned histologically or cleared.

Busch confined his attention mainly to the mucosa and submucosa. Branches from the extramural arteries entered the gastric wall. After a short, but often tortuous, subserosal course they pierced the muscularis externa and entered the submucosa where they anastomosed to form an arterial plexus. This had two discrete sets of components, a net of large (primary) vessels and one of smaller (secondary) vessels. There was a well defined anastomotic line within the submucosa at which vessels originating from the lesser curvature arteries met those from the greater curvature arteries. The smaller arterial components of the plexus tended to be found alone, whereas the larger ones were often flanked by parallel interconnected accompanying veins.

Mucosal arteries arose from the smaller vessels of the submucous plexus and took a tortuous or spiral path through the muscularis mucosae to form a second arterial plexus at

the base of the mucosa. This too had primary and secondary components. Mucosal arterioles arose from this which did not reach the mucosal surface but which gave rise to a mucosal capillary plexus around the glands.

This plexus was drained by perpendicular collecting veins, which were often connected by vascular cross bridges, parallel to the mucosal surface. The collecting veins were tributaries of a venous plexus at the base of the mucosa, again with primary and secondary components, which drained into a submucosal venous plexus which eventually gave rise to the efferent veins.

The microcirculation of the pylorus, fundus and corpus were qualitatively identical. The corpus was particularly well vascularized. By contrast, the diverticulum was hypovascular. Busch found it difficult to make preparations of the lesser curvature, but a few fragmentary corrosion casts seemed to show a number of vessels which entered the mucosa directly, without joining the submucous arterial plexus (personal communication).

Corrosion casts of the mucosal capillary plexus appeared to demonstrate the presence of arteriovenous anastomoses. A few were also found in the dye/gelatine preparations of the fundic submucosa. Busch does not describe the basis on which he identified them He presents only inconclusive photographs of the mucosal specimens.

Trixl (1973) showed significant qualitative differences between the vascular architecture of the pylorus and of the fundus. The arteries of the pyloric submucous plexus tended to run flanked by parallel interconnected accompanying veins. Mucosal arteries were given off which, after a short perpendicular course toward the lumen, gave rise to a few branches parallel to the muscularis mucosae and then pierced this muscle layer, with or without an accompanying vein. An arterial plexus was formed at the base of the mucosa which gave rise to smaller secondary mucosal arteries. These were the origin of perpendicular mucosal arterioles which ran towards, but did not reach, the gastric lumen. They divided extensively to form a mucosal capillary plexus. This was

drained by a subepithelial venous arcade which, in turn, was a tributary of a coarse tree-like system of collecting veins. These were interconnected by vessels parallel to the mucosal surface and drained into a basal venous plexus and hence to the submucous venous net.

The mucosal arteries in the fundus pierced the muscularis mucosae without giving off branches parallel to the layer. The perpendicular mucosal arterioles, arising from then secondary arteries of the mucosal arterial plexus, had an initial coiled segment. The perpendicular collecting veins were longer and more slender than those in the pylorus, with fewer cross connections but still formed a basal venous mucosal plexus. Just proximal to this point, the collecting vein had a distended area, followed immediately by a constriction. The veins in both fundus and pylorus were wider than the corresponding arteries at all levels. The pyloric vessels appeared to be wider than the fundic.

Supplementary information was given by Saito (1974) who investigated the gastric microcirculation in four pigs. He injected contrast media, which he examined histologically or radiographically. He found that the arteries in the nonglandular oesophageal region of the stomach took a tortuous course through the muscularis externa, which was thick at this point. They gave rise to a submucous and a mucosal arterial plexus from which arose the subepithelial capillary plexus. The capillaries in this region were larger than in the remainder of the stomach. The glandular areas of the stomach, the fundus, cardia and antrum, had the same vascular architecture as one another. The arteries took a straight path through the thinner muscularis externa. The mucosal capillary plexus surrounded the glands. In both cases, the capillaries were drained by perpendicular collecting veins. Saito's description is incomplete in many respects and gives only a superficial picture of certain aspects of the gastric microcirculation. His use of only four animals precludes drawing any firm conclusions from his data.
A physiological study giving information on this topic was published by Sales in 1975, who used radioactively labelled microspheres to investigate gastric blood flow in four pigs.An initial bolus of  $6-10 \times 10^5 25+5$ um microspheres labelled with <sup>141</sup>Cerium was followed three hours later by a similar bolus of <sup>51</sup>Chromium labelled spheres. During the course of the experiment, the gastric proportion of the cardiac output fell by 6.7%. No significant activity was found in the hepatic portal vein. This was interpreted as showing the absence, or non-patency, of gastrointestinal arteriovenous anastomoses. However, the validity of extrapolating from microsphere passage to AVA existence has been questioned by several authors (eg Delaney 1975), particularly in cases involving major surgical shock, although the criticisms are of greater significance in relation to positive results. These pigs were in shock, as the cardiac output fell by 40%.

Trautman's veterinary text (1957) described the small intestine of domestic animals, including the pig. This description is homogeneous with regard to species and region of the small intestine and gives no experimental details. He described the branches of the extramural arteries piercing the serosa and muscularis externa to enter the submucosa. Nutritive branches were given off in the muscle which formed a capillary plexus between the two muscle laminae. The arteries ramified in the submucosa to form a plexus, which gave rise to short branches to the mucosal glands and long branches to the villi. The former penetrated the muscularis mucosae and formed a capillary plexus around the glands. The latter divided into several branches after leaving the muscularis mucosae, each of which ascended to the top of a villus where it divided in a 'fountain' pattern to form the capillary plexus. This was drained by villous veins, which arose at various levels and, together with the veins draining the glandular plexus, formed a venous mucosal network. This drained into the submucous venous plexus and then into the extramural veins. Arteriovenous anastomoses were found, but only in the submucosa. They comprised an

artery with an occlusive mechanism which drained into a special venous plexus, communicating with the submucosal veins via non-muscular highly dilatable portions.

Trautman's description was augmented by an experimental investigation of seven pigs by Bellamy (1973). He utilised a dual arterial and venous injection technique, followed by histological examination and gave a single description, encompassing the entire small intestine. The branches of the extramural arteries penetrated the muscularis externa, giving off nutritive branches which formed a capillary plexus between the circular and longitudinal muscle laminae. The main arteries then entered the submucosa, branching to form the submucosal arterial plexus. From this network arose perpendicular arteries to the villi as well as branches to the crypt plexus, the muscularis externa and the duodenal glands. The villi displayed a 'fountain' capillary pattern, the central axial artery giving rise at the villus tip to a subepithelial capillary plexus. Some of these vessels united to form a few venules half way down the villus, which were tributaries of a transverse venule, which ran below the bases of the villi. The remaining capillaries were also tributaries of this venule or they united with components of an adjacent villus plexus or of the crypt plexus. The latter network formed capillary rings around the cryptal tissues. The transverse venule drained into the submucous venous plexus which appeared in some areas to comprise segmented vessels with intermediate dilatations. From this plexus arose the branches to the extramural veins. No arteriovenous anastomoses were seen in either the mucosa or the submucosa. The cryptal plexus was richer in the duodenum than in either the jejunum or ileum.

This work, which uses both injection techniques and histological examination, and in which the injection pressures were carefully controlled within physiological limits, would appear to be capable of giving a comprehensive description of the microvasculature. The main limitation is the implicit assumption that the small intestine is homogeneous and that all the observational data may be

combined into one model. Bellamy does not describe how the spatial positions of his samples were distributed or how the observation of individual specimens related to the final description. It is not, therefore, possible to ascertain whether there were any differences between the regions. However, the fact that the author does describe one, but only one, disimilarity between the duodenum, jejunum and ileum implies that they were alike in all other respects. Bellamy is also one of the few authors to define his diagnostic criteria for arteries, veins and capillaries, based upon the dimensions of the lumen and wall and on their context. Unfortunately, he does not extend this to arteriovenous anastomoses. His description is generally consistent with Trautman's, with the exception of his inability to find arteriovenous anastomoses.

The only other work in this area appears to be that of Noer (1943) who specifically limited his study to the jejunum. It is, therefore, outside the scope of this review.

#### Conclusions

There is comparatively little information available on the pig's gastroduodenal microcirculation and much of this is poorly supported by observational data or technical details. These factors make it difficult to compile an overall picture. The following account appears to be the best synthesis of the gastric vasculature which can be made at the present time.

The branches from the extramural arteries enter the gastric wall and have a short tortuous course through the serosa (Busch). They then pass through the muscularis externa, by a twisting course in the diverticulum and a straight one in the glandular stomach (Saito). Nutrient branches are given off to the muscle which contribute to the recurrent branches from the submucous plexus. The relative importance of these two inputs is disputed. Trautman suggests that the muscular plexus is fed mainly from the submucosal vessels and supplemented by the nutrient branches from the penetrating arteries. Busch describes the converse, and more conventional, system. It is not possible to resolve this question on the basis of the available evidence, but the two statements are not anatomically contradictory. The arteries then leave the muscularis and enter the submucosa.

All authors agree that the arteries enter the submucosa and ramify to form a plexus. Busch notes that there is a primary and a secondary network, based upon a vessel bore differentiation. Within the plexus, there is a well defined line, in which the submucous vessels originating from the 'lesser curvature' arteries anastomose with those from the 'greater curvature' vessels (Busch). The larger submucous arteries tend to be accompanied by parallel flanking veins which are connected by cross bridges. The plexus gives rise to recurrent branches to the mucosa (Trautman) and to mucosal arteries which run perpendicularly towards the muscularis mucosae. They pierce the muscle, with a spiral or a tortuous course (Busch), giving off nutrient branches (Trautman) and enter the mucosa. Trixl agrees with this pattern in the fundus, but notes that, in the pylorus, the mucosal arteries give off small branches parallel to the muscularis mucosae before piercing it.

The mucosal arteries ramify at the base of the mucosa to form an arterial plexus with primary and secondary components. The mucosal arterioles arise from the latter and have a coiled initial segment in the fundus, but not in the pylorus (Trixl). As they rise towards the surface, they break up to form the mucosal capillary plexus around the glands (or beneath the epithelium in the diverticulum). The arterioles themselves do not reach the surface.

The mucosal capillaries are drained by perpendicular collecting veins with a tree like branching pattern in the pylorus (Trixl) which are also supplied by a subepithelial venous arcade at the mucosal surface in the pylorus. The interconnected collecting veins drain into a basal venous plexus. In the fundus, just proximal to the basal plexus, is a distended and then a constricted segment. Drainage is then via the submucous and subserosal venous plexuses and the extramural vessels.

Busch claims to have found both mucosal and submucosal arteriovenous anastomoses in his injected specimens. Sales, on the other hand, was unable to find any radioactivity in the hepatic portal vein after labelled microsphere injection into the arterial system. Busch does not give his diagnostic criteria and his published photographs are inconclusive. It is difficult to provide unequivocal evidence by the observation only of injected and cleared specimens. The validity of inferring the existence of AVA's from the transfer of microspheres from the arterial to the venous system is questionable, but the criticism is mainly applicable to positive results, not negative. It is not possible to make a judgement upon this question on the basis of the limited evidence that is available.

Some spatial variations are evident from these studies. It is generally agreed that the diverticulum is relatively avascular compared with the glandular stomach, the fundus being particularly densely vascularized. Trixl describes different fundic and pyloric mucosal patterns, though he is not supported by Busch. Apart from Busch's suggestion of a possibly anomalous vasculature on the lesser curvature at the submucosal level, there does not appear to be any evidence for significant spatial variations in the submucous plexus anastomotic density.

In summary therefore, an overall description of the gastroduodenal microcirculation, based upon sound experimental observation, is still wanting. This is particularly true of the the existence of arteriovenous anastomoses, end arteries (i.e. mucosal arteries of extramural origin) and spatial variations in the submucous plexus anastomotic density.

# <u>CHAPTER</u> 7 THE GUINEA PIG

# NON-VASCULAR GASTRODUODENAL ANATOMY

Few accounts of the anatomy of the foregut of the guinea pig are to be found in the literature. The principal description appears to be by Breazile & Brown (1976) although its observational basis is unstated. Others authors have addressed particular aspects of this topic, such as Schwalbe (1872) and Kuczynski (1890) who described the duodenum, and Bensley (1902) who studied the cardiac glands. The duodenum was again described by Carleton (1935) whilst Botha (1958a; 1958b; 1962) described the gastro-oesophageal junction. Finally, Oi (1958) studied the gastric parietal cell distribution, and Jervis et al. (1973) the effect of fasting upon the duodenum and its consequent ulceration in an unspecified number of animals.

### External Form

The oesophagus passes through the oesophageal hiatus in the diaphragm and the oesophageal notch in the liver, before it enters the stomach obliquely. According to Botha (1962), there is a small vestibule and an annular thickening of muscle immediately proximal to the cardia.

The oesophageal aperture is slightly to the right of the mid point of the lesser curvature so that the fundus, which extends to the left of and dorsal to the cardia, is large. The long axis of the stomach is orientated transversely and its rostral extremity is more dorsal than the caudal. There is no distinct incisura angularis.

According to Carleton (1935), who examined eight animals, the duodenum normally forms a single loop, the first 1.5 cm of which is expanded, although in some animals a sharp 'kink' after this dilatation converts the loop into a double one. The duodenum has a total length of approximately 125 cm ( Breazile & Brown 1976). There are no external features which enable the different areas to be differentiated. Authors disagree over the point(s) of entry of the bile and pancreatic ducts. Carleton described the bile duct entering immediately after the pylorus whilst the pancreatic duct entered six to eight centimetres below. In five out of eight animals, an accessory pancreatic duct was found between the two. Conversely, both Jervis et al.(1973) and Breazile & Brown described only a common bile and pancreatic duct, which entered less than 0.5cm from the pylorus (Jervis et al.) or about a centimetre the pylorus (Breazile & Brown).

# <u>Mucosal</u> Folds

There is little information on this aspect and that is contradictory. Botha (1958a) described the cardiac mucosa as 'very smooth'. Breazile & Brown described the entire mucosa similarly, except at the pylorus where it was thrown into longitudinal rugae. Conversely, according to Jervis et al. the antral mucosa, defined as the area around the pylorus, was smoother than the rest of the stomach. There is no evidence which enables these inconsistencies to be resolved.

# Gastro-oesophageal Junction

Botha reported the presence of a sphincter at the gastrooesophageal junction ((1959a; 1959b; 1962).There were no mucosal folds around the oesophageal aperture.

# Epithelial Lining

The oesophagus is lined by keratinized stratified squamous epithelium. By contrast, the stomach is entirely secretory, the transition between the two mucosae being abrupt and occuring, according to Botha (1958a), immediately above the cardia. The oesophageal epithelium is often continued as a ridge which protrudes into the stomach (Bensley 1902; Botha 1959a; 1959b; 1962). Cardiac, fundic and pyloric glands are found, the fundic glands comprising the majority. These regions may be differentiated histologically but not by their gross appearance. In all three, the mucosa is invaginated by pits, each of which receives several simple branched tubular glands. The cardiac glands were described by Bensley (1902) and form a ring, 0.3 cm in width, around the oesophageal aperture. They comprise columnar epithelial cells which, according to Breazile & Brown, contain no secretory granules. However Bensley reported that their staining characteristics indicated that they had a mucus secretory function. The fundic glands contain mucous, parietal and chief cells whilst the pyloric glands contain mainly mucous cells. There is no description of the spatial distribution of these mucosal types but it is known that the cardio-fundic and fundo-pyloric transitions are gradual. In the former case, Bensley showed that large numbers of parietal cells appeared suddenly but chief cells increased in number more slowly, so that the interface between true cardiac and true fundic mucosae was extended.

The distribution of the parietal cells was studied by Oi (1958) who took 34 0.132  $\text{mm}^2$  samples from the stomach of each of ten guinea pigs and examined them histologically. He found the maximum concentration in the middle of the corpus on the lesser curvature, decreasing rostrally and caudally and towards the greater curvature. The density of the parietal cells in the pyloric glands was 0% - 6% of the maximum.

Carleton (1935) studied the distribution of Brunner's glands in the duodena of eight guinea pigs. These glands, found beneath the muscularis mucosae, formed a layer of uneven thickness immediately distal to the pylorus, after which they broke up into isolated groups., extending 60 cm from the gastroduodenal junction. This was in contrast to Kuczynski's results, in which they extended only 10 cm beyond the pylorus (1890) and to Schwalbe's, which demonstrated a wedge shaped group of glands, only 1 cm long (1872). It also contradicts Villemin's hypothesis (1922), that Brunner's glands do not extend beyond the major papilla, but this was founded upon observations in species other than the guinea pig.

Jervis et al. (1973) found that the Brunner's glands extended approximately 15 cm from the pylorus. They noted that the character of the mucosa differed above and below

the major papilla. Distal to this point, the mucosa comprised many villi in a 'herringbone' pattern, whereas proximally the villi were short or absent. The circumpapillary mucosa was consistently avillous. Avillous areas were marked by an absence of alkaline phosphatase, normally found in the brush borders of duodenal cells, and by hypertrophy of the crypt cells and the lamina propria. These authors also observed that the gastroduodenal mucosal interface was often distal to the pylorus, with interdigitation of the mucosae. By contrast, Breazile & Brown (1976) found villi throughout the small intestine.

#### Musculature

There is little information available. Botha (1962) described a sphincter at the cardia and Jervis et al. one at the pylorus. Breazile & Brown (1976) reported that the proximal oesophageal muscle was striated whilst the distal was smooth, but did not localise the point of transition. They also described an ampullary swelling on the common bile duct, with a proximal sphincter, which isolated the ampulla when it was full.

### <u>Conclusions</u>

There is a small vestibule where the oesophagus joins the stomach obliquely, to the left of which is a large fundus. The stomach is orientated transversely and does not have a distinct incisura cardiaca. The singly or doubly looped duodenum has an initial dilatation, but the anatomy of the bile and pancreatic ducts is disputed.

The oesophagus is lined by stratified squamous epithelium which changes abruptly to the gastric secretory mucosa immediately above the cardia. From the point of transition, a fringe of oesophageal mucosa projects into the gastric lumen, but there are no gastric mucosal folds. There is a narrow ring of mucus secreting cardiac glands around the oesophageal aperture, the remainder of the stomach comprising a fundic gland region, containing mucous, chief and parietal cells, and a pyloric gland region, containing only mucous cells, with transitional glands between the zones. The pyloric and duodenal mucosae are interdigitated so the mucosal interface is often distal to the anatomical one.

The proximal oesophageal muscle is striated and the distal smooth. There are two sphincters, at the cardia and the pylorus.

Several aspects have not been studied or are contentious. The external morphology is clear, with the exception of the inter-relationship of the bile and pancreatic ducts and the duodenum. Discounting Breazile & Brown's unsupported account, it is not possible to compare the validity of Carleton's and Jervis' results describing, in the former case, separate bile and pancreatic ducts (immediately and 6 - 8 cm after the pylorus respectively) with a frequent intermediate accessory pancreatic duct and, in the latter case, a common duct, less than 0.5 cm from the pylorus. Although histological details of the stomach and duodenum are available, the distribution pattern of the gland types is not known or, in the case of Brunner's glands, is disputed. Similarly, there is an almost complete absence of information on the tunica muscularis throughout the foregut.

#### EXTRAMURAL VASCULATURE

Information upon the vascular anatomy of the guinea pig is only sparsely available. An early dissection based study was carried out by Luther (1923), which was limited to the abdominal aorta and its branches. Somewhat later, Potter et al. (1958) reported on dissections of the entire arterial and venous systems. However, fewer details of the coeliac artery and its branches were given in this work than in subsequent more specific studies. Angiography and dissection was carried out by Morozova (1965) on the iliocecal region, which gave some information on the coeliac artery and its branches. Favre (1967) used dissection, angiography and corrosion casting in his work. A more specific investigation was made by Perneczky (1969) on the abdominal aorta and its

80

Û

branches in 30 guinea pigs. He dissected fixed specimens after vascular injection with latex and heparin. Finally, Shively & Stump (1975) used dissection, angiography and corrosion casting to study the abdominal vasculature of 59 animals.

### Description

Perneczky (1969) confirmed the occurence of the normal mammalian pattern in this region. However, he found in 29 of his 30 specimens (96.7%) that a coeliacomesenteric trunk arose from the aorta, rather than two separate vessels. Two types of branching pattern of this trunk were noted. In 16 animals (53.3%) the first branch to be given off was a gastrosplenic trunk, which subsequently divided to form the splenic and left gastric arteries. Distal to this was the origin of the hepatic artery. The coeliacomesenteric trunk then continued as the superior mesenteric artery. In the other arrangement, found in 13 of the animals (43.3%), the coeliacomesenteric trunk split to form the coeliac and superior mesenteric artery and gastrolienal trunk, the latter dividing as in the previous pattern.

A more detailed account was given by Shively & Stump (1975). These authors also reported the presence of a coeliacomesenteric trunk in all of the 24 animals in which this region was observed. They described four branching patterns. In 13 (52%), the coeliacomesenteric trunk gave off a proximal gastrosplenic trunk and a distal hepatic artery. The gastrosplenic trunk was short, bifurcating to form the left gastric and splenic arteries. (Perneczky found this arrangement in 53.3% of his specimens). In 7 animals (29%), Shively & Stump found the coeliacomesenteric trunk forming a coeliac and superior mesenteric artery. The former then gave off a gastrosplenic trunk and a hepatic artery as before. (This was found by Perneczky in 43.2% of animals). The third pattern, observed by Shively & Stump in 2 animals (8%), comprised a gastrosplenic trunk given off directly from the aorta, proximal to the point of origin of a hepatomesenteric

trunk. The latter continued as the cranial mesenteric artery, after giving rise to the hepatic artery. The final pattern, also in 2 animals, was similar to the first, with the addition of an anastomotic branch between the gastrosplenic trunk and the aorta, proximal to the coeliacomesenteric trunk's origin.

The left gastric artery divided into two branches shortly after its origin in 15 of 18 animals (83%). In the remaining 3 (15%), the two branches arose separately from the gastrosplenic trunk. One branch supplied the cardia, anastomosing with the oesophageal arteries and ramified on the left aspect of the greater curvature, contributing to the left gastroepiploic artery. The other passed along the lesser curvature, supplying both the gastric surfaces and anastomosing with the right gastric artery.

The splenic artery entered the pancreas, giving off branches to it, and forming two to three splenic branches which passed to the splenic hilum. Two to five branches to the greater curvature were also formed within the pancreas. These contributed to the formation of the left gastroepiploic artery, which was supplemented by vessels from the splenic hilum. The splenic artery continued within the greater omentum as the epiploic artery, finally ending by sending branches to the right gastroepiploic artery.

The hepatic artery left its point of origin and passed to the liver, traversing the pancreas. It gave off the gastroduodenal, cystic and right gastric arteries before entering and supplying the body of the liver. The cystic artery supplied the gall bladder. The right gastric artery passed to the duodenum, ascended and finally anastomosed with the left gastric artery on the lesser curvature. The gastroduodenal artery coursed to the descending duodenum where it bifurcated to form the right gastroepiploic and cranial pancreaticoduodenal arteries. Immediately proximal to the pint of splitting, a small pyloric branch was given off. The right gastroepiploic artery, which received contributions from the epiploic artery, anastomosed with the left gastroepiploic along the greater curvature. The cranial

pancreaticoduodenal artery anastomosed with the caudal pancreaticoduodenal, which was formed from the cranial mesenteric.

Although Shively & Stump appear to give more detail than Perneczky, it is uncertain how much reliability than be placed upon their results. They refer, in their introduction, to an investigation of 59 animals, but the observations of the coeliacomesenteric system are only in groups of 24 and 18 animals and it is unclear whether these groups are intersecting or exclusive. The composition and selection criteria for these groups are unstated and it is uncertain which of the investigative techniques were applied to them. These factors make it difficult to assess their results.

# <u>Conclusions</u>

It is agreed that, the gastroduodenal vessels usually originate in a coeliacomesenteric trunk, branching from the aorta. Two uncommon variants may be found in which either the gastrosplenic trunk arises directly from the aorta, proximal to the hepatomesenteric trunkor the branches of the left gastric artery arise directly and independently from the gastrosplenic trunk. The results of Shively & Stump are the major source of information on the detailed branching of vessels distal to this point, but they do not assess the relative importance of the various branches.

# INTRAMURAL VASCULATURE

There appears to be no report on the intramural gastric vasculature . Noer (1949) examined the microcirculation in the wall of the jejunum and ileum but this is beyond the scope of this review.

; ·

# <u>CHAPTER 8</u> <u>THE FERRET</u>

## NON-VASCULAR GASTRODUODENAL ANATOMY

Comparatively few investigations of the foregut in this animal have been carried out, the only comprehensive one being by Poddar & Murgatroyd (1976) on five animals, two of which were dissected and three studied histologically. In addition, the ferret was included in Botha's comparative study of the gastro - oesophageal junction (1958a; 1958b; 1962). However, a number of studies have been carried out on aspects or regions of the digestive tract in closely related species, principally the mink (Mustela vison) and the stoat (M. erminea). The ferret and he stoat have been shown to be able to mate and produce offspring. In addition, the artificial insemination of ferrets by mink sperm resulted in fertilization and the development of the zygote to an advanced stage (Chang 1965). The literature relating to these species has, therefore, been drawn upon in the compilation of the following description.

# External Form

The oesophagus enters the stomach at an acute angle after a short abdominal course of 1.0-5 cm. Its right lateral aspect is continuous with the lesser curvature and it is dilated just proximal to the oesophageal hiatus in the diaphragm (Poddar & Murgatroyd 1976). In addition, Botha (1958a; 1962) described a small vestibule in the oesophagus, immediately before its entry into the stomach, although there was no macroscopically visible muscular thickening. He also described the dilatation at, or proximal to, the hiatus, located 0.3-0.5 cm before the cardia. In a study of 77 minks, Kainer (1954a) found exactly the same ratios of abdominal oesophageal to total oesophageal and body lengths as Poddar & Murgatroyd.

The stomach is elongated along its long axis, the lesser curvature being about 7cm long in the adult animal. The oesophagus enters towards the rostral end so the fundus is quite small, whilst the elongated corpus merges into the antrum as there is no definable incisura angularis. There is, however, a distinct gastroduodenal junction, marked by an external constriction and a thickening of the muscularis to form a sphincter. In the mink, Wood (1950) found the stomach to comprise 0.7% of the body weight, in comparison to Kainer's determination of 0.52%, equivalent to  $3.8 \pm 0.8g$ with a lesser curvature  $8.5 \pm 1.1$ cm long (Kainer 1954a). When maximally dilated, the stomach had a volume of 40.7ml.

The duodenum was described by Poddar & Murgatroyd (1976). It is a 'C shaped' loop, 10cm long, divisible into three parts. The first runs slightly cranially and to the right and is separated from the caudally directed second part by a sharp flexure. A second flexure separates the second part from the third, distally directed and sinistrad, part. The duodenojejunal junction is marked externally by a slight constriction. The common bile and pancreatic duct enters dorsally 2.5cm from the pylorus (i.e. after 25% of the duodenal length), no accessory duct being present. This general arrangement was also found in the stoat (Carleton 1935) and the mink (Kainer 1954b). Carleton examined only one animal in which, although the duodenum was shorter (6cm), the common bile and pancreatic duct still entered after 22% of the duodenal length (1.3cm from the pylorus). She also found an accessory pancreatic duct, 0.3cm proximal to the main duct. In the 103 minks studied by Kainer, the main duct also entered the second part of the duodenum, after 10 - 25% of the duodenal length (1.0 - 2.6cm from the pylorus). When present, the minor papilla was again proximal to the major, in contrast to Villemin's study, in which the accessory pancreatic ducts always entered distal to the major papilla.

## <u>Mucosal</u> Folds

In the oesophagus of the mink (Kainer 1954a), the mucosal folds are longitudinal, whereas in the undistended ferret stomach, there are many fine 'criss cross' folds. This is particularly true in the corpus with some degree of longitudinal orientation in the region of the greater curvature (Poddar & Murgatroyd). No such arrangement was described on the lesser curvature (Botha 1958b; 1962).

# Gastro-oesophageal Junction

Botha showed that distension of the stomach in the living animal eliminated all the mucosal folds, except those at the oesophageal opening, around which they formed several concentric rings. He postulated a sphincteric action for them. These disappeared rapidly after death. He was unable to find any evidence for an anatomic gastro-oesophageal sphincter, although he reported that a band of tonic contraction was consistently found in the oesophagus, immediately proximal to the cardia. There was, however, no structural specialization of this region. By contrast, Poddar & Murgatroyd found a sphincter like muscular thickening in the oesophagus, just proximal to the cardia.

# Epithelial Lining

The oesophagus is lined by squamous epithelium which, is keratinized proximally but not in the abdominal region, with an intermediate length of uneven keratinization (Poddar & Murgatroyd 1976. Submucosal glands and lymph nodes are found throughout the course of the oesophagus. Both Poddar & Murgatroyd and Botha (1958a) agreed that there was an abrupt transition between the oesophageal and gastric mucosae immediately above the cardia. Poddar & Murgatroyd also observed a mucosal rim at this point but it is unclear whether this was equivalent to Botha's peri-oesophageal mucosal folds.

There is no comprehensive account of the mucosal boundaries and gland type distributions in the ferret, but some isolated features have been reported. The oesophageal epithelium is succeeded at the cardia by cardiac glands, which Botha (1958a) described as "typical". These contain tall columnar mucous cells which stain strongly with P.A.S. The bulk of the gastric glands are confined to the fundus and corpus. However a few unusual features have been described. Poddar & Murgatroyd were unable to find any mast cells in the stomach but did observe Brunner's glands in the pyloric antrum. Pfeiffer (1974) found a type of parietal cell with previously unobserved ultrastructural characteristics, mixed with the normal cells. These contained 'giant' mitochondria and uncharacterised nonmembrane bound crystalloids, but they were quite rare.

The histological features of the duodenum were also described by Poddar & Murgatroyd and follow the normal mammalian pattern. The long villi have a muscular core, derived from the mural muscularis mucosae and they bear goblet and other cell types. Tubulo-alveolar Brunner's glands were found in the submucosa but extended only as far as the major papilla, in accordance with Villemin's concept (1922). Conversely, Carleton (1935) found, in the stoat, that the glands stopped abruptly at the accessory duct, none being found at the major papilla, 0.3cm proximal to this point. This was, however, based upon the observation of only one animal.

#### Musculature

The oesophageal muscle, studied in three animals by Poddar & Murgatroyd, comprised an outer longitudinal layer and an inner circular layer and was striated throughout most of its length. It was replaced by smooth muscle near to the cardia. The circular layer was thickened to form a sphincter at the gastro-oesophageal junction. By contrast, Botha (1958a) found the oesophageal muscle in an unspecified number of specimens was all striated and he was unable to find any sign of a sphincteric muscular thickening.

There is very little information available on the gastric and duodenal musculature. Poddar & Murgatroyd found the normal inner circular and outer longitudinal muscle layers in the stomach but were unable to find an oblique layer. The former lamina was thickened at the pylorus to form a sphincter. In the duodenum, the authors described an apparently sphincteric ring of circular muscle around the common pancreatic and bile duct in its intramural course.

#### <u>Conclusions</u>

Comparatively little information is available specific to this species. The oesophagus expands distally to form a vestibule before entering, at an acute angle, the rostral end of the slightly elongated stomach, the fundus of which is small. There is no clear incisura angularis or distinct external demarcation of the different regions. The duodenum is 'C shaped' and is entered by a common bile and pancreatic duct after approximately one quarter of the duodenal length. There is some longitudinal mucosal folding near the greater curvature and annular folding around the oesophageal aperture. The oesophagus is lined by a glandular squamous epithelium which is not keratinized in its abdominal course. This layer changes abruptly you a gastric secretory mucosa just above the cardia. In the duodenum, Brunner's glands extend as far as the major papilla. The muscle of both the oesophagus and the stomach comprises two layers, no inner oblique being present.

An extensive investigation of most aspects of the anatomy of the foregut in the ferret is required. Nothing is known of the arrangement of muscular bundles or of mucosal regions in the stomach, only isolated features having been described. In addition, there is disagreement on the point of transition of striated to smooth muscle in the region of the gastro-oesophageal junction and whether there is an annular muscular thickening at this point.

# EXTRAMURAL VASCULATURE

Despite the widespread use of the ferret in biomedical research (Hahn 1969), there is little data in the literature on the characteristics of the normal ferret. In particular, there appears to be no description of the ferret's vascular anatomy.

### Description

The only relevant work appears to be an investigation of the anatomy of the coeliac trunk in the American Mink, a species quite closely related to the ferret (Wiland 1970). He examined 102 animals, dissecting them after vascular injection with latex and fixation in 5% formalin. A coeliac trunk, arising from the aorta, was present in 100 cases (98%) and absent in the remainder. In one of the latter, the hepatic artery arose directly from the aorta and a gastrosplenic trunk from the cephalic mesenteric artery, whilst in the other, the reciprocal origins were found. The coeliac, when present, varied from two to eight millimetres in length and in 18 cases (17%) it trifurcated into the hepatic, splenic and left gastric arteries. In the other 82, it divided to form a hepatic artery and a gastrosplenic trunk which split, after one to six millimetres, to form the splenic and left gastric arteries. The hepatic artery gave off a number of small proper hepatic arteries before continuing to the pancreas the cephalic as pancreaticoduodenal artery. There appeared to be no right gastric and gastroduodenal arteries. The splenic artery divided after a short course into dorsal and ventral branches. The dorsal branch split into smaller vessels and ran in the greater omentum to the splenic hilum whilst the ventral branch gave off short gastric arteries to the greater curvature before reaching the hilum. The left gastric artery coursed in the lesser omentum, supplying the lesser curvature and cardia and the distal end of the oesophagus.

# INTRAMURAL VASCULATURE

There appears to be no report in the literature on the intramural vasculature in the stomach or duodenum of the ferret.

# <u>CHAPTER 9</u> <u>THE RAT</u>

## NON-VASCULAR GASTRODUODENAL ANATOMY

Early descriptions of the anatomy of the rat were either elementary (Flower 1872; Martin & Moales 1884; Hunt 1924) or were based upon a species other than the laboratory rat (Howell 1926). The first comprehensive and detailed account, based upon the results of dissection, was by Greene (1935) who studied each system of the body in ten or more dissections. Unfortunately, she restricted her observations to structures visible with the naked eye, or under very low magnification, so that much detail was still lacking. This detail has been provided by subsequent authors each of whom examined a particular facet of the rat's anatomy. The definitive description of the gross and microscopic anatomy of the stomach is still that given by Berg (1942) who studied 116 animals. His results were confirmed by Shay et al (1945) and summarised by Lambert (1965). The biliary system was studied by Mann (1920) and McMaster (1922) and the duodenal mucosa by Carleton(1935). Oi et al. (1958) charted the parietal cell distribution, whilst the gastrooesophageal junction and adjoining parts of the stomach and oesophagus were examined in extensive detail by Botha (1958a; 1958b; 1962), El-Ramli (1964) and Fatani (1971). From a consideration of the complementary and supplementary results of all these authors, it is possible to compile a description of the lower oesophagus, stomach and duodenum of the rat.

## External Form

Greene's diagrams show the oesophagus descends initially in the midline, then move to the left in the thorax, pierces the diaphragm at the hiatus and enters the stomach after a long abdominal course. This was confirmed by Botha (1958a; 1962), who stated that the abdominal portion of the oesophagus varies from 1.5cm to 2.5cm in length and enters the stomach slightly ventrally, the incisura cardiaca

being approximately 90°. The oesophagus is firmly fixed by a dense mesentery and has a marked vestibule immediately proximal to the cardia, although Botha maintained that this was only visible in the living animal or within two to three hours of death. El-Ramli (1964) and Fatani (1971) confirmed Botha's findings, whilst also reporting the presence of an externally visible muscular thickening, coincident with the vestibule.

The oesophagus enters the stomach at about the midpoint of the lesser curvature. The stomach is clearly divided into two regions, the demarcation running transversely from the oesophageal entrance to the middle of the greater curvature. The proximal part, which also extends to the left of and rostral to the cardia, comprises 40% of the empty stomach (ratio of wall areas) and has pale thin walls. The distal portion has thick, muscular, red-grey walls and is itself bipartite, as the area around the lesser curvature is thinner walled and paler than the anterior and posterior surfaces and the greater curvature. There is a gradual transition between the two regions. The size of the stomach is very variable, owing to the high degree of distensibility of the proximal portion (El-Ramli 1964). This was also reported by Lambert, who also asserted that the stomach is unusually mobile, as its ligamentous bindings are lax, but he did not cite his sources.

The terminology used by the various authors to name the  $(\tau_a \iota / \epsilon_a)$  regions of the stomach is confused. Particular parts have been given various names and specific terms have been applied to different regions. Berg (1942) used the term "fundus" to describe the area of the distal stomach comprising the greater curvature and its anterior and posterior walls. He was challenged by Shay et al. (1945) who felt that this was anatomically and functionally analogous to the corpus in Man and named it accordingly. Lambert, in his review derived mainly from Berg's and Shay's work, used the former's nomenclature, whereas Oi et al. (1958) used Shay's. An additional complication was introduced by Botha (1958a; 1958b; 1962) who used the term fundus to denote the

ţ

non-glandular stomach and did not distinguish between the two glandular regions, naming the entire area the "pylorus". He was followed in this by El-Ramli (1964) and Fatani (1971).

In this work, I adopt the nomenclature of Shay et al. with the additional terms "cardia" (after Botha), to denote the gastro-oesophageal interface, and "pylorus" (after Berg), to denote the gastro-duodenal interface. This terminology appears to be the least ambiguous and the most consistent with other species (see Table 2).

There are few descriptions of duodenal anatomy. Carleton (1935) described it as a "C shaped loop" with a slight initial dilatation into which the common bile and pancreatic duct entered immediately after the pylorus, in confirmation of McMaster's results (1922)). Conversely, Greene (1935) quoted Morell (1872) "The pancreatic ducts are many and paired" and her diagrams showed the common bile duct entering some distance distal to the pylorus. It is worthy of note that the rat does not possess a gall bladder and cannot, therefore, concentrate its bile (Mann 1920; McMaster 1922).

# <u>Mucosal Folds</u>

All authors agreed that the mucosa of the glandular stomach was thrown into longitudinal ridges, although Berg (1942) maintained that these were less marked in the antrum. Greene (1935) described longitudinal folds an the rumen, but this was denied by Berg and Shay et al. (1945). However, Lambert, in his review (1965) based mainly upon Berg and Shay et al., contradicted both by describing the rumen as "folded". The most recent author to mention the point was Fatani (1971) who, at different points in his thesis, described the rumen as "smooth and unfolded" and "thrown into folds". However, photographs presented by El-Ramli (1964) clearly show ridges in the rumen of an empty stomach, but a smooth mucosa in the distended rumen of a full stomach. Of the above authors, only Shay et al. mention their rats' nutritional status at death (starved for 48 to

( <sup>-</sup> T	GASTRO-OESOPHAGEAL INTERFACE	PROXIMAL PORTION (NON-GLANDULAR)	DISTAL PORTION	(GLANDULAR)	GASTRODUODENAL INTERFACE
	i		LESSER CURVATURE & SURROUNDING AREA	GREATER CURVATURE AND ANTERIOR & POSTERIOR WALL	
		8	1	808	
		Rumen/forestomach	Antrum	Fundus	Pylorus
		Rumen	Antrum	Body / corpus	
		Forestomach	Pyloric antrum	Body	
Cardia		Fundus	P Y I	8 7 7	
		Fundus			
Cardia		Rumen/forestomach	Antrum	Fundus	Pylorus
		Fundus			
Cardia		Rumen	Antrum	Corpus	Pylorus
		Ĩ	Table 2 Terminology Regions of	/ Used by Various Authors the Stomach in the Rat	to Name

72 hours) but it is not clear if this applied to both their 'normal' and experimental groups, or only to the latter. This unstated variable might explain the differing results.

## Gastro-oesophageal Junction

It was generally agreed that there was an annular thickening of the oesophageal circular striated muscle at the vestibule. In addition, Botha (1958a; 1962) claimed that the epithelial ridge between the glandular and non-glandular areas was particularly marked around the oesophageal aperture and, in the living animal, could occlude it (Botha 1958b). There were also well developed muscular sling fibres in the wall of the stomach around the cardia.

## Epithelial Lining

The stomach of the rat comprises a distal glandular and proximal non-glandular region, separated by an epithelial ridge ('Grenzfalte' - Bensley 1902). This runs transversely with a U-shaped diversion around, and caudal to, the oesophageal aperture, which is, therefore, located in the proximal region. Berg claimed that the ridge was raised by a thickened submucosa and Shay et al. described it as "keratinized". Most authors agreed that there was an abrupt change in the nature of the adjoining mucosa at the ridge, from non-glandular to glandular. Only Shay at al. disagreed, describing the transition as "gradual" but, since both terms are subjective, it is difficult to compare them. According to Botha, the ridge was particularly well developed around the cardia.

There is general agreement on the characteristics of the proximal part of the stomach - the rumen. This is lined by keratinized stratified squamous epithelium, continuous with the oesophageal mucosa. It is described as nonglandular or non-secretory by all authors with the exception of Greene who, citing Flower (1872), claimed that it contained mucus secreting glands. The balance of opinion does not support this contention. According to Botha (1958a), the zone of the cardiac glands, normally located

around the oesophageal aperture, was not found in the rat. The sequence of tissue layers in the wall of the rumen from deep to superficial, was described by Berg as :

- a) A superficial thin keratinized layer.
- b) A layer of cells containing eleidin granules. This material is a refractile basophilic keratin precursor.
- c) A layer of nucleated cells.
- d) Thin connective tissue.
- e) a well developed muscularis mucosae.
- f) Loose connective tissue.
- g) Muscle.
- h) Serosa.

This was confirmed by Shay et al.

The glandular stomach is thicker and more muscular than the rumen and is lined by columnar epithelium. Berg and Shay et al. differentiated two regions, the antrum and the corpus. The antrum occupied the lesser curvature and the area immediately proximal to the pylorus. Its walls were thinner than those of the corpus and the glands shorter and wider. According to Shay et al. only columnar mucous cells were found in these glands, whereas Lambert denied the presence of any glands, but did not substantiate this assertion. The antrum also contained Brunner's glands for a short distance proximal to the pylorus. By contrast, the corpus had thicker muscular walls and well developed tubular glands. In addition to mucous cells, prominent parietal (acid secreting) and inconspicuous chief (pepsinogen secreting) cells were found. This region comprised the anterior and posterior walls of the stomach and the greater curvature, with the exception of a small area immediately proximal to the pylorus. The mucosal transition from corpus to antrum was described by Berg as "fairly abrupt.... (occupying an area (sic) of 300 to 400 um". Conversely, Shay et al. described it as "gradual" naming it the Intermediate Zone of Aschoff (after Babkin 1944). It is not possible to ascertain whether the difference is real or semantic.

The distribution of the parietal cells was studied histologically in the glandular stomachs of ten rats by Oi et al. (1958) who took 30 x 0.045 mm<sup>2</sup> samples from each stomach. They found these cells only in a region corresponding to the corpus, with a decreasing concentration gradient from the greater to the lesser curvatures, in a distribution pattern consistent with Berg's and Shay's descriptions. His samples only occupied approximately 0.2% of the total area of the glandular stomach.

There is little information available on the duodenal mucosa. Carleton (1935) described a thin wedge of secretory epithelium containing Brunner's glands which had disappeared less than one centimetre from the pylorus.

## Musculature

The oesophageal muscle was described by Botha (1958a; 1962). It comprised two striated muscle layers, an outer longitudinal and an inner circular, although he reported a degree of oblique orientation of the longitudinal fibres. The circular muscle was thickened over the vestibule to form a sphincter like structure. A few of the striated longitudinal fibres passed onto the wall of the stomach. These observations were confirmed by El-Ramli (1964) who additionally described the oesophageal muscle fibres as forming two opposed spirals. There was a gradual transition in the region of the cardia from oesophageal striated to gastric smooth muscle.

In the stomach, Botha observed very well developed sling fibres around the left and right aspects of the oesophagus at the cardia, which overlapped the dorsal and ventral surfaces. There is little additional information available. Most authors described two muscle layers, an outer longitudinal and an inner circular, which were thickest in the corpus. The circular layer displayed an annular thickening at the pylorus. The muscularis mucosae was also of non-uniform thickness and orientation. According to Botha (1958a), it was thickest at the rumen - corpus interface where it "splayed out", but Fatani (1971) stated that it reached its maximum thickness around the cardia, where, according the Botha (1958b), it penetrated the mucosal ridge.

Finally, in the duodenum, Mann (1920) observed a well developed sphincter around the bile duct.

## <u>Conclusions</u>

The long abdominal oesophagus joins the stomach at 90<sup>0</sup>, and has a terminal vestibule. The stomach is visibly divided into the pale rumen (40%) to the left of the oesophagus, and the darker corpus and antrum, to the right. The C-shaped duodenum receives a common bile and pancreatic duct, although this has been disputed. The rat has no gall bladder.

Descriptions of the mucosal folds in the stomach have varied, probably due to differing degrees of distension of the gastric wall, particularly the fundus. In the empty stomach, the folds are mainly longitudinal. The rumen and corpus are separated by an epithelial ridge which is particularly well developed around the oesophageal aperture. At the gastro-oesophageal junction, there are also well developed sling fibres and a sphincteric thickening of oesophageal circular striated muscle.

The proximal rumen is lined by a continuation of oesophageal non-glandular stratified squamous epithelium and receives the oesophagus. It is separated from the distal glandular area by an epithelial ridge. The corpus contains mucous, chief and parietal cells and occupies the anterior and posterior walls and the greater curvature, except a small area immediately proximal to the pylorus. This is occupied by antral mucosa, as is the lesser curvature, which contains only mucous cells. Brunner's glands are found in the antrum, immediately proximal to the pylorus but extend less than one centimetre into the duodenum.

The oesophageal muscle forms an outer longitudinal and an inner circular striated layer, which change gradually to the gastric smooth muscle at the cardia. The circular striated muscle forms a sphincter at the vestibule and

several longitudinal striated fibres are prolonged onto the stomach. The stomach also has outer longitudinal and inner circular layers, the latter forming a pyloric sphincter.

The anatomy of the foregut in the rat is well documented with few areas of ignorance. The principal requirement is for further study of the orientation of the fibres of the muscularis externa and muscularis mucosae, particularly in the stomach. In addition, a few minor points await clarification, such as the position and number of the bile and pancreatic ducts, the extent of mucosal folding and the details of gastric and duodenal histology. None of these aspects can be satisfactorily described on the basis of the currently available evidence.

# EXTRAMURAL VASCULATURE

Little attention was paid to the detailed patterns of the rat's extramural gastroduodenal circulation until the second quarter of the twentieth century. Early accounts (Martin & Moales 1884; Hunt 1924) were designed to assist dissection and were elementary. A more detailed text was produced by Howell (1926) but this was based upon dissection of the wood rat rather than the white laboratory rat (Rattus norvegicus).

The first comparatively detailed study was carried out by Greene (1935). The vessels were injected with starch or gelatine prior to formalin fixation and dissection or with Murphy's artery red lacquer after intravenous fixation. The structures described were limited to those visible under low magnification and were named according to Emmel's (1927) anglicized version of B.N.A. terminology. No more than ten specimens were studied for each region, a number described by the author herself as "inadequate". This precluded the analysis of variants from the normal pattern. Despite this, Greene stated that the rat's anatomy was similar to Man's, although the pattern which was a variant in man was often the norm in the rat.

#### **Description**

Greene names the three branches of the coeliac artery as the left gastric artery, lienal artery and hepatic artery. The left gastric artery gives branches to the anterior and posterior surfaces of the stomach. The lienal artery supplies the stomach, pancreas and spleen. The hepatic artery gives off two branches, the right gastric artery, supplying the lesser curvature of the stomach, and the gastroduodenal artery, supplying the gastric greater curvature, duodenum and pancreas. More information is implicit in her illustrations. Branching appears to be "tripodal". The left gastric artery is shown to be the largest branch. It is depicted giving un-named branches to the oesophagus. No identifiable short gastric arteries from the lienal artery are shown, nor is a vessel, presumably corresponding to the left gastroepiploic artery, named. The right gastroepiploic artery is shown to supply only the most caudal part of the greater curvature. Conversely, the gastroduodenal artery seems to supply most of the duodenum. A bifid superior pancreaticoduodenal artery is named and shown as the terminal branch of the gastroduodenal artery.

A more detailed description of the rat's gastric circulation was given by Berg (1946) in a paper on the vascular effects of nutritional gastritis. He injected an unspecified number of rats after exsanguination with india ink in gelatine (10% ink in 8% aqueous gelatine at 37°C) via the thoracic aorta. The stomach was then removed, fixed and cleared.

Berg emphasised that the main blood supply to the stomach was derived from a single artery which he does not name but which, from his illustrations and topographical description, is clearly the left gastric artery of other authors. This divided into three branches behind the oesophagus. Two vessels supplied the anterior and posterior surfaces symmetrically, giving off long branches to the fundus and rumen and a few short ones to the antrum. The third vessel ran along the lesser curvature, giving off pairs of dichotomous twigs to the anterior and posterior surfaces of the antrum and ending just rostral to the pylorus. The greater curvature was supplied by short branches from a vessel which also supplied the duodenum and pancreas. The origin of the vessels on the lesser and greater curvatures was unstated.

The details of Berg's technique are unclear but it appears that he examined only cleared stomach preparations. It is not possible to ascertain how much perigastric tissue was retained but his photographs show the stomach with only the intramural, and most closely applied extramural, vessels remaining. He may, therefore, have failed to observe any vascular interconnections occuring at some distance proximal or distal to the stomach. In addition, his photographs also show that he opened it by cutting along the greater curvature which would have hindered his observation of the vessels in this area. Both of these factors reduce the scope of Berg's work.

Schnitzlein (1957) investigated the gastric vasculature as a basis for his study of the pharmacological reactivity of the intramural vessels. He dissected an unspecified number of rats , after injecting thier vascular systems with a starch / chrome yellow suspension.

Schnitzlein presented his results diagrammatically. He showed the coeliac artery arising from the aorta and dividing at a single point into three equally sized branches, the left gastric, common hepatic (un-named) and splenic arteries. The left gastric artery passed anterior to a lobe of the liver and divided into a number of branches on the posterior surface of the oesophagus at the cardia. One branch ran along the lesser curvature, giving off small twigs to the antrum and distal fundus. A number of long branches supplied the fundus, proximal antrum and distal rumen on the posterior surface. Branches encircled the oesophagus to reach the anterior surface, but their distribution was not shown. It is not possible to ascertain whether oesophageal branches were present. The splenic artery gave off a branch to the stomach before beaking up in the splenic hilum to supply that organ. This branch ran to,

and along, the left part of the greater curvature, supplying it by short gastric arteries. The common hepatic artery gave off the hepatic and superior pancreaticoduodenal arteries before it bifurcated and gave rise to the right gastric and right gastroepiploic arteries. The former ran along the lesser curvature, supplying the antrum, whilst the latter ran along the right part of the greater curvature, which it supplied by small twigs. Schnitzlein's use of the dissection technique would have enabled him to see the arteries supplying the stomach in relation to the surrounding tissues and vessels and would, therefore, appear to give a more comprehensive view, at this level, than Berg's technique.

Lambert (1965) in a text on experimental surgery in the rat, reviewed the gastric vasculature, quoting Berg (1946; 1947) and Schnitzlein (1957) as his authorities. However, his account is based, without justification, upon Schnitzlein's rather than Berg's work, and includes addition unsubtantiated facts and undefined terminology. His account is, therefore, of limited value.

Guth (1972), observed the gastric circulation in an unspecified number of exteriorized stomach preparations, using transillumination. The main blood supply was via the left gastric and right gastroepiploic arteries, with secondary supply from the right gastric, left gastroepiploic and short gastric arteries. The left gastric artery was the largest branch of the coeliac trunk. It divided into three behind the oesophagus at the level of the cardia, forming a vessel supplying the anterior surface, one to the posterior surface and a third which ran along the lesser curvature. On the anterior surface, long branches supplied the rumen and proximal corpus and short ones the distal corpus and antrum. Guth's technique did not permt him to observe the posterior surface. The vessel on the lesser curvature gave off four or five parallel branches to the distal corpus and antrum. Similarly, the right gastroepiploic artery (from the gastroduodenal branch of the hepatic artery) ran along the greater curvature and supplied the anterior and posterior surfaces of the antrum and corpus by ten or eleven parallel

pairs of branches. Guth did not describe any of the other vessels which supplied the stomach. His technique would have facilitated the assessment of relative blood flow in various vessels, but did not permit any extensive manipulation and dissection or the observation of the posterior vessels. His description might, therefore, be expected to be incomplete, but correct in the details which were given.

Leneman & Burton (1967) augmented the current description of the coeliac artery's anatomy with an account of a new hepato-oesophageal artery. This was based upon a photographic and radiological examination of an unspecified number of rats, after the injection of their coeliac arteries with a suspension of barium sulphate. The common hepatic artery bifurcated in a 'T-shaped' manner, the descending branch forming the gastroduodenal artery and the ascending branch the hepatic artery proper. These latter vessels gave off four branches to the lobes of the liver and a fifth branch, the hepato-oesophageal artery, to the oesophagus, meeting it at the rostral end of its distal third. This artery gave off ascending and descending branches which supplied the oesophagus. The ascending ones anastomosed with vessels from the right and left mediastinal arteries whilst the descending ones anastomosed with the cardiac branches of the 'gastric artery' i.e. oesophageal branches of the left gastric artery. There is only one previous description of this artery in the literature (Delong 1953) which described the hepato-oesophageal artery as a branch of the oesophageal artery and hence of the gastric artery. However, since Leneman & Burton gave no quantitative information, it is not possible to know if theirs was a variant pattern and, if so, to assess its occurence.

# Arterioarterial Anastomoses

Several authors have investigated the extent to which the extramural arteries of the stomach anastomose with one another. Berg (1946; 1947) maintained that there were no dirct anastomoses, only collateral pathways at the submucosal and capillary levels. This conclusion was based upon his analysis of injected and cleared specimens but was supported by the results of ligation experiments (1947). He tied various vessels in 54 rats under ether anaesthesia and sacrificed them after differing periods in order to examine the gastric mucosa. In 22 animals, he ligated the artery on the lesser curvature supplying the antrum plus its vein. In a futher 20, the artery to either the anterior or posterior wall of the fundus and its vein were tied. In the remaining 12, both these arteries and veins were tied. In each case, the ligated vessels were cut near to their origin.

Ten of the eighteen rats (55.6%), in which the antral vessel had been tied, showed a single ulcer in the centre of the antrum after 24 hours, which had perforated in two animals. Five of eighteen (27.8%) had multiple erosions and three (16.7%) were unaffected. The mucosa was pale and oedematous. However, animals killed after eight days (two rats) and sixteen days (two rats) showed significant healing. Only one of the animals subjected to unilateral "fundic" artery ligation developed an ulcer (5%). However, five of the eight rats with bilateral fundic artery ligation developed an ulcer (62.5%). In two of these, areas of necrotic tissue were seen, which were interpreted as infarcts. The slough separated to leave an ulcer. Again there was significant healing in the bilaterally ligated group after sixteen days. In both the fundic ligation groups, the mucosa was congested. In all cases, ulcers were diagnosed by observation of their penetration below the mucosa, in contrast to erosions, which were superficial.

Berg interpreted these results as an indication of the absence of significant direct anastomoses between the extramural vessels and therefore consistent with his anatomical observations. He hypothesized that the submucosal and capillary collateral pathways were more plentiful in the highly vascularised fundus than in the relatively hypovascular antrum, which accounted for the greater sensitivity of the latter area to the blocking of its normal source of supply. The ulcers and erosions were, he suggested, necrotic areas consequent upon infarction and healing was due to the formation of a collateral supply via the intramural vessels.

However, Berg included no controls among his animals to eliminate the effects of surgical and non-specific shock, although the marked difference in ulceration frequency between the animals with unilateral and bilateral fundic vessel ligation suggests that this was not a significant factor. As the author cut the vessels at their origins, any anastomoses between this point and the stomach wall would have been ineffective, but his anatomical observations suggested that no such communications existed. He also provides no evidence to support his contention that healing was due to the formation of intramural collateral vessels, rather than revascularisation from adhesions.

In contrast, Schnitzlein stated, on the basis of his dissection studies (1957), not only that there was a prominent anastomosis between the left and right gastric arteries on the lesser curvature and a smaller anastomosis on the greater curvature, but also that there were extensive connections between the gastric vessels and the "anastomotic arteries of the intestine".

The problem is further confused by Lambert's review (1965) in which he cites solely Berg (1946; 1947) and Schnitzlein (1957). He was faithful to neither source in his statement, the precise meaning of which is unclear, that the anastomoses were "relatively few and only occur between the very smallest branches". He then cites Berg's work to show that some anastomoses do exist "....for extensive ligatures of the branches of the coronary artery do not cause necrosis the stomach but simply acute ulcers •••", of an interpretation which is the precise opposite of Berg's. In summary, Lambert states that "... the blood supply of the rat's stomach is more precarious than man or dog, and distinctly so in the forestomach". However, since the latter region has no analogue in either of these two species, this has little meaning.

Lambert continues by citing Schnitzlein and states that the anastomosis between the left and right gastroepiploic arteries is of no functional importance and that there is a very rich anastomosis between the branches of the coronary and right gastroepiploic arteries. Neither of these statements is made by Schnitzlein. Thus, in the course of his review, Lambert begins by claiming that anastomoses are few then proceeds to list a functional anastomosis on the lesser curvature, a non-functional anastomosis on the greater curvature and a "very rich" anastomosis between the right gastroepiploic and coronary arteries. The account is, therefore, potentially misleading.

Guth (1972b) attempted to reproduce Berg's results using eighteen rats which were fasted for 24 hours, then anaesthetised with ether. In eleven of these, the antral artery and vein on the lesser curvature were ligated near to their origin, a small portion of the wall of the stomach being included in the ligature. Eight animals were subjected to laparotomy only. In all cases, the subjects were killed after 24 hours and the gastric mucosa was examined. The circulation patterns in a further six rats (three with ligated arteries and three without) were examined by in vivo microscopy and transillumination (Guth 1972a).

Guth found that only three of eleven (27%) of the experimental animals developed an antral ulcer, which was invariably located directly below the suture site, with the surrounding mucosa appearing normal. Two (18%) had a small erosion distal to the point of ligation, but a similarly located lesion was found in one of the controls (14%). In addition, approximately 50% of both controls and the experimental subjects developed linear erosions on the anterior wall of the fundus. In vivo microscopy showed identical patterns of flow in the submucous plexus and capillaries in the control and experimental animals, although the flow in the antral vein was sluggish or reversed in the latter group.

Guth concluded that his results gave no evidence to support Berg's contention that the blood supply of the rat's

stomach was such that ulcers could be produced by ligation of individual vessels. Anastomoses seemed to be adequate to maintain flow in the submucous plexus despite the cessation of the antral vessel flow. He suggested that the antral ulcers were probably a result of local trauma i.e. damage to the gastric wall by its inclusion in the ligature and that the remaining erosions, which were found in equal numbers in the control and experimental animals, were caused by starvation and operative stress. However, Guth's and Berg's works are not directly comparable. The former used fewer animals and only one pattern of ligation. The latter made no mention of starving his rats or of including a portion of the gastric wall in the ligature, but did cut the vessels in addition to tying them. He obtained a higher incidence of ulceration and the lesions were located in an abnormal mucosa. Thus, Guth's results do not necessarily invalidate those reported by Berg.

In a subsequent investigation, Guth (1975) ligated the left gastric and right gastroepiploic arteries and veins of 92 starved rats, before the vessels gave off branches. 22 controls were subjected to laparotomy alone. He found that a single large ulcer, extending into the muscularis externa, developed in the corpus of most of the experimental animals within three to five days. After two to eight weeks, 75% of the animals examined showed healing of the ulcers and all were normal after six months.

These results indicate that the left gastric and right gastroepiploic arteries are the principal supply vessels to the corpus of the stomach. However, it is difficult to accept Guth's suggestion that a more effective intramural collateral system in the antrum and rumen protected these regions from ulceration. Berg (1947) said specifically that the opposite was true. An alternative explanation might be that they are areas predominantly supplied by the right gastric (antrum) and left gastroepiploic (rumen) arteries, neither of which was ligated.
### <u>Conclusions</u>

There is a consensus that the stomach and upper duodenum are supplied by the branches of the coeliac artery. However, there is disagreement on the relative importance of these vessels. It is accepted that the largest, and most important, with probably the most extensive area of distribution, is the left gastric artery, which has two main groups of branches. Firstly, to the anterior and posterior surfaces of the stomach and secondly a single branch along the lesser curvature supplying the pyloric antrum and probably the distal corpus. The only other vessel mentioned by all authors is one running along, and supplying, the greater curvature, which Guth specifies as the right gastroepiploic artery, a branch of the gastroduodenal artery and hence of the common hepatic artery. The left gastric and right gastroepiploic appear to be the two main arteries. There is probably some supply from a lesser curvature branch of the common hepatic artery (right gastric) and from branches of the splenic artery (left gastroepiploic and perhaps short gastric arteries), but this appears to be relatively unimportant. The proximal duodenum is supplied by the superior pancreaticoduodenal artery, a branch of the gastroduodenal. The supply to the distal oesophagus is less clear, but is probably from the left gastric artery with perhaps a branch from the hepatic artery in some cases. However, the more detailed distribution of these branches of the coeliac artery is not subject to a consensus and, owing to often inadequate technical information, It is not possible to assess the validity of these variations. In addition, no quantitative studies on blood flow have been done and all judgements have been based upon subjective estimates of indirect parameters, such as vessel size and degree of filling by injectate. it is, therefore, difficult to compare the results obtained by different authors.

Only Guth, Berg and Schnitzlein discuss the extent of the anastomoses between the extramural arteries of the stomach. Berg's results suggest their total absence and are opposed to Schnitzlein's, in which anastomoses are described at several sites. Guth's work shows only that some collateral pathways do exist, but not at which level they are to be found. It seems probable that such channels do exist between the intramural vessels and probably between the extramural arteries along the lesser curvature, although they cannot totally compensate for an occluded major supply vessel. Any anastomosis which is present between extramural vessels on the greater curvature is probably of no significance.

In summary, the principal features of the anatomy of the extramural arteries supplying the stomach of the rat are clear. Nevertheless, a more detailed study of the branches of the coeliac artery, particularly the right gastric and left gastroepiploic arteries, and their common variants, is required, as is further work on the patterns of flow within these vessels and the extent and location of anastomoses between them.

#### INTRAMURAL VASCULATURE

### **Description**

The first report in the literature appears to be that of Berg (1946). He examined stomachs which had been injected in vivo by a contrast medium as both cleared and histological specimens. He showed that the branches of the extramural arteries pierced the serosa and muscularis externa to form a plexus in the submucosa. They gave rise, en route, to a diffuse subserosal plexus and to a capillary net within the muscularis. The vessels in the latter ran parallel to the long axes of the smooth muscle fibres, which were aligned, and formed long anastomosing rectangular loops. The submucous plexus contributed recurrent branches to the muscularis. It also gave rise to perpendicular mucosal arteries which penetrated the muscularis mucosae and did not anastomose with one another. They passed directly to, and ended in, the capillary plexuses of the mucosa. These plexuses surrounded the bases and openings of the mucosal glands and, in the fundus, their mid-points. They

intercommunicated perpendicularly and were continuous in a plane parallel to the mucosal surface. They drained into perpendicular collecting veins which were tributaries of the submucous venous plexus. No mucosal venous plexus was described.

Berg showed that the rumen, fundus and antrum were dissimilar. The rumen had a very poor blood supply, the surface keratinized epithelium appearing avascular. Only one capillary plexus plane was visible in the deeper mucosa. He estimated that the fundus was more deeply injected than the antrum. It contained more collecting veins than the antrum and its capillary plexuses were smaller and more numerous.

The principal limitation of Berg's work arises from the absence of quantitative data. He does not state the number of animals or the proportion of the total area of each stomach that he studied. These make it difficult to assess the probability that his results are valid. Unfortunately, his observations do not unequivocally demonstrate at what level in the mucosa the mucosal arteries terminate, branch and form capillaries. This is a point of contention.

A series of experiments to investigate the interrelationship of pilocarpine and the occurence of gastric ulceration were published by Basu Mallik in 1955. He studied the microvascular anatomy of the normal stomach in the course of this work, using injection techniques and in vivo transillumination. The injected specimens were examined after clearing, histologically or as  $1 \text{ cm}^2$  blocks.

He distinguished between the results from the injection and transillumination studies. In the former, he observed the branches of the extramural arteries running for a short course through the serosa, then penetrating the muscularis externa to form an arterial plexus in the submucosa. Perpendicular mucosal arteries arose from this plexus to pierce the muscularis mucosae and enter the mucosa. At the base of this layer each artery formed three or four capillaries which ascended perpendicularly to the mucosal surface where they formed loops around the openings of the glands. Adjacent capillaries were linked by transverse

ï

vessels. Unfortunately, the author does not make clear whether capillaries originating from different mucosal arteries were linked to form a continuous plexus, or only those from the same artery. The surface loops drained into perpendicular mucosal veins which were tributaries of the submucous venous plexus. The antrum and the forestomach were less well filled than the corpus.

Basu Mallik's transillumination studies confirmed this description but, in addition, he saw a 'few' arteriovenous anastomoses in the submucosa, although these were normally closed unless the animal had passed into a state of collapse. Likewise, the mucosal arteries were often in a collapsed state, only a proportion being patent at a given time. It was found that opening the stomach along the greater curvature in an attempt to directly observe the mucosal circulation, inevitably resulted in almost complete vascular stasis. The subcutaneous administration of pilocarpine resulted in vigorous peristalsis which compressed the vessels passing through the muscularis, although Basu Mallik does not describe how this affected the blood flow in these vessels. Owing to the respiratory effects of this drug, it was necessary to intubate and artificially ventilate the animals to which it was given.

Certain aspects of the author's technique merit comment. He does not disclose how many animals were used nor what proportion of the stomach was examined. The pilocarpine dosage is unstated, although analogy with a previous experiment suggests that it might have been 80 mg kg<sup>-1</sup>. The use of artificial ventilation in some of the animals would have introduced a number of variables, in contrast to the unventilated rats, owing to unmonitored and uncontrolled variations in blood gas composition and pH, which might affect the vessels (Wiegman 1975).

Only limited reliance may be placed upon Basu Mallik's identification of arteriovenous anastomoses. These were only visible in the transilluminated specimens and were 'few' in number. Not only are the criteria by which they were identified unstated, but also no steps appear to have been

taken to eliminate overlapping vessels, such as varying the angles of illumination and of view, or observing the directions of flow of blood or of injected contrast medium. The necessity for this has been pointed out by both Guth (1972) and Schnitzlein (1957). It is hard to accept that the author could classify with certainty a vessel which was not stained and which, in many cases, contained no blood. His difficulty must have been compounded by the poor resolution which would have been obtained in the submucous plane, since this was observed through the overlying serosa and muscularis.

Schnitzlein (1957) studied the effect of a number of drugs (atropine, pilocarpine, ergotoxine & acetylcholine) and of vagal stimulation and section, on the gastric vasculature of the rat. He also examined the innervation of these vessels. As a basis for comparison, he also studied the gastric anatomy of the normal rat. He rapidly froze the stomach and duodenum of an unspecified number of previously starved normal animals by the in situ application of liquid nitrogen (Persson 1952). Serial sections of the stomach were cut and were stained with haematoxylin and eosin.

The author concentrated upon specific aspects of the microcirculation rather than attempting to give a comprehensive description. He noted the presence of recurrent branches from the submucous arterial plexus to the muscularis externa, although these were 'few' in number. He pointed out that all vessels passing through this layer did so obliquely and suggested that this might be of significance in reducing the muscle's sphincteric action. By contrast, the more thinnly walled mucosal arterioles, which arose from the mucosal plexus, passed through the muscularis mucosae perpendicularly, before branching to form a capillary bed at the base of the mucosa. He noted that only those vessels nearest to the muscularis mucosae had smooth muscle cells in their walls. The collecting veins drained into the venous submucous plexus. Frequent sacculations were seen in this network but they did not appear to contain valves. In several of his sections, Schnitzlein observed

5

cells in the walls of the blood vessels which appeared to be epithelioid. However, adjacent serial sections demonstrated that these were only smooth muscle cells cut in transverse section, located in small arteries or arterioles in the submucous plexus. Such epithelioid cells ("Quellzellen") are a feature of arteriovenous anastomoses, first described by Havlicek (1934).

Schnitzlein's contribution to the literature is small, being primarily limited to his evidence on the absence of arteriovenous anastomoses and on the depth at which the mucosal arterioles end in the mucosa, which is of value. Since he described his diagnostic criteria, and applied them by histological examination, his results are probably valid.

The role of the sympathetic nervous system in the control of the gastric mucosal blood flow, and the spatial variations in this flow, were investigated by Arabehety (1959). He injected india ink into the aortas of fasted and anaesthetised rats, after subjecting them to one of three procedures; laparotomy alone (7), laparotomy plus splanchnic ganglion anaesthesia (5) or splanchnic section by bilateral section of the greater splanchnic nerves and removal of the coeliac ganglion (3). A further two rats were injected post mortem. The stomachs were removed, fixed, dehydrated, sectioned and cleared. Unfortunately, he did not study the vascular patterns but only measured the optical densities of different areas of the mucosa. These were compared with a 'tissue blank' of readings of the comparatively avascular muscularis externa. The blood flow was taken to be proportional to the difference between the two readings.

Arabehety found that the submucous plexus was uniformly injected as was the fundic mucosa, but that the antral mucosa was unevenly filled. This paper's contribution is limited since the author provides little information. Although he invokes submucosal arteriovenous anastomoses to explain his results, he provides no evidence to support his hypothesis.

One of the most frequently cited papers in this field was published by Nylander & Olerud (1960; 1961), who studied

1

. .

the mucosal microcirculation in normal and vagotomised rats. 16 animals were studied (12 vagotomised and 4 controls) by injection of a contrast medium and subsequent clearing. The erythrocyte haemoglobin was stained by immersion in benzidine alcohol. In the controls, the authors observed irregular mucosal filling, comprising uniformly filled regions interspersed with numerous poorly injected areas. In the vagotomised rats, the entire mucosa was sparsely filled. Microscopic examination of the homogeneous regions, in thin sections, showed that the mucosa contained a large number of coiling mucosal arterioles, passing from the base to the Between these was a dense honeycombe surface. of capillaries. Few venules were seen. By contrast, the poorly injected areas contained fewer arterioles and a sparser capillary net, but many more collecting venules. The latter, which ran perpendicularly through the mucosa, drained a venous plexus on the mucosal surface. Delineating the periphery of the poorly injected areas was a dense mesh of mucosal arterioles. In the vagotomised animals only, the authors noted that this venous plexus on the surface of the mucosa was fed directly by the mucosal arterioles, with no intermediate capillary bed i.e. it constituted a system of mucosal arteriovenous communications. Unfortunately, they do not mention whether this was present in the control rats. No such channels could be found in the submucosa.

Although this work is frequently quoted, particularly as evidence for the existence of arteriovenous anastomoses, the results are of limited value. The report of the arteriovenous channels in the vagotomised animals is of diminished significance owing to the uncertainty regarding their presence in the controls, which were too few to enable any firm conclusions to be drawn. The authors support the validity of their observations of mucosal variations by the claim that their combination of techniques visualises the microcirculation either entire by filling the microcirculation or by staining the remaining erythrocytes' haemoglobin. However, this does not exclude the presence of vascular channels which are devoid of red cells and unfilled

by injectate. The apparent paucity of mucosal arterioles and capillaries in the poorly filled areas might be a result of their non-patency at the time of injection and death, and their consequent non-visualisation. Nylander & Olerud are the only workers to describe a surface venous plexus, but they only report it in pathological specimens (vagotomised) or regions (ischaemic).

In summary, it would seem that the case for the existence of mucosal arteriovenous channels and a surface venous plexus, which contradicts with the results of every other worker, is inadequately supported by the authors' evidence.

In 1970, Oka published the results of an investigation of the gastroduodenal microcirculation of normal rats and of those suffering from serotonin induced ulcers. This is of limited value as few technical details are given. Oka only states that the stomachs and duodena of an unspecified number of rats were subjected to in vivo transillumination or to india ink injection, followed in the latter case by clearing.

The mucosal arterioles displayed two branching patterns. In both cases, the parent artery arose from the submucous plexus and pierced the muscularis mucosae, but then it either branched immediately or, turning through 90°, ran parallel to the muscularis mucosae for a short distance before branching. The branches coursed perpendicularly to the mucosal surface and gave rise, throughout their course to a dense capillary network which filled the space between them. This textual description contradicts the accompanying diagram, which shows the mucosal arterioles forming capillaries and ending at the base of the mucosa. Oka also reported that he saw arteriovenous anastomoses 15 to 30 um in diameter in both the submucous plexus and in he muscularis. he does not describe them in the mucosa. They often displayed a sacculated appearance.

In the absence of adequate technical information, little can be said about the circumstances under which the above patterns are demonstrable. Likewise, the description

of the AVA's appears unsatisfactory. No histological studies were carried out and it is unknown by what diagnostic criteria they were identified. It is unclear whether they were visible in the transilluminated preparation, in the injected specimens or in both. the photographs are inconclusive. Thus, on the basis of the published information, it is impossible to assess the validity f these results.

The results of a minor study of the venous drainage of the antrum and corpus were reported by Hubens (1971). he removed the stomachs of 20 unstarved rats and immersed them in benzidine alcohol to stain the haemoglobin of the erythrocytes. The specimens were fixed and cleared, then examined by transillumination as whole mounts. Segments of the antrum and corpus were removed and examined histologically.

In the corpus Hubens observed a dense capillary plexus which drained into perpendicular collecting veins by means of a tree like network of secondary venous tributaries. These collecting veins drained into the submucous venous plexus. In the antrum, however, the collecting veins were absent, the capillary plexus draining into a venous net at the base of the mucosa, which ramified parallel to the mucosal surface. This was supplied by secondary venous tributaries and itself entered the submucous plexus.

This paper presents results which appear to be reliable. The use of benzidine alcohol to display the microcirculation is subject to modification by physiological factors (see above). The demonstration of a venous plexus in the antrum, which runs parallel to the mucosal surface, may be of relevance to Taylor & Torrance's hypothesis of an antrum corpus portal system (see below).

The first comprehensive descriptions based solely upon in vivo investigation were presented by Guth et al. (1970; 1972; 1975). He used two preparations, the exteriorised stomach and the everted pouch. In the first, the stomach was brought out of the abdomen, irrigated with warm Ringergelatine and a quartz light guide inserted via a hole in the

N 11 H 1

duodenum. The stomach was distended by the Ringer-gelatine. A small window of serosa and muscularis externa was removed to allow the submucosa and mucosa to be seen more clearly. This area was then transilluminated. Observations were restricted to the glandular stomach.

The second preparation was designed to improve the visualisation of the mucosa. The duodenum was transected after the second part and the proximal end was drawn through an incision in the rumen, after separating it from the gastroduodenal artery. The cut edge was tied and a light guide was inserted into the pouch so formed.

Guth's in vivo results largely confirmed the descriptions of the previous authors, which had been based upon the investigation of post mortem material. Branches of the gastroepiploic and left gastric arteries pierced the muscularis, within which they gave off branches, 25-35um in diameter, which ran transversely to the long axis of the stomach. These, in turn, gave rise to a superficial and a deep capillary plexus. There were few inter-plexus anastomoses. Within a plexus, the capillaries took parallel paths, 40-80um apart. The venus system was the inverse of the arterial.

The vessels left the muscularis, after giving off the muscular nutritive branches, and entered the submucosa. Here a large number of smaller branches were formed, 35-58um in diameter, which anastomosed among themselves, forming a primary submucous plexus. A secondary anastomotic arcade, comprising vessels 21-41um in diameter, 'filled in' the primary plexus. The venous arrangement mirrored the arterial, the veins at each level being larger than the corresponding arteries. Guth noted that a continuous network was formed in the submucosa on the anterior wall and the greater curvature of the glandular stomach whereas, in the region of the lesser curvature, the arterial and venous plexuses were less extensive. Their branches, which passed to the mucosa, made few arterioarterial or venovenous anastomoses at any level. The posterior wall was not studied. ÷

Mucosal arteries arose from the secondary vessels of the submucous plexus and entered the mucosa by penetrating the muscularis mucosae perpendicularly. In the base of the mucosa, each of these arteries terminated by branching to form three or four vessels, each of which gave rise to three to six capillaries. These ran perpendicularly into the mucosa forming a hexagonal network around the glands. They drained into mucosal veins, which were also perpendicular and which were tributaries of the submucous venous plexus. Arterioarterial and venovenous anastomoses were seen between the mucosal arteries and between the mucosal veins.

Guth was unable to find any arteriovenous anastomoses at any level of the stomach wall. Apparent examples were eliminated by altering the angle of view or of illumination and by observing the pattern of blood flow or of injected contrast contrast media. He did describe thoroughfare channels (Zweifach & Chambers 1946; Zweifach 1957) in the muscularis externa. These structures extended from a metarteriole to a venule and contained groups of smooth muscle cells in their walls. However, they were components of a vascular bed and themselves gave rise to capillaries. They were therefore not true arteriovenous anastomoses.

In vivo observation of the microcirculation only the vascular channels which demonstrates are functioning at that time and not the total number of anatomical vessels. Thus, any factor affecting blood flow may modify the observed anatomy. It is true that Basu Mallik (1955) claimed that he could see close vessels in his living transilluminated specimens, but he provided no evidence or photographs to support this contention. Guth's experimental rats differed from intact rats in several ways which could affect the observed anatomy. These were factors such as surgical shock, the cardiovascular effects of succinylcholine (Goodman & Gillmann 1965), blood composition variations as a result of uncontrolled artificial ventilation (Wiegman 1975), and the deprivation of the submucous plexus under the window of its superficial inputs and outputs.

1

In the previously described exteriorized stomach preparation, Guth studied the glandular stomach. The description of his eversion preparation is ambiguous, but section of duodenal that а mucosa implies was transilluminated, rather than gastric. If this was so, then combining the two sets of observations into a single model is invalid. Also, as it was necessary to separate the gastroduodenal artery from its intramural duodenal branches, the flow characteristics of the area would have been significantly altered. This technique did not permit observation of the posterior wall.

Guth's inability to find any arteriovenous anastomoses cannot be accorded the same degree of significance as the positive results of some other workers., particularly since he does not give any quantitative information. However, his observations of possible examples and their subsequent rejection by the application of further diagnostic criteria suggests that had any AVA's been present and patent, he would have seen them. Several workers have shown that injection of the stomach in Man (Barclay & Bentley 1949a; 1949b; Bentley 1949) and of the rat (Arabehety 1959) at operation produces less mucosal filling than results from injection post mortem. This has been interpreted as showing that submucosal AVA's are open in the living surgicallyshocked animal. These factors increase the weight carried by Guth's results.

Kobayashi(1973) subjected twenty rats to double dye injection in vivo, and cleared 1 cm squares of tissue. His work was designed only as a study of gastroduodenal AVA's. He described three types of AVA, based upon a structural  $(T_a(He))$  classification, All were found in the submucosa. However, the quoted technical details leave several unanswered questions. The nature of the injectate, the injection technique and the mode of killing are not stated. The value of the detailed quantitative results is open to doubt as Kobayashi did not describe the premises upon which he based his identification of the AVA's. The photographs do not unequivocally demonstrate the nature of the structures in question and the methods of examination are not mentioned.

The gastric mucosa was also investigated by Hase & Moss (1973). They injected four rats with ink in vivo, and four with silicone rubber immediately post mortem. 24 further rats were similarly injected after rotational stress for 2, 4 or 6 hours. The specimens were cleared and examined as whole mounts or 50 - 100um sections. The authors confined their work to the glandular stomach. They described the submucous plexus, which was formed from the branches of the extramural arteries, and from which arose perpendicular mucosal arteries ('connecting arterioles'). These penetrated the muscularis mucosae and branched immediately at the base of the mucosa to form the capillary network which filled this layer The capillaries drained into the perpendicular collecting veins which were the tributaries of the venous submucous plexus. A venous plexus at the surface of the mucosa was not described.

	RUMEN	CORPUS	ANTRUM
NUMBER OF AVA's	96	60	46
AVA DENSITY $(cm^{-2})$	30	19	14
AVA LENGTH (um)	564-1058	384-797	426-630
MINIMUM BORE (um)	10.7-12.3	10.0-13.2	10.0-12.2
AVERAGE ART. BORE (um)	22.2 - 26.9		
AVERAGE CAP. BORE (um)	6.0 - 9.6		
AVERAGE VEN. BORE (um)	24.8 - 36.4		

<u>Table 3 Physical Parameters of Gastric Arteriovenous</u> <u>Anastomoses (AVA's) - From Kobayashi (1975)</u>

The authors described AVA's in the submucosa, 20-40um in diameter in all the groups. Their diagnostic criterion appeared to be the presence of channels joining vessels injected with different colours of rubber, the colours mixing in the channel or with ink from arterial and venous sources. These were usually found where the artery and the vein pierced the muscle together (m. externa or m. mucosae is not stated). They were particularly distended in ischaemic areas, consequent upon 2 hours rotational stress. This was only stated in connection with the four silicone rubber injected rats. The antral pattern was essentially the same as the corpus, other than for the presence of a shorter coarser capillary network in the former.

The significance of the above results is limited due to number of control animals studied. the small The observations from each injection preparation cannot be combined into a single model, but must be considered separately, as there is no proof that the two sets of results are comparable. Unfortunately this cannot be done as the authors do not correlate observations and techniques. In addition, some aspects of their techniques require comment. The use of ether to kill the ink injected rats is slow and could lead to ante mortem circulatory changes which would modify the observed vascular patterns. Although Hase & Moss describe their criterion for identifying AVA's this does not appear to be diagnostic as it cannot be assumed that vessels filled with yellow rubber are necessarily arteries or those red, veins. Such identifications can only with be provisional in the absence of histological investigation. The photographs which are presented are inconclusive as it is not possible to differentiate arteries and veins or overlapping and intercommunicating vessels. The authors' identification of mucosal arteries and capillaries is likewise based upon unstated premises, their sizes and histological characteristics being unknown. In conclusion, it is worthy of note that the AVA's were more prominently associated with pathological ischaemic areas than with normal tissues. This finding agrees with those of other

authors.

Kawarda et al. studied 70 starved rats, subjected to cold and immobilisation, with a control group of five normal animals (1975a; 1975b). This injection study demonstrated AVA's in the experimental group where "arteries and veins penetrate the muscle coat". Most of this group showed gastric ulceration and mucosal lesions. There are several limitations to this study. The AVA's were only reported in an experimental group which' showed marked gastric pathological changes. Their presence is not mentioned in the small number of normal rats or other experimental groups. There is no indication of the tissue processing techniques or observational methods or of the AVA characteristics. In particular, the gastric layer in which they were found is unclear. These factors limit the value of the report.

Gannon et al. (1982) have reported extensive examinations of the mucosal vasculature, based largely upon scanning electron microscopy of vascular casts. They confirmed Schnitzlein's finding that the mucosal arteries formed capillaries at the base of the mucosa. Despite careful searching and defined criteria, they were unable to find any arteriovenous anastomoses, although they did report arterioarterial anastomoses and small (< 10um) arteriovenous communications "which were not arteriovenous anastomoses".

### The Duodenum

There has been no specific study of the intramural microcirculation of the rat duodenum. Oka (1970) and Kobayashi (1973) incorporated some observations upon the duodenum. Oka contrasted the densely distributed villi and the thick mucosa of the duodenum with the converse in the jejunal/ileal area. He described three types of capillary circulation in the duodenal villi. A central arterial input might have peripheral or 'tree like' venous drainage, or a peripheral arterial input would have central venous drainage. The second was the most common. He also described the villi as "dam shaped' i.e. crescentic, with the base wider than the height. This is consistent with Spanner's description (1932) of a fountain pattern for the jejunal villous vasculature.

Kobayashi claimed to have found 0-2 AVA's at the duodenal villous tips (0-0.19 AVA  $mm^{-2}$ ). These were slightly smaller (10-11um) than those found in the stomach, compared with average diameters of 16.6, 33.4 and 5.1um for the arterioles, venules and capillaries respectively. This too agrees with Spanner's findings of direct arteriovenous communications at the tip of the jejunal villi.

The remaining reports in the literature either describe the vascular architecture of a part of the small intestine other than the duodenum (Spanner 1932; Baez 1959; Mohuiddin 1966), or appear to regard the small intestine as homogeneous and do not specify which part was studied (Miller 1967). Since there is no evidence to show that this region is uniform, these results are beyond the scope of this review of the duodenum and will be described in outline only.

Spanner, Baez and Mohuiddin described the duodenum, using injection, transillumination and histological techniques respectively. All found a fountain pattern in the villi, with a central arteriole, peripheral vein(s) and an intervening capillary plexus. Only Spanner reported the presence of direct arteriovenous communications which were located at the tips of the villi. However, Baez did find thoroughfare channels at the base of the mucosa and in the mesenteric region of the serosa. Miller's results, based upon injection techniques, also indicated a fountain pattern in the villi, i.e. a central paraxial artery and two peripheral marginal veins with an intervening capillary net. In some cases, venous escape of injectate preceded significant mucosal filling. The author suggested that this could indicate the presence of AVA's, although there was no anatomical evidence to support this.

## Physiological Studies

A number of investigations using radioactive tracer techniques have been conducted by Taylor & Torrance, which

reveal anatomical correlates (1974; 1975a-c). The authors suggest that their work demonstrates a direct transfer of tracer from the antrum to the body of the stomach, independent of the systemic circulation but dependent upon perfusion of the stomach and upon the integrity of the antral/body junction. They hypothesize that this transfer may be mediated by an anatomical vascular portal system.

Their data does appear to support this conclusion. The degree of transfer may have been actually underestimated, since Taylor & Torrance calculated total activity rather than activity per gram of tissue. In the 1976 paper, this inaccuracy was ammended.

### <u>Conclusions</u>

The preceding works give a generally consistent account of the microcirculatory anatomy of the stomach and duodenum of the rat, although they do differ in some respects. In some cases, it is not possible to assess the differences as the evidence is inconclusive.

Only two authors (Berg 1946; Guth 1972) described the serosa and muscularis in any detail, but their accounts are compatible. Additional isolated facets were contributed by Schnitzlein (1957) and Basu Mallik (1955). The cells of the serosa and muscularis externa are supplied with blood by two independent capillary plexuses, one subserosal and the other in the muscularis. These are fed by branches of the arteries penetrating the muscularis and by recurrent twigs from the submucous plexus.

All the workers agreed that the branches from the extramural arteries anastomose in the submucosa to form an arterial plexus. They give rise to vessels supplying the muscularis externa and the mucosa. According to Guth (1972), this plexus was less dense on the lesser curvature than on the anterior wall or the greater curvature.

There is disagreement between authors on certain aspects of the mucosal arterial vasculature. According to Gannon (1982), Guth (1972), Basu Mallik (1955), Schnitzlein (1957) and Hase & Moss (1973), the mucosal arteries from the submucous plexus do not penetrate far into the mucosa but branch near the base to form the periglandular capillary plexus. However Nylander & Olerud (1961) and Oka (1970) maintained that the mucosal arteries reached the epithelial surface giving off the components of the capillary plexus throughout their course.

The origins of these inconsistent results may lie in the variations in technique and hence available diagnostic criteria used by the different authors. Only Schnitzlein defined the characteristics that he used to identify the mucosal arteries and capillaries and only he examined the stomach histologically. All of the results of the other works are derived from injection and/or transillumination techniques. The identification parameters are not given, but the authors can only have made a decision based upon vessel size since the vessel walls were unstained. Thus the weight of defined evidence supports the view that the mucosal arteries branch at the base of the mucosa and do not penetrate beyond this level.

In contrast to the mucosal arterial system, there is general agreement regarding the venous drainage. This is effected by perpendicular mucosal veins which are tributaries of the submucosal venous plexus. These collecting veins are fed by the capillary loops at the surface of the mucosa and/or by the capillary plexus(es) in the body of the layer. Only Nylander & Olerud's observations of a surface venous plexus contradict this model but, as these were based upon only the poorly injected regions of four specimens and used undefined diagnostic criteria, they are of lesser value. Berg emphasizes that there was no plexus formed between the perpendicular mucosal veins whereas Guth describes extensive veno-venous anastomoses between them. The descriptions of the other authors are consistent with Berg.

Two types of variation in mucosal filling by injectate were noted, regular (Berg 1946; Basu Mallik 1955; Arabehety 1959; Hase & Moss 1973) and irregular (Nylander & Olerud 1961). Microscopic examination showed quantitative

variations in the proportions of vascular components present in the differingly filled mucosae, but no qualitative pattern differences. Oka (1970) and Hubens (1971) did show such qualitative differences but their work was poorly documented. The correlation between injection density and microvascular pattern was not the same in the animals showing regular, and those showing irregular, variations. However, these may represent two phenomena and so not be comparable. It was generally agreed that the rumen was less deeply injected than the antrum, which was less deeply injected than the fundus.

The venous system distal to the mucosa closely mirrors the arterial pattern although the veins at all levels are larger than the corresponding arteries. Sacculations in the submucous plexus were only reported by Schnitzlein.

The presence of arteriovenous anastomoses is a major contention. The evidence is solely anatomical. Several authors (Basu Mallik 1955; Nylander & Olerud 1961; Oka 1970; Kobayashi 1973; Hase & Moss 1973; Kawarda 1975a, 1975b; Lakhtina & Kozlov 1975) reported that they did find them in the submucosa, except Nylander & Olerud who found them only in the mucosa and Oka who also saw them in the muscularis. Conversely three authors (Schnitzlein 1957; Guth 1972; Gannon 1982) were unable to find them after a specific search. Their studies defined their identification criteria, did not use pathological material and utilised a number of examination techniques. It is noteworthy that both Schnitzlein and Guth observed AVA-like structures which were subsequently rejected after the application of further defined criteria.

From the literature it is not possible to give an account of the duodenal microcirculation. A few details of villous capillary circulation and AVA occurence are given by Oka (1970) and Kobayashi (1973) respectively, bit no other works are specific to this regior.

The overall model of the gastric microcirculation in the rat is, therefore, detailed and agreed. However, a number of points await clarification, principally the

occurence of AVA's, the spatial variations in anastomotic density of the submucous arterial plexus and the mucosal angioarchitecture. An anatomical explanation for Taylor & Torrance's portal system hypothesis is also necessary. No mucosal arteries of extramural origin have been described. A detailed study of the duodenum, with particular reference to these features is also necessary since few facts specific to this region are available.

# CHAPTER 10 THE DOG

### NON-VASCULAR GASTRODUODENAL ANATOMY

The dog has been included in a number of anatomy texts, notably those of Ellenberger & Baum (1943) and Sisson (1953) and in the general histology text of Trautman & Fiebiger (1957). Additionally, the anatomy of the dog and cat was described in detail by Bourdelle & Bressou (1953). Several other authors have confined their attention to particular aspects of the canine foregut. Mall (1896) gave a general account of the stomach, with particular emphasis on its vascular system. The gastro - oesophageal junction was examined by Botha (1958a; 1958b; 1962), El-Ramli (1964) and Fatani (1971) and histological aspects of the gastric mucosa by Bensley (1902), Oi et al. (1958) and Solcia et al. (1969). Weissflog (1903) described the gastric musculature whilst the duodenum was examined by Villemin (1922) and Carleton (1935).

# External Form

The abdominal course of the oesophagus is very short, no more than a few millimetres, and deviates to the left. According to Botha (1958a; 1952), there was no visible change in its diameter where it joined the stomach, nor was there any palpable muscular thickening. Conversely, El-Ramli (1964) did feel such a thickening. The incisura cardiaca varied from  $0^{\circ}$  to  $90^{\circ}$ , depending on the volume of the gastric contents, but was generally very acute.

The piriform corpus and fundus are divided from the cylindrical pyloric antrum by a sharp incisura angularis. The weight of the stomach was reported by Bourdelle & Bressou (1953) to vary from 0.065-0.251 kg (equivalent to 0.62-1.39% of the body weight), whereas Delaney & Grim (1964) reported values of 0.057-0.137 kg (equivalent to 0.65% of the body weight). The gastric and body weights were poorly correlated (r = 0.55). Delaney & Grim also noted that the antrum comprised 40% of the gastric weight and that, in

the corpus, the relative weights of the tissue layers were mucosa (47%); submucosa (18%); muscularis + serosa (35%). Mall (1896) found that, the fundus was most distensible (1226% of its size when empty) compared with 22% and 188% for the corpus and antrum respectively. Bourdelle & Bressou found the volume varied between 0.6-8.0 1 in different animals, with a mean of 3.0 1, the latter value also being quoted by Sisson (1953).

The duodenum is a single loop, open to the left, with a long descending limb and a shorter ascending limb. Villemin (1922), but no other author, described an initial dilatation. The bile and pancreatic ducts enter separately, often with an accessory pancreatic duct between them. According to Villemin, the two main ducts entered just after the pylorus, whereas, according to Carleton (1935), the bile duct entered 3.0-4.5 cm from the pylorus, with the pancreatic duct 3.0 cm beyond it, whilst Sisson's values were 5.0-8.0 cm and 2.5-5.0 cm respectively.

### <u>Mucosal</u> Folds

Bourdelle & Bressou noted folding in the oesophagus which, Fatani (1971) added, was longitudinal. Bourdelle & Bressou also described a group of longitudinal folds in the stomach along and adjacent to the greater curvature, which disappeared orally and aborally. There are also specialised folds around the cardia and, according to Mall, at the pylorus which are constant in location and occurence and which probably have a sphincteric function.

# Gastro-oesophageal Junction

The oesophagus enters the stomach with no dilatation or narrowing in its terminal segment. Botha (1958a; 1962) was unable to find any muscular thickening at the cardia and although Fatani found a slight thickening of the muscularis mucosae at this point, he stated that there was no sphincter. Conversely, El-Ramli (1964) was able to palpate a thickening at the cardia in 14 dogs which histological examination (in one dog) showed to be due to a smooth muscle sphincter. In the living or recently killed animal, Bourdelle & Bressou noted the presence of irregular short folds around the cardia which were erased by mucosal tension, leaving only a regular mucosal rosette around the oesophageal aperture, coincident with the incisura cardiaca which, they suggested, might be raised by the gastric sling fibres.

### Epithelial Lining

The oesophagus is lined by a stratified squamous keratinized epithelium, beneath which are a large number of submucosal tubulo-alveolar mucous glands. There is an abrupt transition between the oesophageal and gastric mucosae, occuring at, or immediately distal to, the cardia, so that the stomach is entirely lined by a glandular epithelium. Mall estimated there to be approximately one million gastric glands, the density decreasing from rostral to caudal. However, this was based upon samples taken only from a longitudinal strip of the anterior wall. The stomach is divided into three regions, the cardiac, fundic and pyloric gland areas. The cardiac zone forms a narrow collar around the oesophageal aperture with, according to Sisson (1953), isolated glands scattered along the lesser curvature. The cardiac gland bodies contain cells which stain as fundic parietal cells, whereas the cells lining the pits into which the glands drain are mucin secreting (Trautman & Fiebiger 1957). However, Trautman & Fiebiger observed transitional gland types in this area, with and without mucous, parietal and chief cells. Both Trautman & Fiebiger and Ellenberger & Baum (1943) agreed that the fundic gland area (rostral two thirds of the stomach) was bipartite, comprising a smaller thin light region orally and a larger thicker dark region aborally. The aboral region contains true fundic glands, with mucous cells and a few parietal cells in the gland neck, and chief and parietal cells in the body. The parietal cells were often separated from the lumen by a few cell layers. Between the glandular bases and the muscularis mucosae are, superficially, a collagenous stratum compactum and, deeply, a cell rich stratum granulosum, the two together composing the lamina subglandularis. In the oral region however, the glands are shorter and more tortuous and are distributed in groups. There is a deficiency of parietal cells, relative to other cell types, and the stratum compactum is absent. The pyloric glands contain mucous cells, as found in the necks of the fundic glands.

The distribution of parietal cells was studied in ten dogs by Oi et al. (1958) who removed  $48 \times 0.02 \text{ cm}^2$  samples from the stomach of each. They found the greatest concentration of parietal cells in the corpus on the greater curvature, decreasing rostrally and caudally. In a single gland, the concentration decreased towards the base, implying a maximum in the neck, in contrast to other authors' results. However, Oi et al. examined only approximately 0.006% of the total gastric area, which casts doubt on the validity of their distribution patterns.

Solcia et al. (1969) found several types of cell with a probable, though often unspecified, endocrine function in the canine gastric mucosa. Gastrin secretory cells were found in the middle and deep thirds of the pyloric mucosa.

Villemin (1922) found that the gastroduodenal mucosal interface was slightly caudal to the pyloric sphincter i.e. the first centimetre of the duodenum contained pyloric mucosa. He cites Kuczynski(1890) who also stated that the gastroduodenal junction was unclear. Carleton (1935), Villemin, Sisson and Trautman & Fiebiger were in agreement that the Brunner's glands , found in two layers, deep and superficial to the muscularis mucosae, disappeared within 2cm of the pylorus, at or before the entrance of the bile duct. The mucosa is initially smooth, the villi increasing in length distally. The duodenum also contained intestinal glands but, according to Trautman & Fiebiger, Paneth cells were absent.

# <u>Musculature</u>

The oesophageal musculature comprises two striated layers, initially opposite spirals, which become an inner

circular and an outer longitudinal layer near the cardia. According to Botha (1958b; 1962), there is a sudden transition of striated to smooth circular muscle just above the cardia whereas the change in the longitudinal muscle is more gradual, with some striated fibres extending onto the stomach. The muscularis mucosae is also orientated longitudinally but is absent in the first half of the oesophagus and only becomes continuous near the cardia. There is disagreement concerning the presence of a sphincter at the gastro-oesophageal junction (v.s.).

The gastric musculature has been described by several authors, although some appear to have based their accounts upon that of Ellenberger & Baum. The thickness of the wall increases from 1.94mm at the cardia to 3.38mm at the pylorus. This was associated with an increase in the thickness of the muscular component from 0.83mm to 1.80mm. The outer layer of the muscle is a continuation of the oesophageal longitudinal muscle but is incomplete. There are longitudinal bundles along the greater curvature and the right lesser curvature which are united by longitudinal bands at the pylorus. There are also oblique fibres over the fundus, radiating from the cardia. The middle circular layer, which is continuous with the oesophageal circular layer, is complete and gives rise to two pyloric sphincters, one proximal to the pylorus and one at the pylorus, which intersect on the lesser curvature. The internal oblique layer is incomplete, forming well developed sling fibres around the oesophageal aperture as well as contributing to the wall of the corpus. There appears to be no information available on the duodenal musculature in the dog.

## <u>Conclusions</u>

The very short abdominal oesophagus joins the stomach with no change in its diameter, forming a very acute incisura cardiaca. The piriform fundus and corpus are separated from the cylindrical pyloric antrum by a sharp incisura angularis. The duodenum forms a single loop, into which the bile and pancreatic ducts enter separately, often

accompanied by an intermediate accessory pancreatic duct. Both entry points are within 13cm of the pylorus but their precise positions are disputed and cannot be stated on the basis of the present evidence.

There is conflicting, but apparently valid, evidence concerning the presence of a sphincter at the gastrooesophageal junction. Further work is required to elucidate this point, although the weight of evidence favours the absence of such a structure. It is accepted that there is a mobile occlusive mucosal rosette around the oesophageal aperture and, to its left, a mucosal fold, raised by the well developed sling fibres around the cardia. There are also longitudinal folds in the terminal oesophagus and along the greater curvature and a fold around the pylorus.

The oesophagus is lined by a muco-secretory keratinized epithelium whereas the gastric mucosa is entirely nonkeratinized. The latter is divided into a narrow cardiac zone around the cardia, a bipartite fundic zone occupying the rostral two thirds of the stomach, and a caudal pyloric gland area. The cardiac glands contain mainly mucous cells, with some parietal-like cells in the gland body. The fundic glands contain mucous, parietal and chief cells, the former in the neck and the latter two in the middle and base of the glands. Oi's account inverts the accepted intra-gland distribution of parietal cells and may be discounted in comparison with other accounts. The pyloric mucosa contains mucus and gastrin secretory cells. In all cases. transitional cells types are found between the regions, which may explain inter-author discrepancies, as information is rarely given on the number and origin of histological samples. The first part of the duodenum is lined by a pyloric type mucosa, and the duodenal glands (Brunner's) have disappeared by a point two centimetres from the pylorus.

The two proximally spiral layers of oesophageal striated muscle have become an inner circular and an outer longitudinal layer when they reach the cardia. The circular layer undergoes a sudden transition to the smooth gastric

circular muscle at the cardia but the longitudinal layer is more gradually transformed. The incomplete outer gastric layer comprises mainly longitudinal bundles along the greater curvature and right lesser curvature and oblique fibres on the fundus, radiating from the cardia. The complete middle circular layer forms two intersecting thickenings at the pylorus, whilst the inner oblique forms sling fibres around the oesophageal aperture.

### EXTRAMURAL VASCULATURE

The observational literature upon the anatomy of the canine coeliac artery and its branches was sparse until the last decade. The information was almost entirely located in the standard veterinary textbooks (e.g. Bradley 1912; Ellenberger & Baum 1943; Sisson 1953; Bourdelle & Bressou 1953; Miller et al. 1967). These accounts, which were in general agreement, dealt only with the primary and secondary branches of the coeliac artery and gave no details of the evidence upon which the descriptions were based. All the authors described a canine anatomy which was very similar to the 'classical' pattern in man.

### **Description**

The coeliac artery, which arises from the aorta, gives off the hepatic artery, then continues as a gastrosplenic trunk, which almost immediately divides to form the left gastric and splenic arteries. The hepatic artery gives off several proper hepatic arteries to the liver, and the right gastric artery which passes rostrally along the lesser curvature, supply the distal stomach and anastomose with the left gastric artery. The hepatic artery then continues as the short gastroduodenal artery, which divides to form the right gastroepiploic and pancreaticoduodenal arteries. The former passes along the greater curvature, supplying the distal stomach, and anastomoses with the left gastroepiploic artery. The pancreaticoduodenal artery supplies the duodenum and pancreas. The left gastric artery ramifies on the left part of the stomach and forms a branch which anastomoses

ų.

with the right gastric artery. The splenic artery passes to the ventral part of the spleen. En route, it gives off pancreatic branches, the left gastroepiploic artery which passes along the greater curvature and supplies the stomach, and a gastrosplenic branch to the dorsal part of the spleen. This contributes branches to the left extremity of the stomach.

Thus, prior to 1970, there was a consensus upon the anatomy of the larger blood vessels of this region although with little information on variant frequencies. In particular, the literature appertaining to the duodenal supply was sparse. An early explicit description of experimental observations upon the canine gastric vasculature was given by Mall (1896) who used an injection / dissection technique on an unspecified number of dogs. His account described only the anastomoses on the duodenum (hepatic / superior mesenteric arteries), on the lesser curvature (hepatic / gastric arteries) and on the greater curvature (hepatic / splenic arteries). He also described the areas (size and location) supplied by the branches of the three main vessels. He found that the hepatic artery and its branches supplied the pyloric part of the stomach, the gastric artery supplied the middle zone of the stomach adjacent to the lesser curvature plus a ring at the cardia, and the splenic artery supplied the middle zone adjacent to the greater curvature plus the fundus.

The first detailed study of the gastric circulation appears to be by Enge et al. (1972). This was an in vivo angiographic investigation upon 24 subjects using Isopaque 350 as a contrast medium. The process was validated by the comparison of post mortem observation with the radiographic topology in four of the subjects using Colorpaque. The authors quoted a lower resolution limit of 0.2 mm external vessel diameter. On the basis of Mall's measurements this should enable vessels down to the large branches of the submucosa to be imaged, although the authors admitted that, in some areas, the limit was raised due to vessels overlapping. Both anterior - posterior and lateral films were made to facilitate three dimensional reconstruction.

Enge's results are generally consistent with those of other authors. Where discrepancies do arise, they appear to be a consequence of Enge's recognition and quantification of variant patterns as opposed to other authors' description of what they believed to be the 'standard' pattern. In the majority of animals (20/24-83%), the coeliac artery branched tripodally. In only four cases did the left gastric and splenic arteries arise from a single trunk, branching from the coeliac artery distal to the origin of the common hepatic artery. In all cases, the coeliac was a branch of the aorta.

The common hepatic artery was the largest branch of the coeliac. It gave rise to two to four tortuous proper hepatic arteries although in five cases, a fifth was present. These supplied the hepatic lobes although their precise arrangement was sometimes unclear owing to partial overlay by other vessels. The common hepatic then continued as the gastroduodenal artery. In only seven cases was a right gastric artery given off from the gastroduodenal. In a further two it arose from one of the proper hepatic arteries. In all of these the right gastric ran along the lesser curvature. The gastroduodenal artery then gave rise to the right gastroepiploic artery in all 24 specimens, although in three animals the vessel was double. This ran along the greater curvature, supplying it by several short gastric arteries. The gastroduodenal artery ended by forming one or two superior pancreaticoduodenal arteries which supplied the pancreas and duodenum.

The left gastric artery gave off a branch to the upper stomach before continuing to run along the lesser curvature. No hepatic branch was seen. The splenic artery gave branches to the spleen and occasional branches to the pancreas.

Three sets of arterioarterial anastomoses were seen with varying frequency. The left and right gastric arteries anastomosed on the lesser curvature in 5 of 24 cases. Anastomoses between the right gastroepiploic and branches of the splenic artery were seen in the gastrosplenic ligament in 5 specimens. By contrast, the superior and inferior pancreaticoduodenal arteries anastomosed in most of the animals.

There has been little subsequent work on the vasculature of the canine stomach. Zinner (1975), in a pharmacological study, gave a description in agreement with the classical account. Whether this was based upon his own observations or upon the literature was not stated. Piasecki (1975) in a radiographic examination of the canine gastric microcirculation using a contrast medium, reported that the gross blood supply to the stomach was "generally similar to that in Man". He was the first to report that the left gastric artery was double in all his specimens. However, these results were based upon the examination of only six animals (Piasecki 1975).

Gupta et al (1978) defined the areas supplied by the main extramural arteries in 45 dogs, using an injection technique. Their results confirmed the textbook descriptions and that of Mall, but emphasized the importance of variant patterns. Variation was most commonly seen in the relative contributions of the Left Gastroepiploic, Left Gastric and Right Gastroepiploic Arteries.

Experimental observations on the gross blood supply to the canine duodenum have been made by a number of workers, such as Thamm (1941), Rauch (1962), Kondrashov (1964) and Gomercic (1972b). Thamm, in a study of 12 dogs, reported that the descending duodenum was entirely supplied by the cranial pancreaticoduodenal artery. The ascending duodenum and pancreas were supplied by the caudal pancreaticoduodenal artery. These two arteries, branches of the coeliac and cranial mesenteric arteries respectively, anastomosed on the duodenum. No variations were described.

Gomercic dissected 30 formaldehyde fixed dogs after vascular injection. He found that the cranial pancreaticoduodenal artery supplied the entire descending duodenum in only 9 animals (30%). In 18 dogs, only the cranial half was supplied by this artery and in 3 only the cranial third. He found Thamm's distribution of the caudal pancreaticoduodenal in only 14 animals. In the remaining 16, the cranial mesenteric artery gave rise separately to the caudal pancreaticoduodenal and a ramus duodenalis. A coeliaco-mesenteric anastomosis was found on the duodenum in only 33% of cases. In the remaining specimens, the anastomoses were found within the pancreas.

### <u>Conclusions</u>

The consensus is summarised in the diagram. There are, however, a few discrepancies between Enge and previous authors. The former's full experimental description suggests that his results may have the greater validity. Firstly, the branching of the coeliac artery appears to be normally trichotamous, but a dichotomy is a variant, to form the common hepatic artery and a gastrosplenic trunk. Secondly, according to Enge et al., the right gastric artery was present in only 9/24 animals, in two of which it arose from a proper hepatic artery, whereas this artery is a normal feature of the 'text book' account. This point awaits clarification. Thirdly, Enge et al. describe an anastomosis between the left and right gastric arteries in only 5/24 animals (5/9 cases in which the right gastric was present) and between the right gastroepiploic artery and branches of the splenic artery in only 5/24 animals. Anastomoses along the greater and lesser curvatures are described by all other authors and are of importance in explaining the results of selective ligation experiments. This difference should be subject to further examination. Finally, the left gastric artery has been described as double by only one author. Similarly, the right gastroepiploic artery may be double.

#### INTRAMURAL ARTERIAL VASCULATURE

Despite the widespread use of the dog in the study of gastrointestinal physiology, pharmacology and pathology, there are only two detailed descriptions of its intramural angioarchitecture by Mall(1896) and Piasecki(1974). A single description based on a number of species, including dog, was given by Leone(1949), and observations on the vessels of the jejunum and ileum were published by Noer(1943) and Jacobsen & Noer(1952). Beyond this, the only information is in the form of anatomical correlates of physiological studies, particularly those concerning the effects of vessel ligation and the existence of arteriovenous anastomoses, and incidental remarks in studies on a different subject.

### Description

Mall(1896) gave a description of the intramural vessels of the stomach. He based his vascular descriptions upon double injected specimens, which were dissected and macerated and examined in three dimensions. His work has limitations since it is not clear upon how many specimens his descriptions are based and what variants were present. In addition, he confined his samples to a thin strip along the middle of the anterior and posterior walls, and did not describe any other parts of the stomach. Nevertheless, his account is detailed and has been largely confirmed by subsequent authors.

Mall showed that the muscularis externa has three main sources of supply. The most important is by recurrent vessels from the submucous plexus, approximately 250um in diameter. These give rise to an arterial plexus (average diameter 100um) between the longitudinal and circular laminae, which is the sole supply for the former and contributes to the latter. The circular muscle also receives small inputs from the connective tissue plexus in the submucosa, although Mall did not use this term. In addition, the whole of the muscularis receives nutrient branches from the extramural arteries as they pass though the muscle. This latter feature was confirmed by Peters & Womack(1958), but they did not mention the other sources of supply.

Mall showed that the external vessels enter the submucosa and give rise to the submucous plexus. This is located in the middle of the submucosa and is in a single plane. The vessels are largest in the corpus, where they have a mean diameter of 415um. The primary plexus gives rise to a secondary plexus, which in turn forms mucosal arteries. These pierce the muscularis mucosae and branch at the base of the mucosa to form a dense capillary network which surrounds the glands. Mall noted particularly that the mucosal arteries do not form a plexus within the mucosa, and contrasted this with the cat. The mucosal arteries have a mean diameter of 25um and their immediate branches 17um. The capillaries had diameters of 3 - 6um.

This picture was confirmed by Piasecki(1974) who investigated 13 dogs. Eleven of these were injected with a radio-opaque contrast medium and examined by radiography, and two were injected with an optical contrast medium, cleared and examined by stereomicroscopy. Piasecki gives far more information than Mall, since he examined the entire stomach and first part of the duodenum, and is more rigorous in his description of variants. However, the bulk of his evidence comes from the radiographic examinations, which do not give three dimensional information. This was, to some extent, compensated for by correlation with the cleared specimens.

Piasecki did not describe the vessels of the muscularis externa. He confirmed the location of the submucous plexus within the submucous compartment. Both workers emphasised the excellent anastomoses at the submucosal level between the branches derived from the extramural inputs. Piasecki was able to add detail on variations in the pattern of the plexus in different regions of the stomach. Most significantly, he showed that the plexus in the lesser curvature was not significantly poorer than in the remainder of the stomach, as determined by the concentration of anastomoses. He did note, however, that the largest anastomoses on the anterior and posterior walls were larger than the largest lesser curvature anastomoses in three of eight specimens. The anterior and posterior wall plexus was richer in the corpus than in the fundus or pylorus, and the corpus vessels were larger than the pyloric.

Since Piasecki examined the whole stomach, he was able to report regional variations in plexus pattern. In general, the plexus had a scalariform pattern. However, stellate branching vessels were seen in the centre of the lesser curvature, in parts of the greater curvature and in the fundus, derived from the short gastric arteries. Continuity in the plexus was seen at the gastro-oesophageal and gastroduodenal junctions. In the latter, long parallel leashes of vessels were seen in only two of eight specimens. In the first half inch of the duodenum, a complete plexus with a 'diamond' shaped mesh was present, which became scalariform, running completely around the viscus in the second half inch.

Piasecki confirmed Mall's account of the mucosal arteries, arising solely from the submucous plexus, piercing the muscularis mucosae and branching three to six times at the base of the mucosa. In particular, he noted that there were no anastomoses in the mucosa between adjacent mucosal arteries, and that there were no mucosal arteries that had not arisen from the submucous plexus, i.e. no mucosal arteries of extramural origin.

Unlike Mall, Piasecki was able to find a significant connective tissue plexus in only one specimen.

Mall does not mention arteriovenous anastomoses but Piasecki specifically attempted, and failed, to find them, despite injecting the animals a short time after death, which has been predicted to maximise the probability of filling any AVA's (Sewell 1969). Earlier work using microspheres had suggested their existence. Sherman & Newman (1954) found that glass beads up to 140um in diameter passed across the gastric microcirculation and interpreted this as demonstrating the presence of such vessels with this maximum size. Peters & Womack (1958) found 125um shunts and claimed to have observed a few in histological sections. However, subsequent workers, using more sensitive techniques utilising radioactively labelled microspheres, were generally unable to repeat these results. Delaney & Grim. (1964) were unable to find any spheres of 35um or larger passing from the left gastric artery to the venous circulation. Shoemaker & Powers (1966) found no evidence of shunts above 50um, despite using vasodilators such as

adrenaline, which Peters & Womack had shown to allow the larger spheres to be found in the venous effluent. In a review of the area, Delaney (1975) came to the conclusion that the earlier work was methodologically flawed, and that not exist in the AVA's probably did gastric microcirculation. This was supported by subsequent work by Archibald et al. (1975) and Cheung & Chang (1977) which gave similar negative results. Indeed, Milland et al. (1977) showed that the detergents commonly used in microsphere experiments to prevent aggregation of the spheres had a significant effect on cardiac parameters which could affect the interpretation of experiments in which they were used.

A striking feature of the microcirculation described by Mall and Piasecki was the excellent degree of anastomosis between the branches of the various input vessels, in contrast to the limited degree of extramural anastomosis. This is consistent with the results of investigations over many years which have shown that drastic devascularization by tying and cutting fewer than all four of the main arteries supplying the stomach has little effect on the mucosa or on acid secretion. However, when all four were severed, gastric necrosis resulted rapidly (Brenckmann 1932; Bernheim 1932; Babkin et al. 1943; Layne & Bergh 1943). More specifically, even severe devascularization did not result in the formation of ulcers (Sekine & Shiratori 1974). This has been endorsed by a number of workers, who have failed to find chronic ulcers in 'normal' dogs (Mann 1916; Ivy 1919; Volini et al 1938).

Conversely, in a series of publications, Rau proposed the "steal" phenomenon. He suggested that the excellent anastomoses could allow a significant reduction in blood flow at the antrum-corpus interface to be caused by local vasodilatation in the corpus. The blood flow reduction could be sufficient to be ulcerogenic (Rau 1981; 1983; 1986; 1987). This has not been validated or supported by other workers.

### <u>Conclusions</u>

Although there are relatively few direct anatomical studies of the canine microcirculation, the results of Mall and Piasecki, together with a number of physiological stuies are largely in agreement. A number of features of their descriptions are significant. The key role of the recurrent vessels in the supply of blood to the muscularis externa is in contrst to the more usual predominance of nutrient vessels from the penetrating arteries. The exclusive role of the submucous plexus in distributing blood flow is important, as is its richness and uniformity with no areas of plexus poverty.

There are some areas of contention. Mall reported the presence of a connective tissue plexus, whereas Piasecki could find it in only one of thirteen specimens. Mall's lack of methodological information, particularly numbers, and limited examination area, must be weighed against Piasecki's predominant use of radiographic techniques, in which it might have been difficult to differentiate a sparse connective tissue plexus. The case is not proven from the literature. As in other species, the presence or absence of arteriovenous anastomoses is a point of debate. The balance of later evidence seems to be consistent with their absence, and the evidence for their presence in earlier studies is weakened by the methodological criticisms of workers such as Delaney. Rau's theoretical models of a mechanism by which a rich plexus, such as the dog's, could produce local ischaemic ulcerogenesis are of interest, but they have little experimental basis, in comparison to the well documented resilience of the dog's stomach even when extensively devascularised, and absence of spontaneous ulceration.

Ļ
# PART III ORIGINAL OBSERVATIONS

# CHAPTER 11 MATERIALS AND METHODS

### **SPECIMENS**

The species examined were cat, dog, primate, swine, guinea pig, rabbit, rat, ferret and dolphin. The cats, dogs, guinea pigs, rabbits rats and ferrets were standard investigatory species obtained from normal laboratory suppliers The primates were all rhesus monkeys from the Indian subcontinent, obtained via the National Institute of Medical Research, London. The pigs were a standard animal husbandry variety, obtained via the Medical Research Council, Northwick Park Hospital, London. The dolphin was obtained shortly after stranding at Flamborough Head via H.M. Inspector of Wrecks and the Natural History Museum, London.

#### **SACRIFICE**

The method of sacrifice was, as far as possible, kept ( $\tau_{a} \downarrow \downarrow \ell + \ell$ ) constant for each species. All the cats (15) but one were given an overdosage of anaesthetic. Anaesthesia was induced by sodium pentobarbitone ('Nembutal') at normal dosages, then deepened by further anaesthetic administration until respiration and cardiac activity ceased. The one exception was decapitated under anaesthesia.

The canine (8) and rat (13) specimens were similarly sacrificed, with the exception of Dog 2 which received a pneumothorax under pentobarbitone anaesthesia. All the primates (27) but one were sacrificed by exsanguination from the abdominal aorta under pentobarbitone anaesthesia. The exception was given a pentobarbitone overdosage. Exsanguination from the aorta under anaesthesia was used in 5 of the 19 guinea pigs and all 26 swine. The remainder were sacrificed by cervical dislocation except one which died under normal anaesthesia. Three of the six rabbits were

SPECIES	SPECIMEN No.	TECHNIQUE	
Cat (Felis domesticus)	C1 C2-C15	Decapitation under anaesthesia Anaesthetic overdosage	
Dog (Canis familiaris)	D2 D1;D3-D8	Pneumothorax under anaesthesia Anaesthetic overdosage	
Primate (Macacus rhesus)	P3 P1-P2; P4-P27	Anaesthetic overdosage Exsanguination "	
Swine (Sus scrofa)	S1-S26	Exsanguination	
Guinea Pig (Cavia porcellus)	GP1-GP13 GP14 GP15-19	Cervical dislocation Died under normal anaesthesia Exsanguination	
Rabbit (Oryctolagus	Rb1-Rb3	Pneumothorax	
cuniculus)	Rb4 <b>-</b> Rb6	Anaesthetic overdosage	
Rat (Rattus norvegicus)	R1-R13	Anaesthetic overdosage	
Ferret (Mustela putorius)	F1-F12	Carbon dioxide asphyxiation	
Dolphin (Delphinus delphis)	Dn1	Bullet to heart	

# Table 4 Sacrifice Techniques

.

sacrificed by a pneumothorax under pentobarbitone anaesthesia, whilst the remainder were administered an anaesthetic overdose. The 12 ferrets were all sacrificed by carbon dioxide asphyxiation. Finally, the dolphin was put down by a bullet to the heart after stranding.

#### INJECTION STUDIES

#### <u>Method</u>

The stomach, duodenum, spleen and associated blood vessels were removed after death in the pig, dog and dolphin. This was necessary owing to the size of the viscera and the logistics of transport and storage. In the primate, the entire abdominal viscera and associated blood vessels were removed. The remaining species (cat; guinea pig; rabbit; rat; ferret) were injected with the abdominal The arteries supplying the stomach viscera in situ. and the duodenum were cannulated with polyethylene tubing ("Portex") of appropriate bore. In the larger animals (dog;pig;dolphin;cat), the coeliac and superior mesenteric arteries were separately cannulated. In the remaining, smaller species, the cannula was inserted into the aorta from the rostral direction, its tip just proximal to the origin of the coeliac. The aorta was ligated distal to the superior mesenteric origin.

The blood vessels were flushed with hypertonic ---heparinised saline (1.8% sodium chloride;10 I.U./ml Heparin--Weddel Pharmaceuticals) until they appeared pale and bloodless. The hepatic artery was then ligated as were any cut vessels.

Most of the specimens (intact or isolated) were stored in a conventional refrigerator at 4°C for 24 hours to allow any post mortem vascular spasm to disappear. It was necessary to inject primates 1 to 15 immediately after death to comply with quarantine regulations. This was also done in cats 10 to 15 to ascertain if any differences in results were obtained, but none were observed.

The specimens were removed from the refrigerator after

24 hours for injection. The previously implanted cannulae were connected to a disposable hypodermic syringe (10 - 50 ml depending on species size) via a three way tap. One arm of the tap was blocked off by an empty syringe. A 'T' piece was inserted into the circuit near the tap to which an air manometer, previously calibrated from a mercury manometer, was attached via a short length of wide bore tubing.

The injection medium was placed into the syringe and injected into the animal's arterial vasculature via the implanted cannulae. The pressure of the injectate, as monitored by the manometer, was not allowed to exceed 150mm Hg. The injection was continued until the stomach and duodenum appeared to be fully filled, but was not allowed to continue to diffuse capillary filling. If necessary, escape of displaced heparin saline was allowed from the hepatic portal vein or inferior vena cava.

### <u>Media</u>

Two alternative injection media were used to delineate the vessels - gelatine and latex rubber. The majority of the specimens (88) received latex whilst 26 received gelatine. A further three received "Micropaque" a proprietary radio opaque barium sulphate preparation.

#### Gelatine

A 5% (w/v) mixture of gelatine powder in distilled water was made and dissolved by heating (Gelatine powder -Hopkins & Williams 4378000). To this was added sufficient "Pelikan" india ink to make a 25% (v/v) mixture This was maintained at a temperature of 35 -  $40^{\circ}$ C in a water bath and was injected at that temperature.

#### Latex

A proprietary liquid neoprene latex rubber preparation (Du Pont Chemicals) was coloured with 17% (1:6) blue or black dye, supplied by the latex manufacturer. To this was added 0.2M sodium hydroxide (1 part NaOH solution to 2 parts --latex solution). The purpose of the NaOH was to maintain the pH above 7.0 to prevent polymerisation of the latex. This solution was injected into the vasculature at room temperature. In 11 specimens (C10 - C15;Rb6;GP7 - GP10), the NaOH was replaced by a similar quantity of Sorenson's Phosphate Buffer, which maintained a pH of 8.0. It was felt that this was more physiological than NaOH, but no differences in results were observed.

# Micropaque

In three specimens, a solution of radio-opaque barium sulphate ("Micropaque"-Damancy & Co.) was used at room temperature in place of the latex or gelatine based mixture. It was found to have insufficient opacity and these specimens were not used in the analysis.

In all cases, after injection was judged to be complete, the oesophagus and duodenum were ligated, as was the inferior vena cava. The viscera were the removed, if this had not already been done and immersed for 24 hours in cold 10% formalin (from a 37.4% aqueous solution of formaldehyde BDH 2842). This allowed the gelatine to 'set' or polymerised the latex by its acidity.

### **Dissection**

After a minimum of 24 hours, when the injection medium had solidified, the gastroduodenal arterial vasculature was dissected out and recorded. The stomach and duodenum were then removed from the rest of the specimen. The serosa and muscularis externa were carefully removed over the entire surface of the stomach and duodenum. As far as was possible, the muscularis was cut along the mid line of the anterior and posterior walls and the pieces preserved. In the larger specimens, additional cuts had to be made. During this process, the muscularis was lifted away from the submucosa, enabling the dividing plane and the inner surface of the muscle to be clearly defined. Any vessels which were seen to pass between the muscularis and the submucosa were cut on the inner surface of the muscularis so that the entire extra-muscular course of the vessel was preserved with the submucosal component of the specimen. The completeness of this procedure could be checked in the isolated muscle specimens.

This left the submucosal and mucosal components of the stomach and duodenum intact. The stomach was then opened along the anterior and posterior walls and its contents removed. The mucosal surface was washed in running tap water. The pieces were then secured by pins to pieces of card to give an approximately flat surface. This inevitably involved some distortion of the curved gastric wall and, in larger species, it was necessary to cut the stomach and duodenum into smaller fragments. In all cases, the orientation of the pieces in relation to the whole stomach was mapped. The muscularis externa and serosa were also fragmented where necessary and pinned to card. In some cases, the muscle thickness was so great that it could not be flattened and had to be processed whole. The tissue samples were then washed in running tap water for approximately 18 hours and then fixed in formalin for a further 7 days or until fixation was complete, if longer.

# <u>Dehydration</u>

After fixation was complete, the samples were dehydrated. This was accomplished by passing the specimens through a series of solutions of alcohol (industrial methylated spirits ) of ascending concentration, with 24 hours in each. (50%; 75%; 95%; 100%; 100%).

# <u>Clearing</u>

The primary aim of this technique is to render the tissue translucent so that the vessels can be seen. This was accomplished by clearing the tissue, using a modification, devised by the author, of Spalteholz's technique (1914). In the original method, the specimen is initially immersed in pure benzene but, in this modification, this step is replaced by immersion in a 1:1 mixture of alcohol and benzyl benzoate. This was done because of the high toxicity of benzene, particularly its carcinogenicity. The modified version was found to give comparable results to Spalteholz's original technique.

#### **Observations**

All specimens were stored in methyl salicylate after clearing. They were transilluminated when immersed by a horizontal x-ray box and were examined using a Nikon stereomicroscope or a Carl Zeiss (Jena) operating microscope. Magnifications up to 40x were used.

Descriptions were compiled of the appearance of the vascular beds based upon a comprehensive detailed (Figle) examination. The specimens were observed in three dimensions, and the vessels were manipulated, in order to define the tissue planes in which they were located. The correlation between the appearance of the vessels, their manipulability and their location was confirmed by subsequent histology of defined areas.

Measurements of the diameters of vessels, such as mucosal arteries, and their areas of supply were made directly from the specimen, using linear or squared eyepiece graticules, respectively, in the Nikon stereomicroscope.

#### **Photography**

Macrophotography of whole specimens was carried out by a Nikon large format camera using 5 x 4cm FP4 (Ilford), rated at 100 ASA. Microphotography was done using the Leitz Tessovar on Ilfodata-B 35 mm film, rated at 4 ASA. The Tessovar provided magnification in the negative plane of 0.8 - 3.2x at f4 - f1. Some colour photography was done using Agfachrome 50C artificial light film, rated at 50 ASA. Histological thin sections were photographed on the Leitz Ultraphot, using FP4 or Agfachrome 50C artificial light film. Stained, cleared whole blocks were photographed on the Tessovar, immersed in methyl salicylate, on Agfachrome 50C 35mm film.

#### <u>Mapping</u>

Comprehensive maps were made of the entire submucosal vascular topology of several of the specimens. They were immersed in methyl salicylate and placed beneath a Carl Zeiss (Jena) epidiascope. The resolution in the image plane was improved by reducing the lens aperture to f16, using a supplementary diaphragm in the objective plane. The image was projected onto a sheet of card 24 x 36 inches, along with that of a scale bar and the vessels were then traced. A map of a complete specimen might cover several such cards. The completed map was compared with the actual specimen under a stereomicroscope and any necessary corrections made. The diameters of all the inputs to the arterial plexus and of all the arterio-arterial anastomoses were then measured on the specimen by an eyepiece graticule, previously calibrated against a stage graticule. The areas of supply of the input vessels were measured on the map, by overlaying with translucent graph paper and counting the squares. These were converted to real areas by applying the appropriate magnification factor. In Cat 15, this was used as the basis for a detailed network analysis of the entire plexus, by analysing the spatial variations in input diameter, diameter of the anastomoses, area of supply, anastomotic and input density, perfusion and connectivity indices (see below for definition).

# HISTOLOGY

The specimens examined histologically are listed in the appropriate species results sections. They were mostly examined by conventional histological techniques i.e. Haematoxylin & Eosin or Van Gieson staining of paraffin embedded or frozen sections.

One of the major problems in this investigation was to relate the blood vessels to the tissues through which they passed. Conventional histology requires laborious serial sectioning to follow the course of a blood vessel and can only examine a small area. By contrast, the entire course of the blood vessels can be examined in the cleared specimens, but the tissue plane in which they are located cannot be defined. A technique was therefore developed to provide both modes of information. This involved partially rehydrating a block of cleared injected tissue, staining with H & E and counterstaining with Van Gieson, then reclearing. This gave a translucent block, showing the blood vessels and differentiating the connective tissue of the submucosa, the muscularis mucosae and the mucosa in which the vessels were located. It was used to 'calibrate' the correlation between the manipulability of a vessel in the cleared specimens and the plane it which it was located.  $(F_{i}g.2)(F_{i}g.3)$ 

# NETWORK ANALYSIS

Comparative analysis of the observed angioarchitecture requires precise definition of the terms used. These are defined in this work as follows.

1. Plexus/Anastomoses

Whether vessels constitute a plexus, or are arterioarterial anastomoses is a function of their geometry and relative sizes. The criteria to define a plexus are:

- a. The diameters of the plexus vessels are approximately the same as the input vessels forming them.
- b. There is no sharp change in direction between the input and plexus vessels.

Anastomoses are significantly smaller than the vessels which give rise to them, and there is a sharp change of direction.

2. Mucosal Artery

A mucosal artery is the final arterial vessel which supplies the mucosa. Distal to it is only the capillary bed. It may have anastomotic connections with other mucosal arteries, but does not contribute to a plexus.

3. Low Connectivity Vessel.

This is a vessel which has very few and/or very small connections with any plexus distal to the muscularis

externa, before supplying the mucosa.

4. Mucosal Artery of Extramural Origin

This is a vessel which does not make any connections with a plexus distal to the muscularis externa. It is the limiting case of a low connectivity vessel where the number and/or diameter of vessels becomes zero.

5. Arteriovenous Anastomosis

This is an arterial or arteriolar vessel which makes a direct connection between an artery or arteriole and a vein or venule. There is no intermediate capillary bed.

6. Recurrent Vessel

This is a vessel which:

-decreases in diameter superficially.

-has a source of supply deep to its terminal bed.

#### <u>Measurements</u>

Individual Vessels

These were defined by the measurement of:

-internal diameter

-area of supply, defined by the area encompassed by the contour joining the points of minimum diameter on all the anastomoses between that input and its neighbours (analogous to a watershed).

-density i.e. number of vessels per unit area. -perfusion index. This is the ratio of the fourth power of the diameter to the area of supply i.e.  $D^4/A$ . It is an indicator of the adequacy of flow in the input (proportional to the fourth power of its diameter) in relation to metabolic demand (proportional to area of supply).(Fig.4)

# A Plexus

This can be defined by the measurement of:

-the number of anastomoses per unit area. An anastomosis is the point of minimum diameter of a vessel joining two arterial inputs to the plexus.
-the size spectrum of the anastomoses.

-the connectivity index spectrum of the plexus.

Each 'cell' of the plexus is the area supplied by a single input, defined by the line connecting all the points of minimum anastomotic diameter. The C.I. of an input is the sum of the fourth powers of all its anastomotic diameters, divided by the area of supply i.e.  $(\sum (D^4))/A$ . It is an indicator of the adequacy of the potential inward flow through all the anastomoses (proportional to the sum of the fourth powers of their diameters) in relation to the metabolic demand (proportional to area of supply). (Fig.4+).

-the ratio of the connectivity index of an input to its perfusion index. This is an indicator of the extent to which flow through the anastomoses could compensate for the loss of flow through the corresponding input, irrespective of the area of supply.

### <u>Statistical Analysis</u>

When a statistical analysis of the difference between sets of data was required, such as comparing the diameters of mucosal arteries in different areas of the stomach, the Kolmogorov-Smirnov two sample test was used. This tests the confidence with which the null hypothesis, that the samples come from the same populations or populations with the same distribution, can be rejected. It assumes that the two sets of observations are independent, but is more powerful than other non-parametric tests, such as chi-squared and Mann-Whitney. Unlike these, it does not require that both populations are normally distributed and is sensitive to differences in central location, dispersion and skewness. (Siegal 1956). It was used as a two tailed test, making no predictions about the direction of any difference.

#### DISCUSSION

The species used in this investigation were selected to represent the main genera used in biomedical experimentation, particularly of the pathology, physiology and pharmacology of the gastrointestinal tract. The exception to this was the dolphin, included because it experiences chronic gastric ulceration.

The ante-mortem anaesthetic and the method of sacrifice was not always the same since the sources of the specimens varied. These factors could affect the state of the vasculature immediately after death (Baumel 1970; Altura 1975) so injection was delayed for 24hours post-mortem to allow any vascular spasm to subside (Baumel 1970). In species such as the guinea pig and rabbit, where a significant proportion of the specimens were sacrificed by a different technique, no consistent differences in results were seen. The primates might not have been comparable with the other species, since injection had to be carried out immediately after death to meet quarantine regulations. In an attempt to assess the effect of this, five cats were also injected immediately after death, and no difference from those injected after 24 hours was seen.

The injection technique has been very widely used to demonstrate the vascular bed. However, there are certain caveats which must be appreciated when interpreting the results. The pattern shown may not completely reflect the vessels open at any one time in life. Some vessels may contract, and so not fill. Conversely, the pressure of the injection may force open vessels not normally patent. The first is limited by injecting after 24 hours as discussed above. The second is a valid criticism, so that the observed vasculature may represent the maximum potential network, rather than the one normally available in vivo. Piasecki (1975) suggested that injection pressure should be limited to the normal systolic blood pressure in that species. In this investigation, however, a standard pressure was used in view of the uncertain relationship between an externally measured pressure and the actual intravascular pressure. Since injection was continued until arterial filling appeared complete, the intravascular pressure was effectively standardised. Too great a pressure resulted in extravasation which was rare. Too low a pressure resulted in

154

unfilled vessels. Since the injectate was miscible with water, this was visible as the vessel faded away, often with a proximal sphincteric narrowing. Thus, the presence of unfilled vessels was detectable and negative results were significant.

The observed dimensions of the injected vessels may not equal those in life. The diameter can vary for the reasons described above. Stretching or post fixation shrinkage may alter the length. As far as possible the factors which could lead to such distortions were minimised and standardised, to maximise comparability between and within specimens.

The use of any technique entails the production of artefacts and selection of specimen will introduce bias into the results. These problems are addressed by minimising and understanding the artefacts, by comparing the results derived from varying methods and by using as many specimens as possible. An essential component of this study is the validation of the injection results by stereomicroscopy, micromanipulation, histology and cleared edge sections. Micromanipulation was particularly important to distinguish between vessel overlap and anastomosis. Edge sections were essential to validate this use of micromanipulation to define the tissue plane in which vessels were located. It was found in the cleared specimens that some vessels could be moved and crushed, some only crushed and some neither. The tissue planes could not be defined in the cleared specimen, although it was hypothesized that the differences were caused by the vessels' relation to the muscularis mucosae. Conventional histology could only examine a small volume of tissue and was difficult to co-ordinate with the appearance of the cleared specimen. A technique to produce stained cleared blocks of tissue was developed which allowed vessels to be followed in three dimensions, in relation to the tissue planes. Such a facility has not been reported in other works. It validated the hypothesis that movable and crushable vessels were in the submucosa, crushable vessels were within the muscularis mucosae, and vessels which could not be touched were deep to the muscularis, within the



Fig. 13 Illustration of use of parallax to define plane of vessel. Pin is placed on upper surface of submucosa and viewed from left (top), vertical (middle) and right (bottom). Parallax shift shows large vessel is in submucosal plexus and small vessel is in mucosal plexus (C13LC) (16x).





Fig. 2 Illustration of micromanipulation to demonstrate vessel location. SMP & MP shown undisturbed (top). Below, a large vessel is pulled downwards by tweezers (dark bar) demonstrating it is located in the SMP (16x) (C13LC) mucosa. This enabled the plane of any vessels to be easily defined in the total specimen. Such a use of multiple techniques is essential to fully describe the vascular bed and does not appear to have been adopted in other studies. (Fig. 3)

The best injection results were obtained from the use of latex rather than gelatine media, in contrast to the experience of Piasecki (1975). This may be due to the incorporation of buffering agents in the injectate, to maintain the pH above 7 and prevent polymerisation. It was found that this tended to occur during the injection in the absence of the buffer, analogous to the effect of cooling on a gelatine injectate.

The measurement of vessel diameter, area of supply, vessel and anastomosis density as determinants of plexus "richness" has been used in part or whole by other workers (eg Piasecki 1974; 1975). However, these do not describe the ability of a vessel to provide blood in relationship to the requirements of the tissue it supplies. The former is a function of vessel diameter, the latter of tissue volume. The factors determining the flow of a liquid through a tube can be described by Poiseulle's equation:

$$F = \frac{p + r^4}{8 / L}$$

where: F = volume flow per unit time

P = pressure drop in the direction of flow

r = radius of tube

L = length of tube

**P** = coefficient of viscosity.

This is based upon a number of assumptions which are not true of pulsatile blood flow through vessels, but it has been shown to describe such flow with reasonable accuracy (Sherman 1963; Blick 1972). Thus, the flow through a unit length of vessel is proportional to the fourth power of the radius, other factors remaining constant. The blood demand which has to be met by a vessel is proportional to the volume of tissue supplied, assuming that factors such as metabolic activity are comparable. It was not feasible to measure tissue depth over a wide variety of sample sites and specimens, without seriously damaging the specimen, so area of supply was used instead.

A calculated index, the perfusion index, has been derived in this investigation to describe the adequacy of blood supply through an input, and is calculated as the ratio of the fourth power of the vessel input diameter  $(D_i)$  to the area of supply (A).

$$P.I. = \frac{(D_1^4)}{A}$$

In the case of a mucosal artery, this is the diameter of the artery and the area of supply of its branches.  $(F_{i'}g_{i'} + )$ 

Should an input to a plexus become occluded, the tissue within its area of supply, i.e. the area bounded by its anastomoses with the surrounding vessels, must mainly be supplied by the blood flow through these anastomoses (ignoring capillary anastomoses). Using graph and network analysis theory, Ford and Fulkerson (1962) showed that the flow into the output nodes of the plexus (ie the mucosal arteries) cannot exceed the sum of the flow capacities of the plexus anastomoses (the min cut max flow theorem). The plexus can be considered as a number of units, each being the area of supply of one input. The extent to which that unit is connected to the remainder of the plexus is proportional to the sum of the fourth powers of the diameters of all the anastomoses  $(D_a)$ . The ratio of this to the area of the unit (A), the connectivity index, quantifies the adequacy of blood supply to the unit's mucosa, should the input be occluded. i.e.

$$C.I. = \underline{sigma(D_a^4)}(F_{i}g_{4})$$

The CI:PI ratio is an indicator of the extent to which the anastomoses of an input could compensate for the loss of flow through the input, should it become occluded. By removing the absolute level of the original indices and the area of supply it can give information on the adequacy of the plexus to redistribute blood flow, and the sensitivity of the mucosa to disruptions in flow through the inputs to the plexus. However, it does not take into acccount the



Fig. 3 Cleared edge section from area shown in previous figure, (illustrating effect of parallax) to confirm plane of vessels. (Cl3LC) (55x).



Fig. 4 Schematic view of vessels entering plexus to show methods of calculating vessel and plexus perfusion index.

Perfusion Index of vessel =  $\frac{D^4}{A}$ 

Connectivity Index of arterioarterial anastomoses of plexus =  $(\underline{d_1}^4 + \underline{d_2}^4 + \underline{d_3}^4 + \dots + \underline{d_9}^4)$ 

where A = Area bounded by contour joining points of minimum diameter on arterioarterial anastomoses.

margin between the flow through the input and the actual mucosal requirement which may be sufficiently large to tolerate significant flow reductions without damage. Nor it take into account flow through capillary does anastomoses. A Very Low Connectivity Vessel (VLC) is one in which the CI and hence the CI:PI ratio is small. In the limiting case of a Mucosal Artery of Extramural Origin (MAEO) both are zero. Such quantification of the plexus requires a detailed mapping and measurement of the whole plexus which is extremely arduous. In this investigation, it was not possible to do this for all species. This technique has not been previously reported. Further work should be conducted in a further investigation to validate these parameters as indicators of plexus function by actual measurement of both in vivo.

The development of computing capabilities has had a significant impact upon the ability to analyse microvascular networks. Computer-based image analysis techniques will allow the diameters and areas of supply of a vascular bed to be rapidly measured without the requirement to produce maps and measure these parameters manually. The perfusion index could then be calculated and mapped for an entire plexus.

In addition, developments in the field of network analysis allow the topology of a plexus to be taken into account, as well as the dimensions. The effectiveness with which a plexus can distribute blood and compensate for the loss of one or more inputs is a function not only of the dimensions and numbers of the anastomoses, but also of the way in which the vessels are interconnected. Arteriolar anastomoses have been shown to affect the pressure distribution, and hence flow patterns, in microvascular networks (Mayrovitz 1986). Graph theory was developed some years ago, and has been used in the design and analysis of real networks by a number of workers, the seminal work being done by Ford and Fulkerson (1962). Initially this was only possible for relatively simple networks, but the development of more efficient algorithms and more powerful computing facilities means that it should now be possible to analyse

microvascular beds and to quantitatively assess the efficiency and resilience of a plexus. Such a synthesis does not appear to have been reported and would constitute a valuable development of this investigation.

# <u>CHAPTER 12</u> <u>THE CAT</u>

### NON - VASCULAR GASTRODUODENAL ANATOMY

# General Appearance

Five whole stomachs were examined. The stomach narrows gradually distally with an incisura angularis of approximately 150°. The oesophagus enters obliquely, forming an acute incisura cardiaca (terminology after Botha 1958a). Since its point of entry is close to the left lateral aspect of the stomach, the fundus is small.

There is an externally visible narrowing at the pylorus, a few millimetres proximal to the first duodenal flexure of  $90^{\circ}$ . This region of minimum diameter extends over approximately 5mm and is also seen in the cleared mucosal specimens, . A longitudinal section of the gastroduodenal junction, showed a gradual narrowing of the gastric lumen in the vicinity of the pylorus, with no sharply defined junction between the stomach and the duodenum.

#### **Omental Attachments**

The greater and lesser omenta are reflected upon the stomach along well defined lines of attachment. Their position is significant since the blood vessels which supply the stomach run in the omenta and initially contact the gastric serosa along the lines of attachment.

The lesser curvature is delineated by two lines of mesenteric attachment extending from the cardia to the first duodenal flexure. It narrows distally and has a maximum width of about 1cm (equal to approximately 10% of the circumference of the stomach at this point). It is not straight, as in man, but passes onto the dorsal antrum and diverges around the oesophagus reaching the lateral aspect of the cardia in 3 of 5 specimens.

The two layers of the peritoneum, forming the greater omentum, are close together at their attachment to the stomach, so the greater curvature, which they delineate, is only a few millimetres wide. The line of attachment is not coincidental with the caudal edge of the stomach throughout its length, but passes from its original point of attachment at the cardia, across the dorsal aspect of the corpus, to reach the caudal edge of the stomach at the level of the distal corpus. It then continues along the caudal edge of the stomach, extending a few millimetres distal to the gastroduodenal junction.

In addition, there is a line of mesenteric attachment on the dorsal wall of the proximal corpus, between the dorsal layers of the greater and lesser omenta.

# <u>Muscularis</u> Externa

There is an outer longitudinal and an inner circular layer of muscle in the stomach. There are also two longitudinal bands on either side of the lesser curvature, These are below the circular layer and diverge from the oesophagus to the incisura angularis. An outer longitudinal and an inner circular layer is also present in the duodenum which is continuous with that of the stomach.

The thickness of the muscularis externa increases distally, with a sudden increase midway between the incisura angularis and the gastroduodenal interface. At the pylorus there is little noticeable thickening of the circular layer, so that a definite sphincter, such as is present in man, is not evident.

Duodenal muscle is thinner than gastric, and the longitudinal layer is thinner than the circular. In a section of the gastroduodenal junction from specimen C2 the circular and longitudinal layers were respectively 200um and 40um in thickness (formalin fixation and paraffin embedding).

#### <u>Miscellaneous</u>

The above section (specimen C2; section 479) extended along the lesser curvature from the pyloric antrum, through the gastroduodenal junction, to the first part of the duodenum. This specimen displayed a well defined transition between gastric and duodenal tissue. The abrupt disappearance of Brunner's Glands in the submucosa coincided with a localised increase in the submucosal thickness over a length of 1mm. The mucosa appeared to be absent at this point, perhaps due the fortuitous sectioning of a lymphatic nodule. Duodenal mucosa was then found, which was avillous for about 3mm.

In one selected specimen (C15), a block of tissue was removed from the corpus to estimate the thickness of the various layers (section 440). Eighteen sections were cut from this block. Five measurements in each of five sections showed the thickness of the submucous connective tissue to vary between 550 to 650um. Vessels were found throughout its depth, the more superficial ones tending to be smaller, and probably components of the connective tissue plexus. A thick, well defined muscularis mucosa was present, comprising four unicellular layers, defined by the orientation of the spindle shaped smooth muscle cells. It was penetrated by vessels passing from the submucous plexus to the mucosal plexus and contained much smaller vessels. At the base of the mucosa, there was a layer of connective tissue, the lamina subglandularis, approximately 16um thick. The mucosa was 350 - 400um in thickness, but small areas of reduced thickness could be found. Examination of cleared specimens showed an abrupt decrease in mucosal thickness at the base of the oesophagus.

# EXTRAMURAL VASCULATURE (F.g. 5)

The left gastric arterial trunk branches in a "tree like" pattern. Branches run in the lesser omentum and enter the wall of the stomach along the omental lines of attachment, extending to mid-antrum.Further branches run extramurally or within the serosa towards the greater curvature. They enter the muscularis externa two thirds of the way between the lesser and greater curvatures. Very few vessels enter the muscle between the layers of the lesser omentum. Further branches reach the stomach along a line of mesenteric attachment on the posterior distal corpus. One branch of the left gastric artery continues along the lesser curvature to anastomose with the right gastric artery. This vessel gives rise to a single line of inputs on, or ventral to, the lesser curvature. In seven of the nine animals, the anastomosis occurs on the first part of the duodenum, so the right gastric does not supply branches to the stomach but only to the first part of the duodenum. In two specimens, the anastomosis occurs on the antrum, with the right gastric supplying branches to proximal duodenum, distal antrum and pylorus.

An anastomotic vessel, formed from the right and left gastroepiploic arteries runs in the greater omentum, parallel to the greater curvature. Short branches from this chain contact the stomach wall along the line of mesenteric attachment and pierce the muscularis externa almost immediately with no significant subserosal course. The left and right gastroepiploics anastomosed at mid corpus in all nine specimens.

The anastomotic vessels in the antrum continue onto the proximal 3 - 4mm of the duodenum, as far as the first duodenal flexure, and supply inputs. There is a poorlydefined incomplete ring of vessels around the pylorus, which supplies the pyloric sphincter. The inputs distal to the first duodenal flexure are via the pancreatic attachment on the dorsal surface.

The gastric and oesophageal vessels anastomose at the base of the oesophagus, or a few millimetres orally.

#### INTRAMURAL VASCULATURE

11

#### Supply to Muscularis Externa

Branches of the extramural arteries enter the muscularis externa and pass perpendicularly through it, either immediately or after a short subserosal course, to supply the submucous plexus. They do not penetrate obliquely.

A rich subserous plexus arises from branches given off by the penetrating arteries, which ramifies over the entire





- Area (1) Anterior & posterior cardia & fundus Lesser curvature Oesophagus
- Area (2) Greater curvature Anterior & posterior walls Greater omentum

stomach. This network comprises irregularly shaped loops with random orientation. The vessels vary between 10 and 140um in diameter with most less than 60um.

Branches, about 60um in diameter, arise from the subserous plexus and pass, perpendicularly or obliquely, to the deep surface of the muscle, where they give rise to a sparse deep muscular plexus. Some of these branches continue and anastomose with the submucous plexus. The deep muscular plexus is made up of small vessels (less than 35um) which run mainly parallel to the circular muscle fibres. This plexus also receives recurrent vessels from the submucous plexus, which run directly or vía an intervening submucous connective tissue plexus. Recurrent vessels have been previously defined. The criteria which were found to distinguish them in the car were:

- diameter less than 50um
- diameter inversely proportional to the distance from the submucous plexus (ie. larger near their origin from the plexus).
- no linear continuity with vessels which have penetrated the muscularis externa.
- may pass via the connective tissue plexus.

The deep muscular plexus rarely receives branches from the main penetrating arteries.

The vessels between the subserous plexus and deep muscular plexus give off arterioles and capillaries en route which tend to be orientated parallel to the muscle fibres in which they run. In the fundus there is a suggestion of an intermediate plexus between the muscle layers which merges with the subserous plexus in the vicinity of the funduscorpus interface.

#### Spatial Variations in Supply to Muscularis Externa

The external arteries which enter the muscularis externa on the greater curvature invariably pass almost perpendicularly through the muscle after giving off branches to the subserous plexus. By contrast those entering on the lesser curvature often have a significant subserous course before piercing the muscle.

The fundic region of the muscularis externa is atypical in two respects. Firstly the subserous plexus tends to display isolated regions in which the vascular "loops" are elongated and uniformly orientated. This is in contrast to the random pattern found over the remainder of the stomach.Secondly, an intermediate plexus, between the subserous and the deep muscular plexuses, is found in this region. This is a fairly dense network, whose vessels are of similar diameter to those of the subserous plexus. It is supplied from the latter and gives branches to the deep muscular plexus. In the region of the fundus-corpus interface the subserous and intermediate plexuses merge in the subserosal plane.

The pyloric sphincter is poorly developed in the cat, but has a characteristic blood supply. The extramural vessels on the greater and lesser curvatures at the gastroduodenal junction contribute to an incomplete ring of vessels around the pylorus which is completed by the subserous plexus, which here has an annular orientation.

### Submucous Plexus

After the penetrating arteries, have passed through the muscle, they divide near the base of the submucosa to form the submucous plexus. These inputs are normally perpendicular and range in diameter from 80 to 400um. They give rise to a coarse network of primary, secondary and tertiary branches between 13 - 138um in diameter. The plane of the plexus is at a constant distance from the mucosal surface, so the vessels follow the mucosa into folds, rather than bridge them. This feature is more evident distally, where the folds become more numerous. Branches from the submucous plexus give rise to the connective tissue and mucosal plexuses.

# Spatial Variations in Submucous Plexus (Fig. 6-7)

The branching pattern of the submucous plexus is constant throughout the stomach, except in the juxtapyloric

Ļ

169

region, where the vascular loops become elongated along the lumenal axis. Whereas the inputs, branches and arterioarterial anastomoses are narrower distally, they are more numerous per unit area with the consequent reduction in the area of supply of each input. Conversely, there are fewer arterioarterial anastomoses per unit area in the regions bordering the greater curvature, but there is no corresponding reduction in the number of inputs.

Cleared specimens confirm that there are fewer inputs to the central areas of the greater and lesser curvatures, most vessels entering along the lines of peritoneal attachment. A minority of inputs are found on the anterior and posterior walls, corresponding to the subserous branches of the left gastric artery. The pattern in the antrum becomes unclear where the inputs appear to be randomly distributed, owing to the reduced circumference of the stomach.

Longitudinal "leashes" of vessels, approximately 5mm long,run between the stomach and the duodenum, across the gastroduodenal junction in all the specimens examined. These vessels received equal contributions from two lines of gastric and duodenal inputs, which were present on either side of the gastroduodenal junction. The contour joining the points of minimum diameter on the vessels, the anastomotic line, was just on the gastric side of the line of minimal mucosal width in all the specimens.

The gastric submucous and mucosal plexuses merge on the oesophagus to form a single submucous plexus of uniform, longitudinal, large and convoluted vessels which give rise to large mucosal arteries. These branch extensively before giving rise to the terminal capillary bed.

#### Connective Tissue Plexus

This is a sparse plexus of small vessels, with no apparent regularity, located in the submucosa superficial to the submucous plexus. Its component vessels vary between 10 - 40um in diameter, the majority being less than 25um. The plexus is formed by oblique recurrent branches from the submucous plexus and it gives rise to vessels to the deep



<u>Fig. 6</u> Lesser curvature half of C25. Gastroduodenal junction is at centre of field, duodenum to left and oesophagus at right (1x).



Fig. 7 Greater curvature half of C5. Gastroduodenal junction at left edge and oesophagus at right (1x).

muscular plexus of the muscularis externa.

# <u>Spatial Variations in Connective Tissue Plexus</u>

The richness of the connective tissue plexus is approximately proportional to the depth of the submucosal connective tissue and is, therefore, sparser distally. However, the plexus was absent on those portions of the distal oesophagus which could be examined (five specimens).

#### Mucosal Plexus

A second plexus is found deep to the submucous plexus. Examination of cleared edge sections of submucosa and mucosa, taken from a number of specimens and areas and stained by modified Van Gieson's method showed the plexus to be located at the base of the mucosa, below the muscularis  $(F_{ij},...,i)$  mucosae, These vessels could be neither touched nor crushed by micromanipulation. Approximately 500um separates the two plexuses in the corpus of the lesser curvature.

The majority of inputs to the mucosal plexus run perpendicularly from the submucous plexus, either immediately or after a short horizontal course, although a  $(F_{1}(g_{2}, g_{-1}(g_{2})))$  few pass obliquely. It is very rich, composed of vessels smaller and more uniform in size than those of the submucous plexus. They range from 10 - 75um, with the majority less than 50um.

### <u>Spatial Variations in Mucosal Plexus</u>

The richness of mucosal plexus varies with that of the submucosal plexus as the vessels become smaller and more numerous distally although the range is less. The separation of the two plexuses, assessed by stereoscopic microscopy of cleared specimens, appears to be constant throughout the stomach. In three cleared edge sections it was found to be 500um, whilst in five paraffin embedded histological sections it was 200um.



<u>Fig. 8</u> Topological diagram of intramural vessels in stomach wall of cat.

¥



<u>Fig. 9</u> C15GC. Vessel passing through muscularis mucosae. The trilaminar structure of the muscularis is shown (64x).



Fig. 10 C15GC. Vessel within muscularis mucosae (100x).

#### <u>Network Analysis</u>

A comprehensive network analysis of the entire submucous and mucosal plexus was conducted in Cat 15 by mapping as described in 'Materials and Methods'. The results of this analysis were consistent with the overall description from all the specimens, but quantified them. In addition, it revealed some phenomena that were not obvious visually (Table 5). The number of inputs was fairly constant from oesophagus to pylorus, but with more in the greater curvature half. The diameter of the plexus inputs and their area of supply fell distally. However, the total cross sectional area of the anastomoses fell very markedly, particularly in the pylorus, due to a reduction in diameter, rather than in number (anastomotic density increases). As a result of these changes, the Connectivity Index and the CI:PI ratio was particularly low in the pylorus of both lesser curvature and greater curvature halves. This was particularly evident in the greater curvature, where the PI, ratio rose distally, CI and CI:PI before falling dramatically in the pylorus. Despite this, the values in the greater curvature half were consistently larger than in the lesser curvature half. In addition despite the relative poverty in the pylorus and lesser curvature half, the indices were all fairly high, and the plexus was an efficient flow distribution system since the CI:PI ratio was good throughout the stomach, with many vessels have a ratio near unity, and no vessels with extremely low values.

### <u>Mucosal Arteries</u>

The mucosal arteries arise exclusively from the mucosal plexus. They are small vessels, less than 20um in diameter and are present in large numbers. They begin to run towards the mucosal surface, either immediately or after a short horizontal course, but soon ramify near the base of the mucosa to form a large number of arterioles and capillaries. These run superficially to enter the surface venous plexus, which is drained by thick collecting veins (well seen in specimen C12 lesser curvature). The fine arterial vessels,

	PYLORUS PRE-PYLORUS		CORPUS	FUNDUS	
LESSER CURVATURE			· · · · · · · · · · · · · · · · · · ·		
No. Inputs	9	7	10	7	
D <sub>i</sub> (um)	124 <u>+</u> 18	129 <u>+</u> 16	168 <u>+</u> 45	190 <u>+</u> 63	
Area (mm <sup>2</sup> )	24 <u>+</u> 14	26 <u>+</u> 10	93 <u>+</u> 55	106 <u>+</u> 84	
$(D_a)^4$ (um <sup>4</sup> x 10 <sup>8</sup> )	0.3 <u>+</u> 0.3	0.5 <u>+</u> 0.4	2.2 <u>+</u> 1.5	3.1 <u>+</u> 2.7	
Anast.cm <sup>-2</sup>	22	23	12	6	
Mean D <sub>a</sub> (um)	38 <u>+</u> 34	35 <u>+</u> 31	51 <u>+</u> 43	62 <u>+</u> 45	
Connectivity I'x	151 <u>+</u> 102	175 <u>+</u> 113	223 <u>+</u> 78	280 <u>+</u> 86	
Perfusion Index	1522 <u>+</u> 1368	1376 <u>+</u> 797	1434 <u>+</u> 1288	2065 <u>+</u> 1635	
CI:PI	0.14 <u>+</u> 0.08 0.20 <u>+</u> 0.18 0.36 <u>+</u> 0.41		0.35 <u>+</u> 0.46		
GREATER CURVATURE					
No. Inputs	16	18	28	13	
D <sub>i</sub> (um)	121 <u>+</u> 25	149 <u>+</u> 41	178 <u>+</u> 31	211 <u>+</u> 63	
Area (mm <sup>2</sup> )	20 <u>+</u> 14	26 <u>+</u> 20	57 <u>+</u> 52	128 <u>+</u> 67	
$(D_a)^4$ (um <sup>4</sup> )	0.6 <u>+</u> 0.7	2.6 <u>+</u> 2.2	3.4 <u>+</u> 2.1	4.5 <u>+</u> 2.3	
Anast.cm <sup>-2</sup>	25	5	15	7	
Mean D <sub>a</sub> (um)	43 <u>+</u>	27	60 <u>+</u> 46	62 <u>+</u> 54	
Connectivity I'x	328 <u>+</u> 259	1331 <u>+</u> 844	678 <u>+</u> 237	387 <u>+</u> 212	
Perfusion I'x	1639 <u>+</u> 1501	3044 <u>+</u> 2303	2702 <u>+</u> 1943	2597 <u>+</u> 2951	
CI:PI	0.37 <u>+</u> 0.60	0.65 <u>+</u> 0.80	0.41 <u>+</u> 0.44	0.31+0.36	

#### NOTES

 Total number of inputs to submucous plexus.
 D<sub>i</sub> = mean diameter of inputs to submucous plexus (um).
 Area = area of supply of single input, defined by area bounded by contour joining points of minimum submucous plexus anastomotic diameter (mm<sup>2</sup>).
 (D<sub>a</sub>)<sup>4</sup> = sum of fourth power of all anastomoses (submucosal and mucosal) of a single input (um<sup>4</sup>)
 Density = number of all anastomoses per centimetre.
 Mean D<sub>a</sub> = mean diameter of all inputs (um).
 Connectivity I'x. = Connectivity Index - (Da)<sup>4</sup>/Area (um<sup>4</sup> mm<sup>-2</sup> x 10<sup>4</sup>)
 Perfusion I'x = Perfusion Index - (D<sub>i</sub>)<sup>4</sup>/Area (um<sup>4</sup> mm<sup>-2</sup> x 10<sup>4</sup>)
 <u>Table 5 Parameters of Total Plexus (Submucous and Mucosal)</u> <u>in Entire Specimen of Cat 15</u>

derived from different mucosal arteries, appear to anastomose with one another, and no unfilled interval is seen between capillary beds. Stereoscopic microscopy of the cleared specimens, with tilting and micromanipulation, confirmed that anastomosis, rather than merely overlap, between the vessels did occur. No "mucosal arteries of extramural origin" as described in man by Piasecki (1974), were seen in the cat. All the mucosal arteries arose from the rich mucosal plexus. Nor were any very low connectivity vessels (VLC's) found. Additionally, all the input vessels to the submucous plexus were seen to have extensive anastomoses at both the submucous and mucosal plexus levels with the vessels deriving from adjoining inputs. The number and size of the anastomoses varied, particularly in the submucosa, but were always significant. Thus, in the cat there are two plexuses to distribute blood flow, with fewer regional variations than in some species and with no observable vessels excluded from the system. (Figs / (-1))

REGION	FUNDUS			CORPUS (GC)			A	ANTRUM		
Area	1	2	3	1	2	3	1	2	3	
Measurement										
MA's/6.25mm <sup>2</sup> (n)	256	223	269	153	172	184	335	372	321	
Mean (n)	249 <u>+</u> 23			170 <u>+</u> 7			3	343 <u>+</u> 15		
MA density (MA's mm <sup>-2</sup> )	40 <u>+</u> 3			27 <u>+</u> 2				55 <u>+</u> 4		
MA supply area (mm <sup>2</sup> x 10 <sup>-2</sup> )	2.5 <u>+</u> 0.2		3.7 <u>+</u> 0.3			1	1.8 <u>+</u> 0.09			

- <u>NOTES</u>
- 1. 10 measurements were made in each of 3 parts of the fundus, corpus and antrum.
- 2. The number of MA's per 100 graticule squares (=6.25mm<sup>2</sup>) was measured.
- 3. All means were expressed <u>+</u> one standard deviation.
- 4. The number of MA's in the antrum was significantly greater than in the corpus (p < 0.1)

# Table 6 Number, Area of Supply and Density of Mucosal Arteries in Cat 13

. . . .

Spatial Variations in Mucosal Arteries (Tables 6-7)

The diameter and area of supply of the mucosal arteries is not uniform throughout the stomach. The area varies between approximately 0.02 and  $0.16 \text{mm}^2$ , with most less than  $0.06 \text{mm}^2$ . the largest mean area is in the lesser curvature  $(0.06 \pm 0.04 \text{mm}^2)$ . The diameter varies between 6 and 19 um, with most being less than 13 um. The smallest vessels are in the fundus ( $8.4 \pm 2.3 \text{ um}$ ). The region with the poorest perfusion index is therefore the fundus, with a value approximately 30% of the lesser curvature and antrum. The mucosal arteries in the lesser curvature, though sparse, are large ( $12.3 \pm 4.2 \text{ um}$ ). Conversely, those in the antrum, though smaller, are densely packed.

# Arteriovenous Anastomoses

Evidence of arteriovenous anastomoses was sought in all specimens, but no direct communication between the arteries and the veins could be found at any level, even where venous filling had occured. The use of stereoscopic microscopy with manipulation and specimen tilting showed that all apparent anastomoses were actually overlapping vessels.

# Duodenum

In general, the duodenum did not fill well. In only one specimen were the first 7-8cm well filled (C12). In three others, several centimetres were unevenly filled (C5;C10;C11) whilst in two (C13;C14) only the first centimetre was filled. Thus , the following features were present in the first 4-5cm of duodenum.

The duodenal muscle is very thin and it was not possible to describe its vasculature. The connective tissue plexus was not visible. The inputs to the submucous plexus appear to pass directly through the muscle from the external trunks, and are orientated randomly for the first 0.5cm distal to the pylorus. Beyond this point, they become organised into three lines on the dorsal duodenal wall. The two outer lines give off scalariform branches around the duodenum whilst the central line of inputs branch in a
	Diameter (um)	Area of Supply (mm <sup>2</sup> x 10 <sup>-2</sup> )	Perfusion Index (um <sup>4</sup> mm <sup>-2</sup> x 10 <sup>4</sup> )
FUNDUS	6.3 10 6.3 6.3 10 6.3 10 12.5 10 6.3	2.5 6.2 1.9 2.5 1.9 1.9 3.1 10.6 5 2.5	6 16 8 6 53 8 32 23 20 6
MEAN FUNDUS	8.4 <u>+</u> 2.3	3.8 <u>+</u> 2.8	18 <u>+</u> 15
CORPUS (LC)	6.3 12.5 18.8 6.3 18.8 12.5 10 12.5 12.5 12.5	2.5 6.3 15.5 6.3 6.3 6.3 2.5 6.3 5 2.5	6 39 81 3 198 39 40 39 40 39 49 98
MEAN CORPUS	12.3 <u>+</u> 4.2	5.9 <u>+</u> 3.8	59 <u>+</u> 57
ANTRUM	6.3 6.3 12.5 12.5 10 12.5 12.5 12.5 12.5 12.5 12.5	2.5 3.1 2.5 1.9 3.1 1.9 3.8 1.9 3.8 2.5	6 5 6 128 79 53 64 128 64 98
MEAN ANTRUM	10.4 <u>+</u> 2.9	2.7 <u>+</u> 0.7	63 <u>+</u> 47
MEAN WHOLE	10.4 <u>+</u> 3.5	4.2 <u>+</u> 3.0	46.7 <u>+</u> 46.8

	DENSITY OF MA's (mm <sup>-2</sup> )
FUNDUS CORPUS (LC) ANTRUM	26 17 37
WHOLE STOMÁCH	24

<u>NOTES</u>

The diameter and perfusion index are significantly greater in the lesser curvature than the fundus. The area is greater in the lesser curvature than the antrum. (p < 0.05)

Table 7 Parameters of Mucosal Arteries in Cat 14

stellate pattern, having a small area of supply. In contrast, within the first 0.5cm of duodenum, the branching pattern appears to be random.

Although the degree of anastomosis is high over the length of the duodenum studied, virtually all the anastomoses appear to be within the mucosa i.e. deep to the muscularis mucosae. Only the larger, initial branches are within the submucosa. Oblique vessels pass continuously from the submucosa to the mucosa. This was supported by longitudinal sections of cleared duodenum, but the extreme thinness of the layers renders a definitive judgement very difficult.

In those areas which were well filled (especially C12), the villous arterial vasculature had a fountain pattern. The plexus gives off large mucosal arteries, each of which supplies one villus, rising centrally to its tip and branching in a fountain pattern. These vessels drain into a venous plexus at the base of the villus, deep to the arterial plexus.

#### DISCUSSION

The gross structure of the 9 cat stomachs was in accord with the consensus in the literature. However, two principal differences were seen. Firstly, there was no clearly defined pyloric sphincter in any of the specimens. A region of reduced diameter 5cm long could be seen externally but longitudinal sections of the gastroduodenal junction showed only a generalised thickening of the circular muscle, with no clearly defined sphincter. This is in contrast to the texts of Trautman & Fiebiger (1957) and Crouch (1969) which describe a pyloric sphincter. However, the descriptions may be derived from the normal mammalian pattern and not be specific to the cat. Secondly, the lesser omentum diverged onto the dorsal wall in the distal corpus, a feature not noted previously.

The arrangement of vessels immediately adjacent to the stomach was generally in agreement with textbooks typified by Crouch(1969) but some discrepancies and ommisions were noted. The paucity of inputs directly to the lesser -----curvature, between the lesser omental attachments, is new to the literature, as is the dorsal bias of the dorsal line of inputs from the left gastric artery. Indeed, Crouch did not mention any inputs in the omentum reflected upon the dorsal corpus. According to Crouch, the anastomosis between the left and right gastric arteries occurred over the antrum, whilst in 5 of 7 specimens in this work it occurred over the duodenum. Thus, variants may occur. Indeed, since Crouch's text is not based on any experimental observations, this study indicates that the anastomosis is most likely to be on the duodenum. By contrast, it was found over the antrum in man (Michels 1955). Thus, this study suggests that the left gastric artery in cat has a greater area of distribution than in man, where the it usually fails to reach the pylorus.

An important characteristic of the cat's intramural vasculature demonstrated by this study is the presence of two quite distinct arterial plexuses - the well known submucous plexus within the submucosa and a second plexus at the base of the mucosa. The latter was not described by Trautman & Fiebiger and was only mentioned obliquely by Svanes (1975). Previous authors did not recognize the differentiation of two plexuses, since they did not subject the vessels in cleared specimens to micromanipulation. The criteria to differentiate a true plexus from anastomoses of branches have been described previously. On this basis, these vessels constitute a true plexus. It could be argued that this deep plexus comprises anastomotic branches of mucosal arteries. However, since its vessels were wider than their branches to the mucosa, it would be more logical to consider them as a plexus in their own right, with the smaller branches to the mucosa being mucosal arteries proper. This feature is not seen in Man, where only anastomoses between mucosal arteries are seen in the mucosa (Piasecki 1973).

The detailed network analysis of the plexuses in C15 revealed a comparative pyloric and lesser curvature poverty,



Fig. 11 Submucous and mucosal plexuses in corpus of C10GC (9x).



Fig. 12 Same area as above with submucous plexus removed to show presence of extensive mucosal plexus (9x).

which would not have been revealed by an analysis of anastomotic density or area of supply alone. However, even in the sparser regions, the plexus was still comparatively effective as a redistribution system.

Svanes' results (1975) are consistent with this study. He found that most microspheres (15+-5um) were trapped in the mucosa and least in the submucosa. This would be predicted from the extent and size of the plexus vessels and mucosal arterioles, in relation to the spheres, most of which would be too large to enter the mucosal arteries.

The vasculature in the region of the gastroduodenal junction is similar to that in Man, with continuity between the gastric and duodenal plexuses, although there is some degree of duodenal 'dominance' as the anastomosis is on the gastric side of the gastroduodenal junction. In the duodenum itself however, there appear to be few arterioarterial anastomoses in the submucosa, the vast majority being at the mucosal level. This differs from the description by Trautman & Fiebiger.

The dominance of the subserous plexus in the supply to the muscularis externa was not reported by Trautman & Fiebiger. The extensive intramuscular plexus seen in Man (Piasecki 1973) was only observed in the corpus in the this study, and was sparse even there. Similarly, the connective tissue plexus was not described by Trautman & Fiebiger but was shown by this work to be an entity distinct from the submucous plexus.  $(F_i y, \mathcal{F})$ 

# CHAPTER 13 THE PRIMATE

# NON-VASCULAR GASTRODUODENAL ANATOMY

### General Appearance

The primate stomach is at its widest at the fundus, then tapers continually to the gastrodudenal junction. The shape differs noticeably between different specimens. In four of seven specimens, the oesophagus entered on the ventral aspect of the stomach. In three of the remainder, it entered on the lesser curvature, whilst in the fourth, its entry point was indefinable as the stomach and duodenum were not coplanar. The incisura angularis was clearly definable in four of eight of the specimens but diffuse in the remainder. Finally, there was a marked constriction at the gastroduodenal junction.

### Omental Attachments

The omental lines of attachment were consistent in the eight whole stomachs and one isolated muscle specimen examined. The two layers of lesser omentum reach the stomach on either side of its rostral edge, delineating a relatively narrow lesser curvature, about 1cm in width. It extends from the fundus, ventrally around the oesophagus, then continues to a point approximately 1 cm beyond the gastroduodenal junction.

By contrast, the attachments of the two layers of the greater omentum are contiguous, delineating a very narrow greater curvature. This is not coincident with the caudal edge of the stomach, but extends from the dorsal cardia , across the dorsal fundus and corpus, to meet the rostral edge of the stomach at the level of mid-corpus. It then continues distally to a point 0.5-1.0cm beyond the

gastroduodenal junction. The greater and lesser omenta are united at the pylorus by a ring of mesentery which completely encircles the stomach at that point.

### <u>Muscularis</u> Externa

Most of the muscularis externa has a circular orientation. Two wide but poorly defined longitudinal bands run on the submucosal aspect of the muscularis externa, one on either side of the lesser curvature, half way between the lesser curvature and the mid-ventral and mid-posterior walls. In addition oblique fibres radiate from the cardia to the middle of the ventral walls.

The thickness of the muscularis externa gradually increases distally with a sudden marked change at the incisura angularis and between the middle and the last third of the pyloric antrum. There is also a pronounced sphincteric thickening of circular muscle at the pylorus. The duodenal muscularis externa is much thinner and forms a slight ampulla although there appears to be no peri-pyloric recess.

### <u>Micellaneous</u>

The muscularis mucosae and mucosa were examined histologically in three specimens (P2;P21;P23.). The muscularis mucosae consists of approximately five layers of cells, which have a uniform orientation, parallel to the surface of the mucosa. The thickness varied between 35 - 40 um. However, there were isolated "peaks" of smooth muscle and connective tissue cells penetrating the mucosa to a depth of 23-80um. Vessels, could be seen within the muscularis mucosae and were equal to, or less than, the corresponding submucous vessels in diameter. The mucosa was fragmented in these sections, but varied in thickness between 450-575um (447/2) and 616-836um (447/4).

Cleared stained edge sections, taken from the corpus of P21 & P23, were examined. The dimensions were uniformly greater than in the histological specimens, the muscularis mucosae varying between 70-78um and the mucosa being approximately 780um thick.

. -

185

#### EXTRAMURAL VASCULATURE

The lesser curvature and adjoining areas of the stomach are supplied as far as the incisura angularis by the left gastric artery. Individual branches arise from its main trunk in a "tree-like"pattern and pass in the lesser omentum to the stomach. The narrow lesser curvature is delineated by two lines of inputs, just dorsal and ventral to the rostral edge of the stomach, which almost immediately enter the muscularis externa. There appear to be no inputs penetrating the stomach on the lesser curvature ie. between the lines of mesenteric attachment. A second population of input vessels runs subserosally on the ventral aspect from the vicinity of the lesser curvature, to pierce the muscularis externa by a 90<sup>0</sup> degree turn, approximately two-thirds of the way to the middle of the ventral wall. These do not appear to be matched on the dorsal wall. The mesentery around the base of the oesophagus contains few vessels and none appear to pass from the stomach up the oesophagus.

The two lines of input on either side of the lesser curvature continue distal to the incisura angularis, but are derived from the right gastric artery, a branch of the hepatic artery. In only one of thirteen specimens could any extramural continuity between left and right gastric arteries be seen, other than very small branches of the mesenteric adipose tissue. In specimen P25, two chains of inputs were united by an anastomotic vessel whose minimum diameter was immediately proximal to the incisura angularis. In the remainder of the cleared specimens (3), the demarcation between the areas of supply of the left and right gastric arteries was at early or mid antrum, distal to the incisura.  $(Talr/e \vartheta)$ 

The single chain of inputs on the greater curvature pierce the stomach with little or no subserous course along the line of attachment of the greater omentum. This line begins on the dorsal aspect of the gastro-oesophageal junction, crosses the dorsal fundus, and reaches the greater curvature at mid corpus. It then continues along the greater curvature to approximately 1 cm beyond the gastroduodenal

186

junction. The inputs arise from an anastomotic vessel within the mesentery. This runs parallel to the surface of the stomach and is formed by the anastomosis of the left and right gastroepiploic arteries. The latter reaches the duodenum approximately one centimeter distal to the gastroduodenal junction and is derived from the common hepatic artery.

At the gastroduodenal junction, there is an incomplete ring of extramural vessels, derived from the right gastric and gastroepiploic arteries. In only one specimen of thirteen (P26) did the ring appear to be complete. In all specimens, each hemi-annulus gave off branches to both stomach and duodenum.

	P23	P24	P25	P26
Extramural Anastomoses			+	-
Min. diameter of extramural anastomoses (um)	-	-	100	-
Other anastomoses	Few:small Mesenteric adipose tissue	Subserous Small	Subserous Small	Subserous Small

# <u>Table 8 Anastomoses Between Left Gastric Artery & Right</u> <u>Gastric Artery in Cleared Primate Stomachs</u>

#### INTRAMURAL VASCULATURE

# Supply to Muscularis Externa

Four cleared specimens were examined. The extramural inputs turn through 90° at the end of their subserous course and enter the muscularis. Branches arise at this point which give rise to a subserous plexus, whose component vessels are mainly less than 50um in diameter and tend to have a longitudinal orientation. This plexus gives rise to perpendicularly running vessels which ramify throughout the muscularis, parallel to the muscle fibres. The perpendicular branches of the extramural vessels themselves have comparatively few intramural branches, other than those which form the subserous plexus so there is no significant deep muscular plexus. All the penetrating arteries pass to the submucous plexus, which is also supplied by vessels from the subserous plexus. Additional supply to the muscularis is received from recurrent vessels from the submucous plexus.

# <u>Spatial Variations in Supply to Muscularis Externa</u>

The vascular architecture appears to be fairly uniform. The inputs to the proximal part of the greater curvature tend to have a longer subserous course than those on the distal greater curvature, although always much shorter than those on the lesser curvature.

At the gastroduodenal junction, the marked sphincteric thickening of circular muscle is supplied by intramural branches from the extramural hemi-annulus of vessels.

### Submucous Plexus

It proved difficult to obtain uniformly well-filled but not over-filled, specimens of the primate gastric submucosa and mucosa. Many were filled inadequately and unevenly. However, it was possible to compile the following description from the observation of the whole of five good specimens (P23;P24;P25;P26;P27) and from parts of others (particularly P7;P11;P12:P13). (Fiys/3-/6)



Fig. 13 Whole cleared stomach (P24). Duodenum at left. Note area of reduced vascularity on the lesser curvature (centre) field and the gastroduodenal junction to the left (1x).



Fig. 14 Whole cleared stomach (P27). Note the branching of the major scalariform inputs and the stellate vessels in the central lesser curvature (1x).



Fig. 15 Whole cleared stomach (P26). Note the stellate vessels of the central lesser curvature and the gastroduodenal junction at the bottom left (1.4x).



Fig. 16 First part of duodenum of P24. Note central stellate vessels and lateral scalariform branching pattern (3x).

The submucous plexus is supplied by two groups of inputs, penetrating artery branches and vessels from the subserous plexus. These inputs turn through approximately 90<sup>0</sup> in the submucosa then course obliquely through the submucous connective tissue, bridging the mucosal folds, before penetrating the muscularis mucosae to form a second plexus at the base of the mucosa. The sparse submucous plexus they form therefore does not have a distinct plane but is spread throughout the depth of the submucosa. Its constituent vessels are large (most are greater than 40 um in diameter) and there are few nodes and arterioarterial anastomoses. Indeed a large proportion of the vessels enter the mucosa before they have made any arterioarterial anastomoses at all. Thus, in the primate, the submucous plexus is sparse and is <u>not</u> the main blood distribution system in the wall of the stomach. (Figs. 22-23)

# <u>Spatial Variations in Submucous Plexus</u>

Although no extramural inputs are visible within the lesser curvature, some are found in the submucosa. They are less than 140um in diameter, and have a small area of supply. Their few branches are orientated longitudinally, except where they pass around the base of the oesophagus. However, most of these inputs have no anastomoses in the submucosa since, after a short submucosal course, they pierce the muscularis mucosae and enter the mucosa, especially around the incisura angularis.

Dorsal and ventral to the lesser curvature vessels are two lines of larger inputs (approximately 300um diameter) which delimit the lesser curvature. They meet the mid-line of the lesser curvature at the oesophagus and middle of the antrum. The branches of these vessels are mainly orientated circumferentially, mostly away from the lesser curvature. There are few anastomoses within the submucosa, as the main vessels pass obliquely through to reach and penetrate the muscularis mucosae. During their course , they give off smaller branches, many of which turn through 90°, pierce the muscularis mucosae, and enter the mucosal plexus directly, without submucosal anastomoses.

The largest inputs to the submucous plexus have a diameter of approximately 500um, and are found at the middle of the dorsal and ventral walls. Virtually all these vessels' branches are directed towards the greater curvature, and they anastomose within the submucosa with branches from the greater curvature. The mid-dorsal and mid-ventral inputs also supply the fundus.  $(f_iy_i)$ 

The greater curvature inputs comprise two chains, separated by 1-2mm. These have a diameter of approximately 200um and their branches are directed away from the greater curvature. Some of these anastomose in the submucosa with branches from the mid-dorsal and mid-ventral inputs, but others pass directly to the mucosa.



<u>Fig. 17</u> Gastroduodenal junction in P26. Leashes of vessels cross the junction, contributed to equally by gastric and duodenal vessels. Note the transverse arcade of vessels in the stomach (top) (6.5x).

It is noteworthy that an area of greatly reduced vascularity was consistently seen on the lesser curvature around the incisura angularis. The inputs were small, with a few small, or no, submucous anastomoses. Most of the anastomoses were in the mucosa but these too were few and small Although the contrast between the injected vessels and the surrounding tissue was poor, examination of the area at high magnification showed that there were few significant unfilled vessels. Dissection and examination of overlying muscularis externa confirmed that no larger submucosal vessels had been removed with the muscle layer.  $(f_{ij}, ig_{ij}, ig_{ij})$ 

There are few submucous vessels running between the stomach and duodenum or stomach and oesophagus.

# <u>Connective</u> <u>Tissue</u> <u>Plexus</u>

The connective tissue plexus is located between the deep surface of the muscularis externa and the submucous plexus and is very sparse, displaying no obvious regularity of structure. Its vessels are considerably less than 40um diameter and have few anastomoses. Recurrent vessels from the submucous plexus to the muscularis externa pass through the connective tissue plexus and, in some cases, anastomose with its component vessels.

# Spatial Variations in Connective Tissue Plexus

The richness of the connective tissue plexus varies in proportion to the amount of connective tissue present. Thus, it decreases from proximal to distal and is at a minimum on the greater and lesser curvatures. It is virtually absent on the distal lesser curvature.

# <u>Mucosal Plexus</u>

The mucosal plexus is the main vascular network in the stomach, as the majority of arterioarterial anastomoses are at this level. However, the system is comparatively sparse compared to some other species, such as the cat. An examination of stained sections of cleared tissue (15 sections from four specimens) confirmed that the plexus is



Fig. 18 Area of good scalariform anastomoses from posterior wall of corpus (P26) (3.2x).



Fig. 19 Area of poor stellate anastomoses from central lesser curvature of P26 (3.2x).

located deep to the muscularis mucosae and is formed by oblique and perpendicular branches from the submucous vessels. These penetrate the muscularis and supply it with small nutrient vessels. The vessels of the mucosal plexus do not have a uniform orientation and are all less than 40um diameter (usually less than 20um).

# Spatial Variations in Mucosal Plexus

There are no marked spatial variations in the mucosal plexus. The vessel diameters appears to be constant throughout the stomach. Although the density seems to increase distally, it does not vary circumferentially.

The mucosal plexus has a specialised structure at the gastroduodenal junction which could be observed in seven  $(F(\phi)/F(f))$ specimens (P3;P11;P23;P24;P25:P26;P27) [ In all these specimens, a number of longitudinal "leashes" of vessels (approximately 20-40um) crossed the gastroduodenal junction, and all were shown by stereoscopic microscopy, with tilting and micromanipulation, to be constituents of the mucosal plexus. These leashes received equal inputs from the gastric and duodenal vessels which anastomosed at the mid-point of the gastroduodenal junction. However, the supply to the leashes differs in the stomach and duodenum. In the stomach, they arise from the transverse arcade of vessels derived from inputs to the submucosa. In the duodenum, the leashes arise directly from the submucous inputs. The distance between the two sets of inputs is approximately 5mm.

The gastro-oesophageal junction also shows a specialised mucosal plexus, discernible in seven specimens (P7;P11;P12;P23;P24;P25:P26;). The base of the oesophagus is surrounded by circumferential submucous vessels which give off branches to the mucosal plexus. Although no large vessels pass across the gastro-oesophageal junction, there are anastomoses on the oesophagus between vessels of the gastric and oesophageal mucosal plexuses.

195



Fig. 20 Low connectivity vessel from central lesser curvature of P24. Note low connectivity is due to reduced vessel diameter rather than number (50x).



Fig. 21 Low connectivity vessel from antrum of P26. Note stellate branching pattern and very narrow anastomoses (40x)



Fig. 22 Surface view of corpus in P26 showing main input vessel and anastomoses (30x).



Fig. 23 Cleared edge section corresponding to above surface view. Note that all anastomoses are in the mucosal plexus (50x).

# <u>Mucosal Arteries</u>

Mucosal arteries were visible in seven specimens (P2;P7;P12;P14;P22;P25;P26) and varied between 12-25 um in diameter. They arose directly from the mucosal plexus, and ramified near the base of the mucosa to form a capillary plexus. The contrast between the injected vessels and the surrounding tissue was poor, so it was not possible to ascertain whether anastomosis or overlap occured between the vessels derived from different mucosal arteries.

The arterial capillaries drained into a venous plexus at the mucosal surface. This was drained by perpendicular collecting veins (approximately 20um diameter) which gave rise to a venous plexus at the base of the mucosa, parallel to the arterial plexus.

No mucosal arteries of extramural origin (Piasecki 1973) were seen in the primate. However, the extent to which any given submucosal input communicates with other inputs at the submucosal or mucosal level varies greatly. Thus, some vessels communicate with their neighbours only by very narrow anastomotic vessels and only at the mucosal level.

# <u>Spatial Variations in Mucosal Arteries</u>

The diameters and areas of ten mucosal arteries were measured in each of three areas of the stomach of one specimen (antrum, fundus and corpus lesser curvature). No significant spatial variations were seen. The mean diameters varied between 16 and 19 um and the mean areas between 7 and 10 x  $10^{-2}$  mm<sup>2</sup>. None of the areas displayed a significantly inferior perfusion index. (Tab/e 9)

### Arteriovenous Anastomoses

Despite a careful search of the specimens, no evidence of direct arteriovenous anastomoses was found. However, the often incomplete degree of injection rendered it impossible to state definitively that such structures were not present in the specimens studied.

	Diameter (um)	Area of Supply (mm <sup>2</sup> x 10 <sup>-2</sup> )	Perfusion Index (um <sup>4</sup> mm <sup>-2</sup> x 10 <sup>4</sup> )
FUNDUS	25 12.5 12.5 12.5 12.5 12.5 25 12.5 25 12.5	12.5 6.3 6.3 3.1 9.4 6.3 3.1 12.5 9.4	313 39 39 78 78 26 625 78 313 26
MEAN FUNDUS	16.3 <u>+</u> 6.0	7.2 <u>+</u> 3.6	161 <u>+</u> 197
CORPUS (LC)	25 25 12.5 12.5 12.5 25 12.5 25 25 12.5	6.3 9.4 12.5 6.3 9.4 18.8 6.3 6.2 5 9.4	625 416 19 39 26 208 39 635 781 26
MEAN CORPUS	18.8 <u>+</u> 6.6	8.9 <u>+</u> 4.1	282 <u>+</u> 304
ANTRUM	1.3 10 12.5 25 12.5 25 12.5 12.5 25 25	9.4 6.3 6.3 18.6 6.3 21.9 6.3 9.4 12.5 6.3	<0.1 16 39 210 39 179 39 26 313 625
MEAN ANTRUM	16.1 <u>+</u> 8.3	10.3 <u>+</u> 5.7	148 <u>+</u> 197
MEAN WHOLE	17.0 <u>+</u> 6.9	8.8 <u>+</u> 4.6	197 <u>+</u> 238

	DENSITY OF MA'S (mm <sup>-2</sup> )
FUNDUS CORPUS (LC) ANTRUM	14 11 10
WHOLE STOMACH	11

# <u>NOTES</u>

.

There are no significant differences in the spatial variations of any of these parameters.

Table 9 Parameters of Mucosal Arteries in Primate 26

#### Duodenum

There are two lines of inputs, continuous with the gastric greater and lesser curvatures. The branches of these inputs are all directed predominantly onto the ventral walls of the duodenum. The dorsal wall is covered by small inputs whose branches are omnidirectional.

The connective tissue plexus ends just proximal to the gastroduodenal junction and is, therefore, not seen in the duodenum. The submucosal plexus is sparse, most of the visible vessels being located in the mucosal plexus i.e. deep to the muscularis mucosae. This was confirmed by stereomicroscopy and by examination of stained sections of cleared specimens. The mucosal arteries arise from the mucosal plexus and ramify near the base of the mucosa. The relatively few villi which were filled displayed a "fountain" pattern of arterial vasculature i.e. a central arterial vessel which divided to form a capillary network at the tip of the villus. However, there is insufficient evidence to enable this pattern to be described as the sole, or main, vascular arrangement, although no contradictory patterns of filling were observed.

#### DISCUSSION

The overall description of the primate was based upon the observation of thirteen specimens, and so has a good degree of validity. The intramural account was derived from fewer specimens, since it proved difficult to obtain good injections, which were neither under nor overfilled. This problem applied equally to all areas of the stomach, not merely to the antrum as was the case in some other species. The reason for the problem is not known. A factor may be the necessity, for quarantine reasons, to inject all the







<u>Fig. 25</u> Cleared edge section of above specimen. Note central arteriole with capillary ramification at tip of villus (51x).

primates immediately post mortem rather than after 24 hours. However, a comparison between the two injection modes in the cat showed no differences in results.

The extramural vascular pattern was generally the same as in man. The left gastric artery, though important, did not have the dominance it has in some other species, such as the rat. However, one major difference was noted, that the left and right gastric arteries rarely anastomosed (3% in their sample). This was confirmed in the present work, where anastomosis occured in only one of thirteen animals. The left gastric artery supplied the lesser curvature and adjacent regions as far as the incisura angularis or, in three specimens, as far as the mid antrum. The right gastric supplied the remainder of the lesser curvature and the proximal duodenum. By contrast, a clear anastomosis was seen in all the animals on the greater curvature, between the left and right gastroepiploic arteries (as in Osemlak (1968) where an 81% anastomosis incidence was found). A specific pattern was seen at the gastroduodenal junction, where a ring of vessels, derived from the right gastric and gastroepiploic arteries, ran around the junction. However, anastomosis occured in only one specimen, the ring being incomplete in the remainder. This feature does not appear to have been specifically noted in the literature.

The limited success in obtaining good injections has already been discussed but sufficient were available (5 good plus 4+ partial) to give confidence in the validity of the description of the intramural vasculature. Since no data is available in the literature, all the information presented is novel. The most important difference from the classical pattern is the observation that the submucous plexus is not the main blood distribution system in the wall of the stomach and duodenum. This role is fulfilled by the mucosal plexus. The submucosal plexus is coarse, with large vessels and few nodes. It does not have a clearly defined plane, as the constituent vessels pass obliquely through the compartment, after their formation from the input vessels. However, many vessels make no arterioarterial anastomoses at all, before penetrating the muscularis mucosae.

By contrast, most of the arterioarterial anastomoses are within or deep to the muscularis mucosae - it is the main blood distribution system, although it is still comparatively sparse compared to some other species, such as the Cat. It is noteworthy that an area of distinct reduced vascularity was seen on the lesser curvature around the incisura angularis. In both the submucous and mucosal plexuses, anastomoses were smaller and fewer. By examining the area under high magnification, it was possible to see poorly filled vessels, giving confidence that the observed real manifestation of phenomenon was a a reduced vascularity. The inputs in this area are derived almost exclusively from the small vessels which penetrate the muscularis externa between the lines of attachment of the lesser omentum. The branches derived from the two lines of larger vessels running in the omentum are mostly directed towards the greater curvature. Thus, both these factors tend to reduce the input of blood to, and distribution within, the lesser curvature. However, as is discussed below, the mucosal arteries in this area are no different from those in the other regions of the stomach. Although vessels which made no contacts with either plexus before ramifying in the mucosa (mucosal arteries of extramural origin - Piasecki 1973) could not be definitely identified, several possible candidates were seen in the area of reduced vascularity. There did not appear to be any unfilled branches, but this could not be stated with certainty. However, many very low connectivity vessels (with few and/or small anastomoses) were seen, mainly along the mid and distal lesser curvature. These had both smaller and fewer anastomoses.

The bulk of the 'true' mucosal arteries arose from the mucosal plexus, but a small number arose directly from the submucous plexus. This is in contrast to, for example, the Cat, where all mucosal arteries arise from the mucosal plexus. However, like the cat, the mucosal arteries were small, and had a poor perfusion index. Although there were no differences in mucosal artery characteristics between regions of the stomach, within the regions there was considerable heterogeneity of arteries, with a wide spectrum of perfusion indices.

Significant venous filling occurred in some specimens.Nevertheless, no evidence of any arteriovenous anastomoses could be seen, although there were many examples of vessels overlapping, which might be interpreted as communication without stereomicroscopic manipulation.However, since it has been suggested that AVA's are not patent immediately post mortem, this could explain their apparent absence in this species.

A final point of interest is seen in the muscularis externa, where no deep muscular plexus is seen. The bulk of the nutrient vessels arise solely from the subserous plexus, with some contribution from submucous recurrent vessels.

Examination of the duodenal specimens appeared to confirm the 'fountain' pattern within the villus described in the literature. However, no evidence of the direct arteriovenous communication at the tip, described by Spanner, could be seen. This could be for the same reason as the absence of gastric arteriovenous anastomoses, discussed above.

Ż,

# CHAPTER 14 THE RABBIT

### NON-VASCULAR GASTRODUODENAL ANATOMY

### **General Appearance**

The whole stomach was observed at laparotomy in all sixteen preparations. The stomach of one specimen (Rb1) was examined in detail, as was the uncleared isolated muscle of two specimens (Rb5;Rb6) and the cleared isolated muscle of one specimen (Rb4).

The stomach is elongated laterally with its long axis perpendicular to the animal's rostral-caudal axis. The fundus is particularly large as the oesophagus enters midway along the lesser curvature. The incisura cardiaca is approximately  $90^{\circ}$  and there is no clearly defined incisura angularis. There is a visible external constriction at the gastroduodenal junction, but no duodenal ampulla is visible.

### Omental Attachments

The attachments could be delineated in the whole stomach (Rb1), and the three isolated muscles (Rb4;Rb5;Rb6). There appears to be a diffuse fatty mesentery on the lesser curvature around the oesophagus which does not appear to extend as far a the gastroduodenal junction. In one specimen (Rb6), it appeared to extend further onto the ventral than the dorsal aspect. A second line of mesenteric attachment is located on the dorsal aspect of the fundus, reaching the G.C. at approximately the level of the fundus/corpus interface. From here it passes along the G.C. to reach the posterior wall of the duodenum.

### <u>Muscularis</u> Externa

The muscularis externa was examined in the two uncleared muscle specimens (Rb5;Rb6), the cleared specimen (Rb4) and the histological specimen from the corpus of Rb3. The muscularis externa over most of the stomach is very thin. The thickness from ten determinations in the corpus of Rb3 (TUVIEIU)

was 57um. h In two of the specimens, thickening was seen in the fundus at the incisura cardiaca. In all specimens there was a marked thickening in the distal pyloric antrum. This was more gradual on the G.C. aspect (over 1.3cm.) than the (746/4/1) L.C. aspect (0.6cm.) h At the pylorus the muscularis externa is approximately 4 mm thick with the diameter of the canal also being about 4 mm.

The muscularis externa consists of both longitudinal and circular layers, although both could not be discerned in all histological specimens. No longitudinal bands of fibres could be seen on the inner aspect of the muscle along the greater curvature. However it was very difficult to separate the muscle and mucosa in this region and to obtain intact muscle specimens. In one specimen (Rb4) it was noted at dissection that slips of muscle ran from the under surface of the muscularis externa to the submucosa.

### <u>Miscellaneous</u>

The gastro-duodenal junction was observed externally in all 16 specimens. In all cases a constriction was visible followed by a 180° turn by the duodenum. A lateral section showed a marked thickening of gastric muscle proximal to the pylorus particularly on the ventral aspect. There was also a pronounced circular band of muscle at, and immediately proximal to, the point of minimum diameter. No ampulla or juxta pyloric gutter was visible and the duodenal wall was much thinner than the gastric.

The overall structure of the gastric wall was examined in 17 histological sections taken from the anterior corpus of Rb3. (The mean of five measurements in each of two sections was determined. The serosa was thick (47um), comparable with the muscularis externa (57um). In some sections (eg section 5) both circular and longitudinal fibres could be seen, whereas in others (eg section 4) only fibres of one orientation could be seen. The measurement of the submucous thickness are misleading, as separation occurred in this plane. The muscularis mucosae was particularly thin (14um) but displayed a definite structure

	Serosa (um)	Muscularis Externa (um)	Submucosa (um)	Muscularis Mucosae (um)	Mucosa (um)
Rb3 Corpus	47	57	[150]	14	280
Rb6 Corpus			88		750

# <u>NOTES</u>

- 1. Serosa and muscularis externa removed from cleared edge section Rb6.
- 2. Submucosa fragmented and therefore inaccurate in histological section Rb3.

# Table 10 Thickness of Stomach Layers in Corpus of Rb3 (Histological) and Rb6 (Cleared Edge) Specimens

	Length of Thickening on LC Aspect (mm)	Length of Thickening on GC Aspect (mm)	Mean
Rb4	8	6	7
Rb5	6	14	10
Rb6	4	19	12
MEAN	6	13	10

Table 11 Length of Thickened Muscle Proximal to Gastroduodenal Junction despite extensive fragmentation . Two or three layers of differentially orientated muscle cells could be seen. They were interspersed with connective tissue cells and penetrated into the submucosa.

Sixteen whole edge sections were prepared from Rb4, Rb5 and Rb6. These permitted a poorer resolution than histological sections, and the muscularis externa and serosa had been removed. The dimensions of the submucosa, muscularis mucosae and mucosa (average of five sections-(7ak/e,o)specimen of greater curvature corpus of Rb6) were measured. Some submucosa may have been removed with the muscularis externa but nevertheless, the dimensions appear to be greater than the histological sections perhaps due to a lesser degree of dehydration and shrinkage.

The entrance of the bile duct was measured in Rb4, Rb5 and Rb6. This was greater than 5.8cm, 1.1cm and greater than 5.9cm from the pylorus respectively in the three specimens.

# EXTRAMURAL VASCULATURE (Fig. 26)

The extra-mural vessels run in the gastric mesentery. The left gastric artery approaches the stomach from its rostral-dorsal aspect and branches in a tree-like formation distal to the oesophagus. These branches run subserosally into the lesser curvature aspect of the stomach and around the oesophagus. In one (Rb5) the dorsal aspect appeared to be better supplied than the ventral, whereas the converse was true in Rb6. In the cleared muscle specimen (Rb4), it could be seen that the left gastric artery vessels stopped at the line of muscularis thickening in mid-antrum. The stomach distal to this point was supplied from the duodenal input. It was not possible to confirm or refute this in the other specimens.

There appeared to be no peri-gastric vessels running along the greater curvature. This aspect is fed by a relatively small number of vessel stems which reach the stomach on or near the greater curvature and then branch sub-serosally. A large proportion on the greater curvature half of the stomach is supplied by the left gastric artery



Fig. 26 Topological diagram to show consensus on extramural arterial supply in rabbit.

NOTES

(1) Coeliac artery may bifurcate to form splenic artery and gastrohepatic trunk <u>or</u> bifurcate to form splenic, left gastric and common hepatic arteries.

(2) In splenic hilum.

(3) First part of duodenum only.

branches. In Rb5 it could be seen that some inputs at the distal end of the stomach were supplied by the duodenal vessels.

#### INTRAMURAL VASCULATURE

# Supply to Muscularis Externa

The intra-mural arterial supply to the muscle is described mainly on the basis of the cleared muscle of Rb4, with some additional and confirmatory evidence from the uncleared muscle of specimens Rb1;Rb3;Rb5;Rb6. The branches of the extramural arteries pierce the serosa and run subserosally for varying distances, before penetrating the muscularis, either obliquely or almost perpendicularly. During their sub-serosal course, they give off lateral branches. Between entering the sub-serosal plane and penetrating the muscularis there is little reduction in vessel diameter, so the penetrating arteries are relatively large and few.

In all specimens, the arterial plexus was sparse or absent. Very small vessels were given off, which mainly ran parallel to the fibre orientation, but which made few arterioarterial anastomoses. Owing to the thinness of the muscle, it was difficult to define the depth of these vessels.

A striking feature in all the specimens were isolated stellate groups of branches on the external surface. Examination of cleared muscle (Rb4) showed that vessels arose from the deep aspect of the muscle and travelled superficially becoming smaller in diameter. These were interpreted as recurrent vessels. At the surface they branched subserosally in a stellate pattern, giving rise to a number of smaller vessels. These anastomose, if at all, only by very small vessels with branches derived directly from the extra-mural inputs.

210

### Spatial Variations in Supply to Muscle

The paucity of plexiform vessels was uniform over the entire stomach. The vessels arising from the lesser curvature had a longer mean subserosal course than those from the greater curvature. If they penetrated the muscle curvature, they near the lesser did so almost perpendicularly, whereas those which had a significant subserosal course, penetrated obliquely. The lesser curvature branches extended well beyond the mid-point of the ventral and dorsal walls. Conversely, the vessels which arose from vessels the gastroepiploic penetrate the muscle perpendicularly, on or near the gastric curvature.

In the antrum, where the muscularis becomes thicker, it was possible to define the level of the vessels. It was evident that here also, there was no significant planar plexus, with vessels branching in a tree like pattern throughout the depth of the muscularis.

### Submucous Plexus

There is no distinct planar submucous plexus in the rabbit, as there is in some other species. Large vessels pass perpendicularly and obliquely through the muscularis externa and enter the submucous compartment. They continue on their course with little deviation until they penetrate the muscularis mucosae to enter the mucosal compartment. A few large lateral branches run between these large vessels, but the majority of arterioarterial anastomoses, when examined by micromanipulation and edge sections, are within or deep to the muscularis mucosa. In most cases, large anastomosing branches are given off within the submucous compartment but at some point in their course they enter the mucosa.

In one specimen (Rb5LC) small recurrent vessels could be seen running from the submucous vessels, turning through  $90^{\circ}$ and running superficially giving rise en route to a diffuse, connective tissue plexus. In the other specimens, it was not possible to identify these vessels.

# Spatial Variations in Submucous Plexus

In all lesser curvature specimens, the pattern of inputs  $(f_{ij}, \lambda_{ij}; 2, q)$ and their branches was similar. A number of large and uniformly distributed inputs could be seen on the fundus, which tended to be orientated radially away from the oesophagus. In Rb4 and Rb5, a ring of inputs could be seen immediately at the base of the oesophagus. In Rb5 the branches ran both away from and up the oesophagus, whereas in Rb4, they only ran away from the oesophagus. The inputs on the lesser curvature had randomly orientated branches, whereas those on the dorsal and ventral walls of the corpus tended to be orientated around the circumference of the stomach and ran as far as the greater curvature itself in the region of the corpus/antrum interface. The inputs on the proximal antrum were smaller and had a stellate branching pattern. In the distal antrum they were directed rostrally i.e. they seemed to be duodenal in origin.

The pattern on the greater curvature aspect is very different. In Rb5 and Rb6, virtually the entire greater curvature, as far as the antrum/corpus interface, was supplied by branches of the lesser curvature inputs. Rb5 had only two greater curvature inputs in the corpus and Rb6 had three, all with a stellate branching pattern. Distal to the corpus/antrum discontinuity, there were more small vessels though still few in number. The distal ones were directed rostrally. By contrast, in Rb4 there was a chain of inputs along the proximal greater curvature with branches directed towards the lesser curvature as far as mid-corpus. More distally there were a few inputs with stellate branches, then an area with no greater curvature inputs, supplied from the lesser curvature. In the antrum were smaller inputs, which tended to be near the Greater Curvature, the most distal ones being directed rostrally.

# Connective Tissue Plexus

Vessels of the connective tissue plexus could be seen only in the Lesser Curvature half of Rb5, mainly in the fundus. The plexus was very sparse.

# <u>Mucosal</u> <u>Plexus</u>

In all the specimens examined, the majority of the arterioarterial anastomoses were located in the mucosal plexus at the base of the mucosa. Vessels passed obliquely or perpendicularly from the submucosal compartment, through the muscularis mucosa, to the base of the mucosa where they anastomosed with branches derived from the same or other input trunks to form a plexus. The location of these vessels was confirmed by micromanipulation and the examination of stained histological and cleared edge specimens. The plexus is coarse with a relatively small number of large vessels. The filling of the primary cells of the plexus by many small vessels seen in some other species, was largely absent.From the plexus vessels arose smaller branches which penetrated superficially into the mucosa, either directly or after a short horizontal course. These are the mucosal arteries.

A very marked feature of this plexus is a "failure to anastomose". On very many occasions two vessels which could, on the basis of size, be components of the mucosal plexus, approach one another within 5-10um, then, before touching, both turned downwards into the mucosa and ramify i.e. they are mucosal arteries. That this was not due to an unfilled intermediate vessel, was demonstrated by micromanipulation under microscopic observation and the examination of cleared edge specimens.  $(F_1, \mu_2, \mu_4, \mu_5)$ 

# <u>Spatial Variations in Mucosal Plexus</u>

The orientation of the mucosal plexus is not constant throughout the stomach. It tends to be scalariform on the dorsal and ventral walls of the corpus, and stellate, though not necessarily over  $360^{\circ}$  on the Lesser Curvature, Greater Curvature, fundus and antrum (see SMP above). There is a marked discontinuity around the corpus/antrum interface where the branching pattern changes from scalariform to (Fig3)stellate (Rb5;Rb6) and the plexus becomes sparser. This was partly due to a reduction in the number of anastomoses. From an extensive plexus into which all the input trunks are well connected (corpus), the pattern changes to isolated inputs



Fig. 27 Whole cleared stomach - Rb4. Lesser curvature. Note the change in anastomotic pattern in the antrum. Duodenum to left (1x). Diagram shows location of MAEO's no. 6 & 7


Fig. 28 Whole cleared stomach - Rb4. Greater curvature. (1x). Diagram shows location of MAEO no. 8.



Fig. 29 Whole cleared specimen - Rb5. Lesser curvature (above). Narrowing indicates gastroduodenal junction (0.7x). Below diagram showing location of MAEO no. 1.



<u>Fig. 30</u> Whole cleared specimen - Rb5. Greater curvature Narrowing shows GDJ. (0.7x). Diagram shows location of MAEO's no. 2 & 3.



Fig. 31 Whole cleared specimen - Rb6. Duodenum. GDJ to right. Note scalariform branching pattern (2.2x). Diagram shows location of MAEO's no. 5a & 5b. with a few, one, or no anastomoses with adjacent trunks (see mucosal arteries below).

The mucosal plexus has a specialised structure in the region of the gastroduodenal junction, which was seen in Rb4 Rb5. Running between the stomach and duodenum are approximately 20 leashes of vessels in the mucosal plexus. These are contributed to unequally by the duodenal and gastric parent trunks. The branches of both the gastric and duodenal vessels are mainly directed rostrally. Thus the leashes are composed of the main branches of the duodenal vessels and, if they anastomose, they do so with subsidiary backward running branches of the gastric vessels. The anastomotic point is several millimetres proximal to the line of minimum pyloric diameter. In both Rb4 & Rb5, some of the branches turned down into the mucosa before anastomosing with a gastric vessel. It should be remembered that the trunks on the antrum are directed rostrally and may have an extra-mural duodenal origin. This specialised area extends over 60-120um.

#### <u>Mucosal Arteries</u>

The mucosal arteries arise from the vessels of the mucosal plexus and run "perpendicularly" towards the mucosal surface, either immediately or after a short horizontal course. In Rb5 & Rb6, two populations of mucosal arteries could be observed. In one, ramifications occurred in a tree like pattern within the mucosa, as they ran to the surface. In the second, much less ramification occurred, with only a few branches being formed and reaching the mucosal surface. This did not seem to be due to differential filling. Only the first type could be seen in Rb4. The capillaries supplying the mucosa arise from the mucosal artery branches.

The diameters and areas of supply of the mucosal arteries were measured as the mean of 50 determinations in the greater curvature in Rb5. The average diameter was  $40.0\pm16.0$ um with an average area of supply of  $31.8\pm27.5$  (mm<sup>2</sup> x  $10^{-2}$ ). Thus while there is comparatively little variation in the diameter, the large standard deviation of area of



Fig. 32 Fundus of Rb5GC. Shows large vessels with extensive anastomoses (in mucosa) (5x).



Fig. 33 Antrum of Rb5GC. Shows plexus with fewer anastomoses and narrower vessels (5x).

supply indicates that both large and small areas were present. The overall perfusion index, an indication of flow per unit volume was  $1531\pm1497 \times 10^4$  (um<sup>4</sup> mm<sup>-2</sup>).

By definition, the branches derived from a single mucosal artery did not anastomose with those derived from another. As in the mucosal plexus the "failure to anastomose" phenomenon is seen as vessels approach to within a few micrometres, then turn downwards into the mucosa  $\{F_{i}y_{i}, i \neq 2\}_{i \neq 5}$  without anastomosing. Thus, unfilled intervals are seen between areas of distribution of the mucosal arteries. However, in some areas of some specimens, diffuse capillary filling occurs (e.g. Rb6 LC-fundus). The unfilled interval cannot then be seen. It was, however, not possible to determine whether capillary anastomosis, or interdigitation without anastomosis, occurred. Unfilled intervals were seen in areas of capillary filling, eg. Rb4, but closer examination showed this to be an artefact due to mucosal unevenness.

As previously described, the mucosal arteries arise largely from the mucosal plexus which is, in turn, supplied by extramural arteries. However, the mucosal plexus is very sparse in some areas and some input trunks have only one or two anastomoses with adjacent vessels. These may be termed "low connectivity vessels" (LCV's). In some cases, the input trunks make no anastomoses at all at SMP or MP level and are, therefore, mucosal arteries of extramural origin (MAEO's). In these specimens (Rb 4;5;6) five unequivocal gastric MAEO's were seen, in addition to numerous possible MAEO's (i.e. they did not meet all the defining criteria) and many LCV's. There were also four duodenal MAEO's. Their characteristics are described in the next section but they were all located distally in the stomach on both lesser and greater curvature aspects. The mean diameter was 88 + 29 um and mean area of supply  $325 \pm 286 \times 10^{-2} \text{ mm}^2$  (a perfusion index of 2264 + 1325 x  $10^4$  um<sup>4</sup> mm<sup>-2</sup> ).

221

#### Spatial Variations in Mucosal Arteries

No spatial qualitative variations in mucosal artery structure could be seen. The values for the areas of supply and the diameter of MA's (mean of 50 determinations per (74/4, 7)) area) are shown. The area of supply was greatest in the antrum (48.9  $\pm$  33.4 x  $10^{-2}$  mm<sup>2</sup>) and least in the corpus (  $16.8 \pm 11.7 \times 10^{-2}$  mm<sup>2</sup>). The intermediate fundus value was  $29.7 \pm 21.8 \times 10^{-2}$  mm<sup>2</sup>. The vessel diameter was greatest in the fundus. The perfusion indices were 594, 1666 & 2333 x  $10^4$  um<sup>4</sup> mm<sup>-2</sup> in the antrum, corpus and fundus respectively.

Unequivocal MAEO's were seen in all the specimens. There were three in Rb4, one in Rb5 and one in Rb6. In all cases, they were located in the caudal part of the stomach, distal to the corpus/antrum mucosal interface. There seemed to be no preferred location with reference to the curvatures. The average values of area, diameter and perfusion index are shown and are all much greater than the (Table 74) values for mucosal arteries in the antrum. MAEO8 seems to be anomalous and may be a VLC. The values are, therefore, also shown excluding this vessel. In addition, the specimens also displayed numerous very low connectivity vessels, which were also all located distally.

# Table 12 Diameter, Area and Perfusion Index of MucosalArteries in Rb5 (Greater Curvature Half)

NOTES

D = Diameter of mucosal artery (um) A = Area of supply of mucosal artery (mm<sup>2</sup> x  $10^{-2}$ ) PI= Perfusion Index (D<sup>4</sup>/A) (um<sup>4</sup> mm<sup>-2</sup> x  $10^{4}$ )

The area of supply of the mucosal arteries in the corpus was significantly less than in the fundus and antrum (p<0.1).

(See Table overleaf)

	FUNDUS			CORPUS			ANTRUM			
	D	A	PI	D	A	PI	D	A	PI	
	63 75 38 25 50	38 31 16 9 31	4146 10207 1303 434 2016	38 38 25 25 38	22 16 29 13 13	948 1303 135 300 1604	50 63 25 38 38	94 88 19 25 44	665 1790 206 835 474	
	38 38 75 63 13 25	22 13 44 25 6 19	947 1604 7191 6301 48 206	38 13 13 25 38 63	13 9 6 13 56	1604 32 48 651 1604 2813	25 25 25 50 25 25	19 22 13 56 31 25	206 176 300 1116 126 156	
	38 13 38 38 88 75	13 6 13 16 81 69	1604 48 1604 1303 7404 4586	50 50 25 38 38 50	13 28 6 13 22 22	4807 2232 651 1604 948 2841	50 50 25 38 38 50	72 134 19 19 50 100	868 466 206 1097 417 625	
	25 25 38 25 25 88	6 13 19 9 16 103	651 300 1097 434 244 5822	38 38 25 25 88 50	9 13 6 9 66 25	2317 1604 651 434 9086 2500	13 13 25 38 38 25	13 13 53 69 60 43	22 22 74 302 348 91	
	63 50 38 25 75 50	56 31 41 16 59 34	2813 2016 509 244 5363 1838	38 50 50 38 38 13	13 22 22 9 9 6	1604 2841 2841 2317 2317 48	38 25 38 38 38 38 50	83 41 34 34 53 97	251 95 613 613 393 644	
	38 38 38 25 50 25	13 16 13 9 22 13	1604 1303 1604 434 2841 300	25 38 50 25 38 63	9 13 25 13 25 31	434 1604 2500 300 834 5082	13 38 63 50 25 38	6 35 81 72 16	48 596 1945 868 244	
	50 38 50 38 38 38	53 25 47 16 13	1179 834 1330 1303 1604	50 38 25 38 25	28 25 6 9 19	2232 834 651 2317 206	50 38 38 50 50	50 25 16 91 85	1250 834 1303 687 735	
	38       63       63       75       63       63	41 41 50 34 56	1097 3842 3842 6328 4633 2813	38 50 12 38 38 25	16 25 6 9 16 6	1303 2500 35 2317 1303 651	75 38 38 25 25 25 25	163 16 41 22 25 22	1941 1303 509 176 156 178	
	50 38 38 63	19 13 44 75	3289 1604 474 2100	38 38 25 38	16 9 13 13	1303 2317 300 1604	25 38 50 38	28 75 84 25	140 278 744 834	
MEAN SD	46.0 <u>+</u> 18.8	29.7 <u>+</u> 21.8	2333 <u>+</u> 2280	37.0 <u>+</u> 14.0	16.8 ± 11.7	1666 <u>+</u> 1559	37.0 ± 13.2	48.9 <u>+</u> 33.4	594 <u>+</u> 524	

	FUNDUS	LC	GC	ANT WALL	POST WALL	DUODENUM	ANTRUM
Rb5		1 <sup>(5)</sup>				2 <sup>(1)</sup>	(2)
Rb6	? <sup>(5)</sup>	?(5)	?(3)	?(5)	?(5)	2 <sup>(10)</sup>	1(4)
Rb4	?(6)	?(7)				?(8)	<sub>3</sub> (9)
Rb3	?	?	?	?	?	?	?
Rb16	?	?	?	?	?	?	?

#### <u>NOTES</u>

"?" indicates an area where it is not possible to definitely exclude the presence of MAEO's.

"--" indicates an area where the presence of MAEO's can be excluded.

- 1. Immediately scalariform pattern on LC. 2 MAEO's on GC.
- 2. Several VLC's on LC.
- 3. Unlikely approximately 80% filled. All scalariform and stellate.
- 4. Several possibles and VLC's on LC.
- 5. Unlikely most filled and do anastomose.
- 6. Damaged but approximately 80% of vessels filled and do anastomose.
- 7. Poor filling Several possibles.
- 8. Poor filling but non-scalariform pattern.
- 9. Several VLC's.
- 10.Several possibles within 10mm of GDJ.

Table 13 Occurence of MAEO's and VLC's in Rabbit Specimens



Fig. 34 GDJ of Rb5. Duodenum to left, antrum to right and pyloric canal central. Note dominance of duodenal inputs which give branches distally to duodenum and proximally to antrum. The plexus is continuous between stomach and duodenum (5x).



Fig. 35 MAEO no. 5. From duodenum of Rb6 (35x).



Fig. 36 MAEO no. 1 in LC of Rb5 (35x).



Fig. 37 MAEO's no. 6 & 7 in LC of Rb4 (35x).



Fig. 38 MAEO no. 8 in GC of Rb4



<u>Fig. 39</u> MAEO no. 2 from duodenum of Rb5GC. The two large trunks arose from a common parent trunk in the muscularis externa (35x).

REGION	SPECIMEN	No	LOCATION	AREA	DIAMETER	PI	NB
STOMACH	Rb4 " "	6 7 8	LC antrum " GC mid- antrum 17mm from GDJ	219 181 831	75 63 138	1448 870 4364	<pre>{1} {1} {1} {2}</pre>
	Rb5	1	LC corpus/ antrum	138	75	2293	
	Rb6	4	GC antrum Post GC 2.5 mm from GDJ	256	88	2343	{3}
MEAN SD				325 286	88 29	2264 1325	
[MEAN] [SD]			Minus maeo no.8	199 51	75 10	1739 710	{4}
DUO	Rb5	2	Post GC 5mm from GDJ	213	100	4695	
	11	3	Ant-GC 13mm from GDJ	375	138	9671	
	Rb6	5a	47mm from GDJ	25	338	5220677	{5}
	11	5 <b>b</b>	11	25	113	65219	{5}
MEAN SD				156 169	172 112	20027 30359	
[MEAN] [SD]			Minus maeo no.5a&b	294 115	119 27	7183 3519	

<u>NOTES</u>

Area of supply in  $mm^2 \times 10^{-2}$ Diameter in um Perfusion Index in  $um^4 mm^{-2} \times 10^4$ 

- {1} Several other possible MAEO's in this area. Also several VLC's.
- {2} Possibly a VLC.
- (3) Continues onto LC half. There is no corresponding vessel with anastomoses.
- {4} MAEO no. 8 seems anomalous in its area and diameter. May be a VLC. A mean and standard deviation has been calculated excluding it.
- {5} MAEO's no. 5a & 5b are anomalous in their areas. There are several nearby small trunks which may be from the same parent trunk. A mean and standard deviation has been calculated excluding it.

Table 14 Area, Diameter and Perfusion Index of MAEO's in Rabbit

# Arteriovenous Anastomoses

Venous filling was seen in several specimens and areas at capillary, mucosal plexus and submucous vessel level. However, in no instance could any direct arteriovenous anastomoses be seen.

# <u>Duodenum</u> (Figs. 27,29-31)

The vessels of the duodenum could be seen in all or part of three cleared specimens - Rb4,5 & 6. In all of these, there was a double line of inputs to the submucous compartment. This was located on the posterior duodenal aspect and gave rise to scalariform branches running circumferentially around the duodenum. In Rb4 the first centimetre of duodenum distal to the gastroduodenal junction did not display the scalariform pattern, nor the two input lines, but rather a random distribution of inputs and branches. In the greater curvature half of Rb5, inputs to the first 1.4 cm were random whereas, in the lesser curvature half, and in Rb6, the scalariform pattern was manifest immediately distal to the gastroduodenal junction. There was, however, some damage to the initial part of Rb6.

The plexus pattern was not the same as in the stomach since the input vessels gave rise to a significant submucous plexus. The vessels continued to the base of the mucosa where a second, mucosal, plexus arose. From this, perpendicular vessels penetrated the mucosa - the mucosal arteries. Each villus appeared to be supplied by one or two central arterial vessels which travelled to the tip, to be drained by a network of vessels on the surface of the villus. This drained into an intermediate venous mucosal plexus, superficial to the arterial plexus.

The phenomenon of VLC's was less evident in the duodenum than in the stomach, although 'failure to anastomose' could be seen. Only two MAEO's could be unequivocally identified, both on the greater curvature half of Rb5 and both in the randomly orientated, non -  $(F''_{2,3})$  scalariform region. Two more, which appeared to meet the defining criteria, could be seen in Rb6. However, their

distance from the gastroduodenal junction (47 mm ), small diameter (25 um ) and large area of supply are suspicious, as is the presence of a number of small adjacent inputs in a scalariform area. As in the stomach, there are several other probable MAEO's, all within 10 mm of the gastroduodenal junction. (Table / 3 - 14)

#### DISCUSSION

The observations of the gross anatomy and extramural vasculature were in agreement with the literature. The only significant difference lay in the absence of a continuous anastomotic vessel along the greater curvature. This was feature was described by Craigie, and comprised the left and right gastroepiploic arteries, which met and anastomosed. This anastomosis was not seen in this study, although the number of specimens (16) should be sufficient to demonstrate the pattern. It was not due to cutting the vessels proximal to the anastomosis, as these were followed to the parent vessels. It is possible that Craigie was describing the general mammalian pattern, rather than the result of specific observations on the rabbit.

Two features of the muscular intramural vessels are of note. The stellate branching of recurrent vessels on the serosal surface is a feature which was not observed in other species, with the exception of the guinea pig, and is not described in the literature. Their significance, as a source of nutrient vessels to the serosal region, may be related to the paucity of the arterial plexus, derived from the penetrating arteries, which would be the more normal pattern. The absence of an significant arterial plexus, with a defined plane, was seen throughout the stomach. This could be an artefact in the corpus and fundus, as the muscle is extremely thin here. However, it is also seen in the distal antrum, where the muscle is sufficiently thick to define the plane of the plexus, if it had one.

The majority of the specimens consistently showed the dominance of the lesser curvature inputs to the submucosa, which supplied the majority of the gastric area. This was not universal however, as the greater curvature inputs were dominant in one specimen (Rb4). There was insufficient data to assess variant frequencies. Although the connective tissue plexus was seen in only one specimen, the degree of filling in the other specimens may have been too poor to demonstrate existing vessels in this plexus, in which the vessels are narrow.

The paucity of anastomoses in the submucosa was quite clear, as was the presence of an extensive mucosal plexus. The latter plexus was reported by Bulkley, but he did not specifically describe or refute the presence of a submucous plexus. The 'failure to anastomose' phenomenon, where mucosal arteries approach one another to within a few microns, but turn down at the last moment, is distinct and seen in few other species. Close stereoscopic examination confirmed that this was real and not due to inadequate filling of the vessels.

The result of the presence of a mucosal plexus and of the 'failure to anastomose' is that the actual density of the submucous plexus is considerably less than would be indicated by a superficial examination. Only a high power, stereoscopic examination with micromanipulation will show these features. The duodena also showed these features, though to a lesser degree. There were too few good specimens to show whether there was a real difference in incidence between the stomach and duodenum in the relative importance of the submucous and mucosal plexuses and in the occurence of mucosal arteries of extramural origin, very low connectivity vessels and failures to anastomose.

The finding of MAEO's is an important result, as they are found in man, but in few other species. The definition given in "Materials and Methods" was rigorously applied and equivocal candidates were rejected. The values for their diameter, area of supply and perfusion index suggest that they are qualitatively different from mucosal arteries, from which they differ significantly. The MAEO's were twice the diameter, serving four times the area and therefore with a better perfusion index, three times greater than the mucosal



<u>Fig. 40</u> Corpus of Rb6GC. Shows a central vessel with very high connectivity. Note the small mucosal arteries with definable areas of supply (5x).





Fig. 41 Sections along course of a single large (diameter 400um) anastomosing artery from rabbit stomach. Actual distance from (b) to (c) was 7mm. (a) Artery in submucosa. (b;c) Artery ensheathed in split muscularis mucosae. (d) Beneath muscularis mucosae (80x).



Fig. 42 Cleared edge section showing 'failure to anastomose' in fundus of Rb5GC (centre field). Vessels approach very closely but do not anastomose (51x).



Fig. 43 Cleared edge section of antrum in Rb6GC. Note all anastomoses in mucosal plexus (51x).



Fig. 44 Low connectivity vessel in antrum of Rb6LC (16x).



Fig. 45 Multiple 'failures to anastomose' of above vessel (51x).

arteries in the same area. Careful dissection and examination showed that there were no proximal anastomoses which had been lost during the dissection process.

# CHAPTER 15 THE PIG

#### NON-VASCULAR GASTRODUODENAL ANATOMY

# General Appearance

All the porcine stomachs were examined. The oesophagus enters the stomach approximately two thirds along the lesser curvature, nearer to the fundus, forming an acute incisura cardiaca in all but three specimens, in which it was 90°. The terminal portion of the oesophagus has no ampulla (diameter 1.4 + 0.3 cm.). To the left of the oesophagus is the fundus, which ends in a partially separated pouch, the diverticulum ventriculi, which, in most of the specimens, was bound down to the dorsal aspect of the fundus by mesentery and had a diameter of 3-4 cm. The stomach, had a generally constant diameter (9.9 + 2.0 cm.) and was approximately 17 cm. long. It turned sharply to form the antrum, forming an incisura angularis of 69+11°. The antrum tapered continuously to the gastroduodenal junction, although this was only marked by a slight external constriction. In half the specimens, the gastroduodenal junction was followed by a perceptible duodenal ampulla. In the remainder, the antral tapering continued for a few centimetres, until the duodenum reached a constant diameter. The first duodenal flexure, which was normally in excess of 270°, occurred almost immediately after the gastroduodenal junction.

ŧi.

### Omental Attachments

In general, distribution of the mesentery was fairly constant in all the stomachs and duodena which were observed. The lesser curvature was defined by the reflection of the lesser omentum upon the stomach. In all but three specimens, the omentum formed a complete ring around the base of the oesophagus, with lines of attachment running up its ventral and dorsal aspects. In the remainder, there was only an incompletely formed mesenteric ring. From this point, the mesentery diverged to run along either side of the lesser curvature but in all except one (S26) the attachment was asymmetrical. The ventral branch ran fairly close to the edge, whereas the dorsal branch was significantly further away i.e. the mesentery displayed a dorsal bias. This was most evident in the corpus, as the mesentery began to converge on the edge in the antrum, reaching it in the vicinity of the gastroduodenal junction. The space between the mesenteric lines ie. the width of the lesser curvature, had a maximum value (in the corpus) of 3-5 cm, falling to about 1 cm in the antrum (distal to the incisura angularis).

The greater omentum originated at the oesophageal ring, from whence it passed onto the dorsal aspect of the diverticulum ventriculi. From here, it traversed the dorsal fundus, reaching the caudal edge of the stomach in the vicinity of the fundus-corpus interface. It then passed along the greater curvature to unite with the lesser omentum at the gastroduodenal junction. For the duration of its corporo-fundal course it was slightly ventral to the greater curvature. Unlike the lesser curvature, the greater curvature, which is delineated by the two layers of the greater omentum, was narrow since the distance between the two lines of attachment was only 1 - 2 cm. This maximum value is reached in the corpus. In the fundus and antrum, the omentum was virtually linear.

The greater and lesser omenta united at the gastroduodenal junction to form a complete mesenteric ring (partial in S18). From this point, a single line of mesenteric attachment passed along the dorsal aspect of the proximal duodenum.

### <u>Muscularis</u> <u>Externa</u>

The tunica muscularis of the pig stomach was examined by external inspection and during the course of dissection in eight specimens (S9;S14;S17;S19;S21;S23;S24;S26). It was difficult to define the structure of the duodenal muscularis, as it was very thin.

The basic structure of the muscular layer followed the



Fig 46 Muscularis externa of S31 (frozen section). Note the vascular bundle containing a penetrating artery (13.6x).



<u>Fig. 47</u> High power view of above. Note the discontinuity in orientation of muscle cells forming a "sheath" around the vascular bundle (80x).



Fig 46 Muscularis externa of S31 (frozen section). Note the vascular bundle containing a penetrating artery (13.6x).



Fig. 47 High power view of above. Note the discontinuity in orientation of muscle cells forming a "sheath" around the vascular bundle (80x).

normal pattern of the mammalian stomach, comprising an inner circular and an outer longitudinal layer of smooth muscle fibres. The circular layer was complete, extending between the lesser and greater curvatures whilst the longitudinal layer was thinner and discontinuous. There were two welldeveloped parallel bands of sling fibres, deep to the around the layer, which ran circular cardia and symmetrically along either side of the lesser curvature, as far as the incisura angularis. Running between these bands were transverse fibres. In two specimens (S14;S24) the bands diverged from the cardia. In one specimen (S19), the sling fibres were poorly developed. In S23, there was a distinct ventral bias to the fibres. In addition, there was an incomplete layer of oblique muscle fibres, running from the cardia onto the dorsal and ventral aspects of the corpus, as far as the proximal antrum.

There were distinct sphincteric thickenings of the circular layer at the cardia and the pylorus. In addition, in seven of nine specimens there was a thickening of circular fibres around the diverticulum ventriculi, partially separating it from the main gastric cavity. The overall thickness of the circular layer, and, to a lesser degree the longitudinal, showed a sudden increase in the vicinity of the fundus - corpus junction. At this point the thickness increased from approximately 0.3 cm to 1.0 cm. At the gastroduodenal junction, it decreased equally suddenly to its previous value.

A feature unique to the pig, the torus pyloricus was located in the antrum. This was a localised mass of fatty tissue, approximately 0.8 x 2.0 cm, interspersed with muscle fibres of no obvious orientation, which protruded into the gastric cavity, causing a swelling of the mucosa. Its long axis was parallel to the stomach's, and it was sufficiently close to occlude the gastroduodenal junction.

#### <u>Miscellaneous</u>

The muscularis was examined in histological sections (corpus of S2). Its mean thickness was 41um, calculated from

20 measurements in each of 5 sections using an eyepiece graticule. It appeared to have a three layer structure, with the middle layer at  $90^{\circ}$  to the other two. Stereomicroscopic examination of the cleared S25, suggested that the muscularis mucosa was significantly thicker in the antrum and the torus pyloricus than in the corpus.

The point of entry of the bile duct to the duodenum was a mean 1.85 cm from the gastroduodenal junction (1.0 -3.2cm). In one (S23) it could not be seen at all in the preserved 11 cm of duodenum.

An interesting feature was also seen in the sections of S2 corpus. A region of damaged mucosa extending into the muscularis mucosa was found, about 300 um wide by 125 um deep. This had the histological appearance of a chronic ulcer, but was very small.  $(F_{ij}, \tilde{y})$ 

#### EXTRAMURAL VASCULATURE

The arterial supply to the stomach and duodenum reached the organs along the lines of mesenteric attachment and , in some cases, independently of them. The following description is derived from an external inspection and dissection of 12 fixed whole stomachs and three cleared isolated muscle specimens. In general, the patterns of supply were similar, although there were differences in some features.

In all cases, the gastric vessels originated from the coeliac trunk, a short vessel coming off the abdominal aorta. This trifurcated after a very short course in all but two of the specimens, to form the left gastric, splenic and hepatic arteries. In S12 and S14, the parent trunk bifurcated to form splenic and gastrohepatic arteries, the later almost immediately splitting to form the left gastric and hepatic branches.

The majority of the lesser curvature was supplied by the left gastric artery, whose main branches ran in the lesser omentum and contacted the stomach along the lines of mesenteric attachment. These were widely separated on the lesser curvature with a dorsal bias in most cases. The main dorsal and ventral branches had a prolonged subserosal course, they gave off horizontal branches to the serosal plexus and vertical branches to the muscularis externa. These branches were spread evenly over the area supplied, and did not show any tendency to form 'bands' of inputs as on the greater curvature. The serosal vessels eventually anastomosed with branches from the greater curvature inputs. This occured on the dorsal and ventral walls, 60% to 80% of the distance from the lesser to the greater curvatures. Thus, the left gastric artery supplied significantly more than half of the dorsal and ventral aspects of the stomach, with the dorsal branches tending to be longer than the ventral.

The serosal branches from the lesser curvature supplied the posterior fundus, corpus and proximal antrum, as far dorsally and ventrally as the anastomotic line with the greater curvature vessels. The left gastric artery also gave off isolated branches to the lesser curvature of corpus and proximal antrum, i.e. between the lines of attachment of the lesser omentum. These tended to be small and sparse, especially in the proximal antrum. The left gastric artery normally bridged the incisura angularis. It also gave rise to a complete annulus around the base of the oesophagus, giving off subserosal branches which ran a few centimetres rostrally up the oesophagus to anastomose with branches from the oesophageal artery (derived from the hepatic artery).

The lesser curvature of the distal antrum was supplied by branches of the right gastric artery, derived from the hepatic artery. This was much smaller than the left gastric artery, and gave branches to the anterior and posterior walls of the stomach, anastomosing subserosally with vessels derived from the greater curvature supply. In only four of the 15 specimens was it possible to see any extramural anastomosis with branches derived from the left gastric artery. In the remainder, there was a clear demarcation between the two, and a paucity of inputs at the interface between the areas of supply in the proximal antrum. The right gastric artery supplied the gastroduodenal junction,

241

but there was no lesser curvature annulus at this point in any of the specimens, and it was not possible to see any extramural anastomosis with the greater curvature annulus.

The diverticulum ventriculi was not supplied by the left gastric artery, but by a branch of the splenic artery, the diverticular artery. This was a large vessel which supplied isolated inputs to the diverticulum and to the proximal fundus. It did not anastomose with either the left gastric or the left gastroepiploic arteries.

The proximal greater curvature was supplied by another branch of the splenic artery, the left gastroepiploic artery. This reached the greater curvature in the greater omentum at about the proximal corpus. From here, branches ran subserosally as far as the mid point between the greater curvature and the middle of the dorsal and ventral walls where they anastomosed with subserosal branches of the left gastric artery. During their subserosal course, they contributed to the serosal plexus and gave off vertical branches to the muscularis externa. In none of the 15 specimens were any anastomoses seen between the left gastroepiploic and diverticular arteries. Where they interfaced in the distal fundus, there were very few input vessels.

The distal part of the greater curvature was supplied by the right gastroepiploic artery, derived from the hepatic artery. This passed rostrally along the proximal duodenum, crossed the gastroduodenal junction and supplied the greater curvature as far as the distal corpus. Its subserosal branches anastomosed on the dorsal and ventral walls with the subserosal branches of the right gastric artery. The right and left gastroepiploic arteries anastomosed in only five of the specimens. In the remainder, the corpus-antrum junction, where they interfaced, was hypovascular.

The right gastroepiploic artery gave rise to a partial annulus at the gastroduodenal junction which ran dorsally and ventrally around 40 - 50% of the circumference. In no case were anastomoses with the branches of the right gastric artery seen.

> . .

#### INTRAMURAL VASCULATURE

# Supply to Muscularis Externa

The supply was defined in 12 fixed and three cleared specimens, supplemented by paraffin, frozen and cleared edge specimens. The branches of the extramural arteries entered the serosa and coursed in this layer, parallel to the serosal surface, for some distance. They supplied the subserous plexus, and gave rise to penetrating vessels which passed into the body of the muscularis. At the end of their course, they themselves turned downwards into the muscularis. The subserous plexus was a coarse network of small vessels which ramified over the stomach, parallel to the serosal surface. It gave rise to small nutrient vessels which supplied both the serosa and the underlying superficial muscularis externa.

The penetrating arterioles (diameter 200-300 um) passed obliquely through the muscularis before entering the submucosa to join the submucous plexus. During their intramuscular course, they gave rise to nutrient capillaries which formed 'leashes' running parallel to the bundles of muscle fibres. There was also a deep muscular plexus at the base of the muscularis which was formed by branches of the penetrating arterioles and, to a lesser extent, by recurrent vessels from the submucous plexus.

Penetrating arterioles were often accompanied by one or more venules. They passed through the muscle in a vascular bundle, which was a space in the muscle containing the vessels and surrounding connective tissue. The latter was birefringent, suggesting that elastin fibres might be present. Some cells with large vacuoles were adipose in appearance. The bundle cavity tended to be oval, with its long axis orientated parallel to the predominant muscle fibre orientation. A typical bundle would have dimensions three to four times the diameter of the arteriole it contained. A interesting feature seen in a number of bundles (e.g. section 31 of S20) was the presence of a ring of muscle fibres running circumferentially around the bundle

243

cavity, with a thickness of approximately 100 um. This transverse circumferential orientation was constant despite the arrangement of the surrounding fibres. The peripheral cells of the sheath appeared to be intercalated with the surrounding cells.

### Spatial Variations in Supply to Muscularis Externa

The subserous and intramuscular course of the arterioles tended to be longer in the corpus and shorter in the fundus and antrum. Similarly, the small number of vessels which entered the serosa directly on the curvatures serosa and muscularis penetrated the virtually perpendicularly. In general, the density of subserous and deep muscular plexi and of nutrient vessels tended to be greater the more oblique the course of the parent vessel i.e. greatest on the anterior and posterior aspects of the corpus and least on the antral and fundic curvatures.

# Submucous Plexus (F195.48-5)

The vasculature in the submucosa was examined in fifteen cleared specimens and 5 paraffin, frozen or cleared edge sections. In the cleared surface specimens, all the features described could not necessarily be seen in every specimen, but sufficient examples were present to validate the description given.

Arterioles passed through the muscularis externa and entered the submucosal compartment, usually obliquely, where they ramified to form an extensive plexus, parallel to the mucosal surface, which appeared to be within the submucosa. The vessels of the plexus varied between approximately 50 and 150 um in diameter. From the plexus, smaller vessels were given off perpendicularly which passed superficially towards the mucosal surface.

The location of the plexus was defined by micromanipulation in the cleared specimens and in histological and cleared edge specimens. It was found that all the plexus vessels could be touched and crushed. Some were very mobile, whilst others were less so, but not

: .



<u>Fig. 48</u> Lesser curvature half of S16. Gastroduodenal junction at left edge. (0.8x).



Fig. 49 Anterior surface of S11. Oesophagus at top right. GDJ at top left. Incisura angularis at centre (0.4x).

annipulated respective dest

ersisting, and a character and

The second in a penetrated in a but occasionally 5 also gave rise 15 ves also supplied veterit.

Fig. 50 Greater curvature half of S26.Duodenum to left (0.5x).

completely immobile. Histological examination of previously manipulated vessels showed that all were located within the submucosa. Most were near the base of the compartment, above the muscularis mucosae but within the submucous connective tissue. Other plexus vessels, which tended to be smaller, but which did not constitute a separate plane of plexus, were still within the submucosa but at the very base, with their lower aspects adherent to the muscularis mucosae, and hence were less mobile.  $(F_1, 5_4)$ 

Three types of branching pattern were seen scalariform, radial and stellate. Their distribution throughout the stomach is discussed in the following section.

The submucous plexus gave rise to vessels which penetrated the muscularis mucosae, usually perpendicularly but occasionaly obliquely, to supply the mucosa. The plexus also gave rise to a diffuse connective tissue plexus, which was also supplied by branches from the pre-plexus submucous vessels.

#### Spatial Variations in the Submucous Plexus

The submucous plexus varies spatially in its topology and density, the latter defined by the number and density of the anastomoses. The inputs to the submucous plexus largely corresponded to the extramural vessels. On the anterior and posterior corpus, the derivatives of the vessels from the lesser and greater curvatures branched in a scalariform pattern. Those originating from the lesser curvature were orientated radially about the oesophagus and anastomosed with the vessels from the greater curvature vessels on the G.C. half of the stomach. The plexus in this area was quite dense, with numerous large anastomotic vessels located within the submucosa. The lesser curvature was defined by two lines of input vessels, within which was an area of inputs with a stellate branching pattern. In all specimens, the submucous plexus was much poorer here, as far as the proximal antrum, since the anastomoses were much fewer and were narrower. By contrast, the greater curvature showed few



Fig. 51 Lesser curvature half of S26. Duodenum to left (0.4x).



Fig. 52 Aglandular mucosa from corpus of S2. Submucosa at top (10x).



Fig. 53 Glandular mucosa from the corpus of S2. Note the central feature with the appearance of a chronic ulcer (10x)



Fig. 54 Cleared edge specimen showing large submucous plexus vessel in fundus of S25GC. Muscularis mucosae runs horizontally across centre field. Note vessel beginning to penetrate Muscularis mucosae and mucosal arteries with anastomoses at base of mucosa (55x).
stellate vessels, as the scalariform vessels, directed dorsally or ventrally, were almost on the greater curvature itself. The plexus in the cardia was also sparse with leashes of vessels and scalariform branches. By contrast the diverticulum, supplied by scalariform branches of the diverticular artery, displayed a good submucous plexus, as did the proximal fundus. More distally though, the fundic submucous plexus became sparser as the number of anastomotic branches declined. In the pyloric antrum, the branching pattern was less regular, though it generally followed the pattern of central stellate and lateral scalariform vessels. However, the plexus was less dense, though better than on the lesser curvature.

The corpus and diverticulum had the best developed submucous plexus, the antrum less so, as vessels become (Fry 55-56) fewer and smaller. The lesser curvature and perioesophageal cardia had the leanest plexus, mainly as a result of reduction in vessel size.

The topology of S11A was examined by mapping. Typically, each input anastomosed with 3 - 4 other inputs. However, connectivity, defined by the proportion of inputs having mutual anastomoses, was significantly higher in the corpus (3.5%) than in the antrum (2.6%). This was consistent with the other modes of investigation.

There were several areas in the stomach where a specialised pattern in the submucous plexus had developed. Around the gastro-oesophageal junction, large vessels radiated branches onto the cardia and fundus, with intermediate scalariform branches. Smaller branches ran in the submucosa onto the oesophagus. In five specimens, there were anastomoses 0.5-1.0 cm rostral to the gastrooesophageal junction between these branches and those of the oesophageal vessels. In two specimens there was no anastomosis whilst the remainder were indeterminate.

A second specialised area was the torus pyloricus, located on the lesser curvature of the pyloric canal, which was well shown in four specimens. The mucosa was significantly thicker and there was a local proliferation of



Fig 55 Surface view of submucous plexus vessels in corpus of S25LC. Note primary and secondary anastomoses and mucosal arteries (17x).



Fig. 56 Surface view of diverticulum ventriculi in S25LC. Note the helical structure of many mucosal arteries (17x). submucous (and mucosal) vessels. The region was supplied by anastomoses from the surrounding antral submucous plexus and, in S26, by its own inputs.

The gastroduodenal junction was clearly seen in S25, and the details confirmed in S10;S11;S19;S23. In many of the other specimens this section was poorly filled or damaged in dissection. The location of the gastroduodenal junction was defined by a discontinuity in mucosal filling at the beginning of the duodenal ampulla. Proximal to this, leashes of longitudinal vessels ran on either side of the torus pyloricus in the submucosa which gave rise to few lateral branches. A ring of small inputs was located immediately proximal to the mucosal discontinuity. These gave rise to longitudinal branches directed mainly proximally, but some distally. About 0.8-1.0 cm distal to the gastric ring, on the duodenum, was a ring of duodenal inputs. Most of their branches were directed distally, but some ran proximally. They anastomosed with the gastric branches mid way between the two lines of inputs (separated by about 40 um) . Thus, the anatomical gastroduodenal junction was supplied mainly by gastric vessels, as the anastomosis was on the duodenum, but there was continuity between the gastric and duodenal submucosae. The duodenal vessels gave rise to a better annular submucous plexus than did the gastric ones. Although the degree of anastomosis varied, for example being dominated by the duodenal vessels in S10, in no filled, undamaged specimen was a contradictory pattern seen.

The duodenum was even more difficult to fill and dissect than the gastroduodenal junction. The details of the arterial vasculature in the first few centimetres could be observed in four specimens (S1;S11;S25;S26). The first one to three centimetres showed a random pattern of submucosal inputs, giving rise to a fairly dense submucosal plexus of small vessels in a thin submucosa. In S11 the plexus was poor, perhaps due to inadequate filling. After a few centimetres, two lines of inputs were seen with their scalariform branches directed dorsally and ventrally. On the anterodorsal aspect was an area of small inputs, showing a <u>Fig. 57</u> Cleared edge specimen showing submucous plexus vessel in fundus of S25GC. Note penetration of muscularis mucosae and vessels at base of mucosa with plexiform characteristics. Submucosa at top of field (55x).



<u>Fig. 58</u> Cleared edge section of mucosa of corpus in S25GC. Note the ramification of the mucosal arteries Muscularis mucosae at top of field (43x). random branching pattern. The plexus gave rise to large mucosal arteries. In two specimens, the entry of the bile duct and its associated vasculature could be seen (2.2 cm and 3.2 cm beyond the gastroduodenal junction). In both cases, duodenal submucous branches supplied the first part of the duct. It was not possible to determine whether anastomosis with the ductal vessels occured distally.

In several specimens, there was some degree of venous filling within the submucosa. A good example was S1C, where venae comitantes could be clearly seen, running parallel to the arterioles.

# Connective <u>Tissue</u> <u>Plexus</u>

The connective tissue plexus could be seen clearly in seven specimens. It comprised a three dimensional plexus of variable density, present throughout the submucosa of the stomach and proximal duodenum. The plexus of vessels, generally less than 10 um in diameter, was fed by recurrent vessels from the submucous plexus and its feeder vessels.

# Spatial Variations in Conective Tissue Plexus

The density of the plexus tended to match that of the submucous plexus, being greatest in the diverticulum ventriculi, posterior fundus and corpus.

#### <u>Mucosal Arteries</u>

The mucosal arteries were the final supply vessels to the gastric mucosa. They were filled to some degree in most specimens and could be clearly examined in eleven of the fifteen specimens. In general, the vessels arose from the submucous plexus, pierced the muscularis mucosae and ran vertically into the gastric mucosa, to end approximately two thirds of the way to the surface. During their course, they gave off small arterioarterial anastomoses and nutrient  $(F_1)_{y_2}^{(F_1)} (F_2)_{y_3}^{(F_2)} (F_3)_{y_3}^{(F_3)}$  and supplied an area of about  $29\pm40 \times 10^{-2}$ mm<sup>2</sup>. The mean perfusion index was  $314\pm372 \times 10^4$  um<sup>4</sup> mm<sup>-2</sup>. The anastomoses had a diameter of approximately 10 um.

Y

254

However, in some areas of the stomach, the size and topology of the anastomoses was such that they effectively formed a plexus at the base of the mucosa, from which the mucosal arteries arose. This is discussed more fully in the following section.

In six specimens, the details of venous vasculature within the mucosa could be seen, unobscured by too great a perfusion. The collecting veins ran from the mucosal surface, fed by a surface capillary honeycombe. They eventually drained into the submucous venules. In two specimens (S1;S3), the collecting veins had a bulbous sacculation at the mucosal end. In the remainder they did not.

# Spatial Variations in Mucosal Arteries.

The general arrangement of the mucosal arteries, as described above, was constant throughout the stomach. In most parts of the stomach, the mucosal arteries rose only from the submucous plexus, and, near the base of the mucosa, gave off arterioarterial anastomoses about 10 um in diameter. In some areas though, these anastomoses were approximately the same diameter as the penetrating vessels from the submucous plexus (> 30 um), and appeared to form a definite plexus at the base of the mucosa. This plexus then gave rise to the mucosal arteries. This was most evident in the fundus and the lesser curvature and least in the cardia, diverticulum ventriculi and duodenum. The corpus was transitional between the two. In the corpus of S3, all the mucosal arteries came from the mucosal plexus and none from the submucous plexus. This is the only specimen where this was seen.

The number of mucosal anastomoses was greatest in the fundus and corpus and least in the lesser curvature, antrum and duodenum. The fundus had the richest mucosal supply,  $(F_{F}, S^{3})$  which was plexiform rather than anastomotic. The vessels on the lesser curvature formed a plexus, rather than anastomoses, but they were very few in number. The diverticulum antrum and duodenum had the fewest and smallest

255

anastomoses.

The size and area of supply of the mucosal arteries themselves was examined in S25. Ten mucosal arteries were selected at random from each of three areas and their diameters and areas of supply measured under a stereomicroscope, using an eyepice graticule. The average parameters for all the thirty arteries, measured in the lesser curvature, pyloric antrum and diverticulum ventriculi were a diameter of 26 um supplying an area of 0.29  $mm^2$ . They

	DIVERTICULUM			CORPUS (LC)			ANTRUM		
	D	A	PI	D	A	PI	D	A	PI
	13 13 25 13 13 25 25 25 13 25	25 9 9 9 9 22 9 5 13	11 48 32 651 32 32 178 434 57 300	25 25 25 25 25 13 38 25 19	19 38 16 56 9 13 19 47 13 19	206 103 18 68 434 300 15 444 300 69	50 13 25 38 75 38 19 38 38 25	47 6 34 63 225 44 6 47 19 19	1330 48 115 331 1406 474 217 444 1097 205
MEAN SD	17.8 <u>+</u> 6.2	$11.3 \\ \pm \\ 6.8$	178 <u>+</u> 218	23.3 $\pm 7.2$	24.9 	196 <u>+</u> 165	35.9 <u>+</u> 17.6	51.0 	567 <u>+</u> 514
WHOLE	25.7	29.1	314						

<u>NOTES</u> D = Diameter (um) A = Area of supply  $(mm^2 \times 10^{-2})$ PI= Perfusion Index  $(um^4 mm^{-2} \times 10^4)$ The diameter of vessels in the antrum is significantly greater than in the diverticulum and lesser curvature. (p<0.05) The area of supply in the lesser curvature is significantly greater than in the diverticulum (p<0.05).

# Table 15 Diameter, Area of Supply and Perfusion Indexof Mucosal Arteries in the Piq

; .

were significantly larger in diameter in the antrum than the lesser curvature and diverticulum (p<0.05). In addition, the supplied area in the antrum (0.51 mm<sup>2</sup>) was significantly greater than in the lesser curvature. As a result of these differences, the perfusion index was greatest in the antrum (567 x  $10^4$  um<sup>4</sup> mm<sup>-2</sup>), significantly more than both lesser curvature (196) and diverticulum (178).

In addition to the variations in density described above, other specialised regions could be discerned. There appeared to be a discontinuity in mucosal filling at the gastroduodenal junction. In the duodenum itself, the mucosal arteries were large, with few anastomoses. The arteries rose almost to the surface, where they splayed out. In only two of seven specimens were villi seen (1.0 and 2.2 cm distal to the gastroduodenal junction). In the remainder, the mucosa appeared smooth for the full extent of the duodenal specimen (5 - 11 cm). Where villi could be seen, the mucosal artery rose to the surface then formed a fountain pattern of vessels.

At the torus pyloricus, there were large mucosal arteries with small but numerous anastomoses. In the five specimens where the mucosal filling of the gastrooesophageal junction could be defined, three showed some degree of continuity between the gastric and oesophageal vessels at mucosal level. This was not present in the remaining two. In seven specimens, a specific feature was seen, almost completely restricted to the diverticulum, fundus and cardia. Under low power, large numbers of 'holes' could be seen in the mucosal filling in these areas. Closer examination under stereomicroscopy showed these circular features to be 'domes' of mucosa about 300 - 350 um in diameter, with relatively avascular centres. The vessels of the submucous and mucosal plexuses went around them. These were well seen in for example, S1. (Fig.59)

All the stomachs were examined in detail for the occurence of Very Low Connectivity Vessels (VLC's) or Mucosal Arteries of Extramural Origin (MAEO's). VLC's were seen in three specimens (S11;S19:S25). Several of these incomplete filling made it impossible to identify them as such with certainty. The degree of filling in S3 and S4 made it possible to say with a high degree of confidence that there were no VLC's. In the three specimens quoted, 23 VLC's were seen. In S25, one apparently unequivocal MAEO was seen on the lesser curvature in the antrum.

The VLC's were all seen on or near the lesser curvature in the distal corpus or antrum. Their low connectivity was a result of both reduction in size and number of anastomoses, but was predominantly a result of the latter. A typical vessel (vessel 4 in S11A) had only 2 12.5 um anastomotic vessels. In no case was the 'failure to anastomose' phenomenon seen. This is the situation seen in rabbit and guinea pig where two vessels approach to within a few micrometres, then turn into the mucosa without anastomosing.

SPECIMEN	Di	AREA	INDEX	INDEXa	I <sub>a</sub> /I <sub>i</sub>
S25LC	138	12	3022	0	0
	125	34	718	34	0.05
	100	4	2500	24	0.01
S11A	125	4	6104	29	0.005
	150	6	8438	7	0.0008
	125	5	4883	55	0.01
	125	5	4883	1	0.0002
	138	3	12089	2	0.0002

<u>NOTES</u>

D<sub>i</sub> = Diameter of input vessel (um)

Area of supply in mm<sup>2</sup>

INDEX<sub>i</sub> = Perfusion index of input vessel  $(D_i^4/\text{Area um mm}^2 \times 10^4)$ 

INDEX<sub>a</sub> = Connectivity of input vessel anastomoses  $((d_1^4+d_2^4+\ldots+d_n^4)/area \text{ um mm}^2 \times 10^4)$ where  $d_n$  = diameter of nth. arterioarterial anastomosis (um)

I<sub>a</sub>/I<sub>i</sub> = Ratio of connectivity index to perfusion index. It
is a measure of the ability of flow through the
anastomoses to replace the flow through an occluded
input trunk.

### Table 16 Perfusion Index and Connectivity of Very Low Connectivity Vessels in the Pig

The one MAEO (vessel 2 S25LC) was seen on the lesser curvature in the proximal antrum. It had an input diameter of 138 um and supplied an area of 12 mm<sup>2</sup>. This gave it a perfusion index of 3022 x  $10^4$ . This is five times greater than a typical antral mucosal artery. The ratio between the connectivity index and the perfusion index of the VLC's varied between 0.0008 and 0.05 (mean = 0.01). This is in comparison to a typical value of 0.2 for an antral vessel of visually 'normal' connectivity. (The MAEO had a ratio of zero). (Tab/4/6)

# Arteriovenous Anastomoses

Significant venous filling occured in six specimens (S1;S3;S4;S6;S10;S25). A rigorous examination of these failed to find any direct arteriovenous connections above capillary level. Several apparent cases could be seen, but upon stereomicroscopic examination and micromanipulation, these all proved to be due to overlap.

#### DISCUSSION

The non vascular anatomy of the stomach and duodenum were in accord with the literature. Several features described only by Sloss were confirmed in this study; the middle oblique muscular layer, which was shown to be incomplete, and the irregular muscle fibres within the torus pyloricus. The trilaminar muscularis was also confirmed. The entry position of the bile duct (1.8 cm beyond the pylorus) was slightly proximal to the values from the literature (2 -5cm). The sudden increase in muscular thickness at the fundus-corpus is unusual as this usually occurs more distally in other species.

It is interesting that a feature with the histological appearance of an ulcer was seen in one specimen, since ulcers are not thought to occur in pigs. This lesion penetrated into the muscularis mucosae, but was an isolated feature in an otherwise normal mucosa and was less than 0.5mm in diameter, and may therefore have been artefactual. $(F_{e}^{s}, S_{e}^{s}, S_{e}^{s})$ 

The discrepancy in the literature over the coeliac

branching pattern was clarified. In ten of twelve animals, the coeliac trifurcated, as described by Busch. In only 2 was bifurcation into splenic and gastrohepatic seen (as Sisson). Trixl's hepatic and gastrosplenic bifurcation was not seen in any specimens. The supply of the diverticulum ventriculi by a specific vessel, the diverticular artery, derived from the splenic, was confirmed. This study did not agree with the literature on the presence of extramural anastomoses on the curvatures. Busch specifically failed to find anastomoses between the left and right gastric arteries on the lesser curvature whereas they were present in 4 of 15 specimens here. Conversely, the left and right gastroepiploic arteries anastomosed in all of Busch's specimens but only in 5 of 15 here. In none of these specimens was anastomosis between right gastric and gastroepiploic arteries seen at the gastroduodenal junction, as described by Busch.

The intramural muscular vasculature was as described in the literature, although a specific deep muscular arterial plexus which received most of the recurrent vessels from the submucous plexus had not been previously noted. The specialised arrangement of muscle fibres and adipose tissue around the penetrating vascular bundle was not seen in any  $\langle F_{ij}^{\mu} \psi_{i} \cdot \psi_{j} \rangle$  other species in this investigation. It may be hypothesised to form a specialised structure to resist compression of the vessel as it passes through the particularly thick porcine muscle. This might be achieved by the circumferential muscle fibres forming a more rigid cylinder around the bundle.

The results of this investigation did not fully support the description in the literature of the submucous and mucosal plexuses, particularly based on the work of Busch, Trixl and Saito. These workers described a clear arterial plexus at the base of the mucosa, in addition to the submucous plexus, from which arose the mucosal arteries. This is not the case in this study, where stereomicroscopy, micromanipulation and histology showed that all the anastomoses were, in most areas, in the submucosa. Some were free within the compartment and some partially adherent to the muscularis mucosae, but none were entirely within or deep to the muscularis. In these areas, there were only anastomotic vessels at the base of the mucosa, as defined elsewhere. In the fundus and lesser curvature of some specimens, there were areas of mucosal plexus rather than anastomosis, but only in one, the corpus of S3, did all the mucosal arteries arise from the mucosal plexus. In most they arose from the submucous plexus. This difference may be due to the fact that the authors did not define their concept of plexus, or may be a result of inadequate rigour in the localisation of the arterioarterial anastomoses.

As in other species, the submucous plexus in the lesser curvature was found to be less dense than in other parts of the stomach, although in the pig this was mainly due to a reduction in vessel size, rather than the number of anastomoses. Vessel diameter is is less reliable parameter in injection studies since it may be affected by local perfusion pressure. However, two different measures of plexus density showed that the antral plexus was less dense than that in the corpus. A specific phenomenon, seen in some other species, was the low connectivity vessel, a vessel with very few and/or small anastomoses. These were not specifically described in the literature, but were seen in this study, mainly on the lesser curvature in the distal corpus and antrum. However, they were not present in all, but were specifically seen in 3 specimens and absent in 2. They differed from those of the guinea pig in that 'failure to anastomose' was not seen but, as in the guinea pig, the low connectivity was due mainly to a reduction in vessel number, rather than diameter. Only one probable mucosal artery of extramural origin was seen, although several possible examples were present. This is in accord with Busch, who found one such possible vessel, located, as in this study, in the distal lesser curvature (personal communication). As in other species where VLC's and /or MAEO's are found, the discrepancy between these vessels and 'normal' plexus inputs or mucosal arteries, in terms of their diameter, area and perfusion / connectivity indices,

261

suggests that they are qualitatively different sets of vessels.

No arteriovenous anastomoses were found. This is not in agreement with Busch, but since he does not define his criteria, a firm assessment cannot be made.

No clear explanation for the mucosal domes, seen in the diverticulum, cardia and fundus, was obtained. Histological sections of the region showed the mucosa to be distorted in these areas, but with no other specific features.

of a sircle, whereas the lesser curvature was allost linear. The cosophague entered the stonach at the mid point of the lesser curvature, forming an inclusive cardians of 50° and a large fundus. There was an south inclusive angularis, followed by a sudden reduction in dissetar in the distal antrus and an externally constraine constriction at the gestreductenal junction. The functionization at the pyloric canal was approximately preside to the built of the



Fig. 59 Surface view of fundus in S1C. Note the "dome" structures near the centre of the field (17x). CHAPTER 16

GUINEA PIG

ł

# NON-VASCULAR GASTRODUODENAL ANATOMY

#### General Appearance

The external appearance of the stomach was observed at dissection an all 19 specimens. The mean length and width of the stomachs were 5.4 and 3.3cm respectively. When distended by food, the greater curvature of the stomach formed the arc of a circle, whereas the lesser curvature was almost linear. The oesophagus entered the stomach at the mid point of the lesser curvature, forming an incisura cardiaca of  $90^{\circ}$  and a large fundus. There was an acute incisura angularis, followed by a sudden reduction in diameter in the distal antrum and an externally observable constriction at the gastroduodenal junction. The longitudinal axis of the oesophagus and at right angles to that of the bulk of the stomach. Shortly after the gastroduodenal junction, the duodenum turned through  $90^{\circ}$  to the right.

# Omental Attachments

These were defined in the cleared whole stomach (GP2), the uncleared muscle (GP7: GP10), the cleared muscle (GP14) and the uncleared whole stomachs (GP9: GP11; GP12). The lesser curvature mesentery was visible on the stomach to the left of and dorsal to the oesophagus. It then ran along the lesser curvature to the gastroduodenal junction. The greater curvature mesentery ran down the posterior aspect of the fundus, along the greater curvature to the gastroduodenal junction, which it bridged to pass along the posterior aspect of the duodenum.

# <u>Muscularis</u> Externa

The muscularis externa in the guinea pig is uniformly very thin. It was, therefore, very difficult to obtain satisfactory specimens. The muscle was thickened over the last 0.5cm of the stomach, by fibres with a circular orientation. No fibre orientation was visible in other parts of the stomach.

# <u>Miscellaneous</u>

The structure of the gastric wall was examined histologically in a specimen taken from the anterior corpus of GP6. The serosa and the muscularis externa had been removed prior to sectioning and so could not be observed. There appeared to be little submucous connective tissue, but, since some may have been removed along with the muscularis, this may be anomalous. The muscularis mucosae was also thin (approx 35um) and was composed of two layers of muscle cells of differing orientation. The cells in the two layers did not appear to interdigitate. The mucosa was also thin (approx. 400um). Cleared edged sections taken from the fundus of GP7 GC were also examined. Although the resolution obtainable from these was lower than those from the histological specimens, the dimensions of the layers seemed to be comparable.

The gastroduodenal junction was examined in detail in GP12. The duodenum was at approximately 90° to the body of the stomach. Although no external constriction was visible, a marked sphincteric thickening was visible on the interior, especially on the greater curvature aspect, extending over approximately 5mm. Immediately proximal to this was a region of longitudinal mucosal folding. Distally, was the duodenal ampulla, though there was no peri-junctional recess. The duodenal lumen then narrowed to a diameter of approximately 3mm.

#### EXTRAMURAL VASCULATURE

This was examined in seven specimens, with a detailed examination of the coeliac artery in one. In this specimen a true coeliac (i.e. gastro-spleno-hepatic) trunk was absent. It was replaced by a proximal gastrosplenic and a distal hepatomesenteric trunk arising from the aorta. The gastrosplenic trunk divided to form the left gastric artery and the splenic artery. The left gastric divided into a number of small branches well before reaching the stomach, some of which passed up the oesophagus, although it was not clear whether anastomosis with the oesophageal vessels occured. The splenic artery also supplied branches to the liver. The hepatomesenteric vessel gave rise to the hepatic artery and the pancreaticoduodenal artery.

The features of the arterial supply to the stomach were broadly similar in all the seven specimens studied. The main supply to the lesser curvature was via the left gastric artery. The artery reached the stomach dorsal to the oesophagus, where it gave off branches to the dorsal fundus and the proximal corpus. In 5/7 specimens, it gave rise to a branch running laterally around the oesophagus to supply the ventral fundus and proximal corpus. In 1/7 (GP7) this was absent, whereas in GP11 there was a complete ring around the oesophagus. The main trunk of the left gastric artery then ran along the lesser curvature, but, in all cases, gave off no branches until the distal corpus/antrum, where it



Fig. 60 Topological diagram of coeliac and mesenteric trunks in GP3.





supplied vessels to the dorsal and ventral walls. In GP11, there were two vessels running along the lesser curvature. An anastomotic vessel was seen in GP14, which united ventral antral and fundic branches of the left gastric artery. In GP2,GP9 & GP11, the left gastric artery crossed the gastroduodenal junction to give branches to the first part of the duodenum. There did not appear to be anastomosis with any vessels derived from the superior mesenteric artery.

The greater curvature was supplied by the left gastroepiploic artery which ran in the mesentery along and parallel to the greater curvature. In 2/7 (GP2;GP14), the left and right gastroepiploic arteries anastomosed, whereas they did not do so in the remainder. The anastomoses occured in the region of the gastroduodenal junction. In 4/7, the left gastroepiploic artery gave off alternate dorsal and ventral branches to the greater curvature, the ventral tending to be longer than the dorsal. In the remainder, "triple" branches to the dorsal, antral and ventral greater curvature occured. The left gastroepiploic artery ended 1cm proximal to the GDJ in GP7, whereas it crossed it in GP2 & GP9 to supply the dorsal duodenum and pancreas.Where the gastroduodenal junction was intact, a partial ring of branches around the pyloric canal could be seen, matching, but not anastomosing with, that from the lesser curvature. In GP9 & GP11, the fundus was also supplied by three or four short gastric arteries from the spleen. These were not seen in other stomachs.

#### INTRAMURAL VASCULATURE

#### Supply to Muscularis Externa

The branches of the extramural trunks mainly penetrated the muscle obliquely, but, owing to the thinness of the muscle, they did not have a long "horizontal" intramuscular course. The pattern, seen in some species, of a prolonged subserous course, followed by a turn through  $90^{\circ}$  to penetrate the muscle, was rarely seen. During their intramural course, the vessels gave off branches which gave rise to a superficial and a deep plexus.These were composed of small vessels (approximately 5um diameter), and the two plexuses were united by small communicating vessels. The superficial plexus tended to have transverse orientation, and the deep a longitudinal or oblique one. Any vessel arising from the inter-plexus vessels tended to be orientated parallel to the muscle fibres through which they ran.

In most of the specimens, recurrent vessels were seen on the exterior of the stomach. These were stellate groups of vessels, having no connection with the extramural trunks other than by the sparse subserous plexus, but which were continuous with a larger vessel travelling obliquely through the muscle from its deep surface. These recurrent vessels, which were absent in GP9, had an internal diameter of approximately 40-50um, and supplied both the deep and superficial plexuses.



53%	P E R N E C Z K Y	43%
54%	SHIVELY & STUMP	29%

<u>Fig. 62</u> Topological diagram to show the two most common modes of origin of the gastrosplenic vessels in the guinea pig and their incidence as reported by Shively & Stump and Perneczky.

268

# Spatial Variations in Supply to Muscularis Externa

The limited number of injections and degree of filling makes it difficult to draw any conclusions on this topic. The extra and intra mural courses of the main branches tended to be longer on the lesser curvature aspect than the greater, so that the derivatives of the left gastric artery supplied more than half of the stomach. In addition, the components of the superficial plexus tended to be larger on the lesser curvature aspect than the greater.

# Submucous Plexus

The ensuing description of the submucosa and mucosa in the guinea pig is based largely upon three specimens (GP7;GP10;GP14) and, to a lesser extent, upon three less well filled stomachs GP1;GP6;GP8). Although, in some cases the filling is uneven and the specimen fragmentary, it is possible to define the characteristics of the arterial vessels within the submucosa. The vessels entered this layer, usually obliquely, after passing through the muscularis externa. They continued to travel obliquely, until they left the submucosa by penetrating the muscularis mucosae. During their submucous course, they give rise to two groups of branches. Firstly, relatively large vessels, which remained within the submucous plane, some of which anastomosed with branches derived from other input trunks. Secondly, relatively small vessels which travelled perpendicular to the submucous plane and penetrated the muscularis mucosae. In a few instances, small vessels running back towards the muscularis externa could be seen. Thus, branching and arterioarterial anastomosis did occur within the submucosa, but it is of limited extent. The submucous plexus is coarse and sparse - the majority if anastomoses occurred within the mucosal plexus. Where submucous branching did take place, it was scalariform, The "failure to stellate or irregular. anastomose" phenomenon, described in the rabbit, was also seen at this level. The plane in which anastomoses were located was established by micromanipulation, "calibrated" by the



Fig. 63 Greater curvature of GP7. Duodenum to left (1x).



Fig. 64 Diagram of GP7GC. Position of mucosal arteries of extramural origin (MAEO's) shown. Numbers refer to vessels described and tabulated in text.



Fig. 65 Lesser curvature of GP7. Duodenum to left (1.6x).



Fig. 66 Diagram of GP7LC. Position of MAEO's shown. Numbers refer to vessels described and tabulated in text.

cleared edged sections. (GP7;GP10). The muscularis mucosae was particularly thin and flexible, rendering the differentiation of submucous and mucosal vessels more difficult than in the rabbit, but possible and consistent.

Some venous filling was seen in GP8;&GP14, in which the veins, usually single, ran parallel to the arteries.

### Spatial Variations in Submucous Plexus

Few of the specimens of the submucous plexus were uniformly filled and undamaged. The ensuing is therefore based largely upon the best specimen,GP7, the features being confirmed or refuted wherever possible in GP8;GP10;GP14.

A definite pattern of inputs to the submucosa was visible on the lesser curvature of the specimens.Surrounding the oesophagus were two distinct rings of input vessels, one immediately at the base of the oesophagus, and a second, concentric one, located more distantly. Distal to this ring, very few inputs could be seen on the lesser curvature, or for some distance dorsally or ventrally, until the antrum. On the corpus, dorsal and ventrally, near the midline of these aspects, a double or triple line of inputs was seen on each aspect, which combined in the antrum to form an apparently randomly orientated group of vessels.

The arrangement of inputs to the submucosa on the greater curvature was simpler. A double line of inputs extended from the middle of the dorsal fundus to reach the greater curvature at approximately the fundus/corpus interface. In this region, it became a triple line and continued along the greater curvature as far as the gastroduodenal junction. In the antrum the pattern became more random.

The inner ring of inputs around the oesophagus had a stellate or radially orientated scalariform branching pattern with branches running mainly peripherally. The branches of the outer ring were larger and scalariform. Those on the lesser curvature ran towards the oesophagus, the remainder centrifugally. The antral vessels had a random branching pattern. The outer components of the double chain



Fig. 67 Vessels making linear anastomoses in submucous / mucosal plexuses - fundus of GP14GC (12.8).



Fig. 68 Vessels making stellate anastomoses in corpus of GP14GC (12.8).



Fig. 69 Group of low connectivity stellate branching vessels on greater curvature of GP7 (8.8x).



<u>Fig.70</u> Linear and stellate branching vessels in corpus of GP10LC (3.2x).

on either side of the lesser curvature had a predominantly scalariform arrangement, directed towards (upper line) and (Fig.65;i)) away from (lower line) the lesser curvature. Where a third, inner, group of vessels was found, they had a stellate (Fig.65) branching pattern. The antral greater curvature vessels had a random branching pattern, whereas the outer components of the greater curvature chain had a scalariform pattern directed towards the lesser curvature. Once again, the central vessels of the chain, where present, were stellate.

Anastomoses occurred between the scalariform branches of the greater curvature chain vessels and the scalariform branches of the outer oesophageal ring and of the outer component of the lesser curvature chains. These anastomoses were mainly in the submucosa. Many of the trunks, particularly the stellate vessels, had no submucous anastomoses with vessels derived from the other input trunks. Since in the fundus and proximal corpus the greater curvature inputs are found on dorsal wall, the ventrally directed branches had a longer submucous course than the dorsally, before anastomosing with the derivatives of the lesser curvature inputs.

The gastro-oesophageal junction could be seen in two specimens - GP7 & GP10. In both, there was a ring of inputs around the immediate base of the oesophagus. In GP7, small branches of these vessels ran up the oesophagus before anastomosing with the oesophageal arteries. Conversely, in GP10, no branches ran up the oesophagus.

The gastroduodenal junction could also be seen in these (Fig.F2)two specimens. In GP7, the junction was poorly defined, whereas in GP10, it was sharply defined, extending over only 1.5mm. In both, the vessels crossing the gastroduodenal junction were the main rostrally directed branches of a ring of vessels in the duodenum, immediately distal to the junction. Some of these anastomosed with the minor, caudally directed branches of a few isolated gastric vessels, immediately proximal to the junction. Thus, although significant leashes of vessels were not present, the vessels supplying the perijunctional region were mainly of duodenal



Fig. 71 Vessels of mucosal plexus in transverse section. Submucosa above, mucosa below. (40x).



Fig. 72 Gastroduodenal junction of GP7. Duodenum to left. GDJ supplied by duodenal vessels (1.8x). origin.

# Connective Tissue Plexus

A thin layer of connective tissue was demonstrable in the submucous compartment of GP7 & GP10. However, in neither of these specimens was it possible to see any components of a connective tissue plexus. All vessels given off from the main vessels passing through the submucosa either travelled parallel to the muscularis mucosae or travelled towards it. No branches were given off towards the deep surface of the muscularis externa. Similarly, in four sections taken from the fundus of GP7, and five from GP10, the very thin connective tissue did not appear to contain connective tissue plexus vessels.

#### Mucosal Plexus

The mucosal plexus was seen in GP7 & GP10 and parts of GP8 & GP14. It was formed from two sources, the vessels which passed obliquely through the submucosa, penetrated the muscularis mucosae and entered the mucosal compartment, and also from perpendicular branches given off by vessels within the submucosa. Once within the mucosa, the vessels ramified to form a plexus at the base of the mucosa. The plexus vessels were of the same approximate diameter as the inputs. They gave rise to vessels, the mucosal arteries, which ramified perpendicularly within the mucosa. These were smaller than, and non-continuous with, the plexus inputs. The majority of the arterioarterial anastomoses that are seen from the surface of the cleared mucosal specimens, other than the large ones within the main lesser and greater curvature inputs, are located within the mucosal, rather than the submucous plexus. The position of these vessels may be determined by micromanipulation, stereomicroscopy and the cutting and microscopic examination of cleared edge sections.

The branching pattern of the mucosal plexus was scalariform, stellate or irregular in different regions. However, the plexus, although richer than the submucous plexus, was sparser than that in many other species. In addition, the phenomenon of 'failure to anastomose' was very commonly seen. Many of the inputs to the mucosal compartment made only a few anastomoses with branches derived from adjoining trunks, and some made none at all (see below).

# Spatial Variations in Mucosal Plexus

The variations in the branching patterns of the plexus mirrored those of the submucous plexus. The plexus appeared to be less dense distally, and less dense where the branching pattern was stellate as opposed to scalariform.

# <u>Mucosal Arteries</u>

The characteristics of the mucosal arteries are described on the basis of their observation in GP7;8;10 & 14, the former being largely complete, the three latter being, to a greater or lesser extent, partial.

The mucosal arteries arose from the mucosal plexus at the base of the mucosa. In most cases, they had an initial horizontal course, in the plane of the plexus , before turning through 90° and branching within the mucosa in a tree-like pattern. This ramification occured near the base of the mucosa in most cases, only small arterioles and capillaries reaching the mucosal surface. However, in GP14LC, large mucosal arteries appeared to travel to the mucosal surface. There was also extensive branching of the mucosal arteries in their horizontal course in all the specimens observed. The diameter of the vessels became less distally. The previous section emphasized the sparse nature of the mucosal plexus - there were relatively few arterioarterial anastomoses. As a corollary, the mucosal arteries were large and supplied a large area of mucosa. The mean of thirty determinations in GP7 gave an average diameter of 32.4 + 14.6um and an average area of supply of  $31.0 + 28.8 \times 10^{-2} \text{ mm}^2$  with a corresponding perfusion index of 690 + 787 um<sup>4</sup>mm<sup>-2</sup> x 10<sup>4</sup>. (Tuble 17)

In the guinea pig, the low connectivity vessels had fewer, but normally sized, anastomoses. As described in the  $(f_{1g5}, \delta q_{1}; s)$  previous section. many of the inputs to the mucosal plexus had few anastomoses with their neighbours. This was reflected in the phenomenon of 'failure to anastomose' of the mucosal arteries. In very many cases, mucosal arteries approached one another to within a few microns, then turned through  $90^{\circ}$  and penetrated the mucosa without touching. It often required careful high power examination, with micromanipulation, to confirm that no anastomosis was present. This phenomenon was seen in all areas of the specimen though with varying frequency.

Many inputs to the mucosal plexus were visible which had only one anastomosis. In GP7, seven unequivocal mucosal arteries of extramural origin (MAEO's) could be identified (79343-46) which had no anastomoses with the surrounding vessels. In addition, there were possible examples in other specimens which could not be unequivocally identified due to poor filling. All these vessels had a stellate branching pattern (Figs.74-36)and showed multiple failures to anastomose. The mean internal diameter, area of supply and perfusion index of these vessels were 133  $\pm 20$ um, 518  $\pm$  318 x 10<sup>-2</sup> mm<sup>2</sup> and 8003  $\pm$  4703 x 10<sup>4</sup> um<sup>4</sup>mm<sup>-2</sup> respectively. (7a6/45 18-79)

Unfilled intervals between inputs to the mucosal plexus were very commonly seen. However, in many cases, these were artefactual, caused by incomplete mucosal filling. When complete filling was present, an unfilled interval was sometimes seen between the areas of supply of mucosal arteries, but in many cases it was not evident, as the mucosal artery branches interdigitated, although they did not anastomose.

Some of the features of the venous supply to the mucosa could be seen in GP10 & GP14. The arterioles and capillaries derived from the mucosal arteries drained into a surface honeycombe venous plexus. This was drained by large collecting venules running perpendicularly from the surface to the base of the mucosa where they gave rise to a venous plexus.

5

279



Fig. 73 Low connectivity vessel (central field) in antrum of GP7GC (12.8x).



Fig. 74 Mucosal artery of extramural origin in corpus of GP7GC (central field) (12.8x).



Fig. 75 Mucosal artery of extramural origin (central field) in corpus of GP7GC (12.8x).



Fig. 76 Mucosal artery of extramural origin (central field) in corpus of GP7GC (12.8x)

# Spatial Variations in Mucosal Arteries

The diameter and area of supply was measured ten mucosal arteries in each of three areas in GP7. The subjective impression that the vessels were smallest in the antrum was not borne out. There were no significant differences in diameter, area of supply or perfusion index between the fundus, corpus and antrum. (Table 17)

Owing to the incomplete filling in some areas of some specimens, it was not possible to describe comprehensively the distribution and occurence of the MAEO's. All of those which could be unequivocally identified were located in GP7, probably because this was the best filled specimen. However, observation of all the cleared specimens enables certain predictions to be made concerning the occurence of the MAEO's. This phenomenon was related to the occurence of

	FUNDUS			CORPUS (LC)			ANTRUM		
	D	A	PI	D	A	PI	D	A	PI
	38 38 63 25	56 53 48 91	372 393 3282 43 353	38 38 50 38 25	34 34 53 16 22	613 613 1179 1303 176	25 38 25 25 38	13 16 25 19	300 1303 156 206 2317
	13 25 25 13 63	59 6 13 9 9 72	48 300 434 32 2188	25 50 25 25 38 13	44 6 22 25 9	1420 651 178 834 32	25 13 13 63 25	13 9 3 128 13	300 32 95 1231 300
MEAN SD	34.1 <u>+</u> 17.8	41.6 <u>+</u> 30.3	745 <u>+</u> 1091	34.0 <u>+</u> 11.8	$26.5 \\ \pm \\ 14.9$	700 <u>+</u> 488	29.0 $\pm$ 14.6	24.8 <u>+</u> 36.7	624 <u>+</u> 748
WHOLE	32.4	31.0	690						

NOTES

D = Diameter (um)

A = Area of supply  $(mm^2 \times 10^{-2})$ PI= Perfusion Index  $(um^4 mm^{-2} \times 10^4)$ 

٤,

There were no significant differences in any of these parameters.

#### Table 17 Diameter, Area of Supply and Perfusion Index for the Mucosal Arteries of the Guinea Pig

#### 659.57-00)

'failure to anastomose'. FTA could be seen in all areas of all specimens, between both large and small vessels. However, those inputs which had a scalariform or linear branching pattern always had a significant number of anastomotic branches. Conversely, the stellate branching vessels made fewer anastomoses, particularly the more distally located ones. Thus, in the relatively well injected GP7, it could be seen that virtually all the stellate branching vessels were low connectivity vessels i.e. they made few anastomoses with their neighbours. The converse was also true - the majority of LCV's were stellate vessels. Whilst filling was less complete in other stomachs, nothing was seen which contradicted this observation. It should be noted that although the areas of supply were not measured, they were usually subjectively less for the stellates than for the scalariform vessels.

Seven unequivocal MAEO's were visible in GP7. One of these was an irregularly branching vessel lateral to the oesophagus, whereas all the remainder were the central stellate branching vessels of the greater curvature chain. All of these stellate vessels were either low connectivity vessels, having only one or two anastomoses, or were MAEO's.Such vessels could have been present in the corresponding areas of other stomachs, but incomplete filling made it impossible to be certain. The MAEO's seemed to have a smaller area of supply than the VLC's but, although there was no significant difference in diameter, the perfusion index was significantly lower (worse) for the MAEO's. (Table 19)

### Arteriovenous Anastomoses

Extensive venous filling was seen in GP10 and GP14. In neither of these could any direct arteriovenous connections be seen.

	FUNDUS	LC	GC	ANT WALL	POST WALL	DUODENUM	ANTRUM
GP1 <sup>bh</sup>	?	?	?	?	?	?	?
GP2 <sup>b</sup>	?	?	?	?	?	?	?
GP6	?	?	?	?	?	?i	?
GP7		1+?	6+?			j	?a
GP8 <sup>f</sup>	5a	.sa	? <sup>e</sup>			ŝа	<sup>5</sup> a
GP10	?	?	,q	5p	5p	?	?
GP14	?	?	?c	/b	,p	?	?

#### **NOTES**

"?" indicates an area where it is not possible to definitely exclude the presence of MAEO's.

"---" indicates an area where the presence of MAEO's can be excluded.

- (a) Random branching area. Several unfilled, but many do anastomose.
- (b) Scalariform branching area, therefore MAEO's unlikely.
- (c) Mainly scalariform branching areas, but some fairly well filled stellates present.Some do anastomose, five do not.
- (d) Four fairly well filled stellates which do not anastomose. Others have 1+ anastomoses. All scalariforms do anastomose. In GP10 better degree of anastomosis with fewer FTA's but many vessels unfilled or partially filled (might expect a greater proportion of FTA's to be poorly filled?).
- (e) Most of central GC unfilled. A few unfilled stellates visible. Scalariform vessels which are well filled.
- (f) Largely masked by venous filling. FTA less common.
- (g) Some damaged areas but most of specimen present. All visible vessels anastomose.
- (h) Very heavy muscular filling. Submucous details not visible.
- (i) Poorly filled but most show more than one anastomosis.
- (j) First centimetre of duodenum only.
- (k) Distal part of duodenum.

Table 18 Occurence of MAEO's in the Guinea Pig



<u>Fig. 77</u> Mucosal artery of extramural origin (central field) in corpus of GP7GC (12.8x).



<u>Fig. 78</u> Mucosal artery of extramural origin in corpus of GP7GC (25.6x).
SPECIMEN	MAEO NO	AREA OF SUPPLY $(mm^2 \times 10^{-2})$	DIAMETER (um)	PERFUSION INDEX (um <sup>4</sup> mm <sup>-2</sup> x10 <sup>4</sup> )
GP7GC GP7LC	1 2 3 4 5 6 1	238 463 913 194 275 575 969	138 113 150 113 138 113 163	15238 3522 5545 8405 13188 2836 7285
MEAN		518	133	8003
SD		318	20	4703
	VLC No	Γ		
GP7GC	7	806	150	6281
	8	631	175	14864
MEAN		719	163	10573
SD		124	18	6069

There is no evidence that the MAEO's and the VLC's are from different populations with respect to their areas, diameters or perfusion indices.

#### Table 19 Diameter, Area and Perfusion Index of MAEO's and VLC's in the Guinea Pig

#### Duodenum

It proved to be very difficult to obtain adequate duodenal injections, due to the fragility of the tissue and incomplete filling. In GP1, the filled duodenum was overlaid by opaque muscle. Some information could be derived from GP6 and GP8 and again, the best specimen was GP7.

The inputs formed a double line along the lesser curvature or posterior aspect of the duodenum. Branches arose from these in a scalariform manner although on the lesser curvature half of GP7, the branches appear to be random in orientation. Micromanipulation of the cleared specimens and the examination of cleared edge specimens from GP7 showed that both a submucosal and a mucosal plexus was present, as in the stomach, but it was not possible to demonstrate the presence of a connective tissue plexus. Only the first centimetre of GP7 was well filled and no MAEO's were present here.It was not possible to determine their presence in the remainder of the duodenum, but the predominance of the scalariform pattern militates against this.

The villi were filled in GP7. There appeared to be a single central arteriole which branched into a fountain pattern at the tip to give rise to peripheral venules which drained into a basal venous plexus.

#### DISCUSSION

The non-vascular characteristics of the 19 specimens were as described in the literature. It was noticeable that the muscularis mucosae was thinner and simpler than in other species, as shown by both histological and cleared edge specimens. Only one dissection was made of the coeliac and this mesenteric vessels, as is well documented. Interestingly, this one specimen was not consistent with the common patterns, but was an example of a minor variant, only reported by Shively and Stump and seen by them in only 8% of cases. The extramural vessels of the seven stomachs examined were not in accord with the patterns described by these authors. According to Shively & Stump, all the specimens showed anastomoses between the left and right gastric arteries, and between the left and right gastroepiploic arteries. The former was seen in none of the seven specimens, and the latter in only two, when it occured at the gastroduodenal junction rather than on the greater curvature. Thus, since the left gastric artery ran onto the duodenum and the left gastroepiploic often reached the duodenum, all the specimens in this study showed a greater degree of gastric dominance than indicated by Shively &

Stump. Similarly, the contributions to the left gastroepiploic artery by the left gastric artery on the fundus were not seen in this study. Although these authors examined more specimens (24 vs. 7), it is difficult to account for the clear differences seen.

Since the literature contains no descriptions of the intramural vasculature in this species, there are no standards against which to compare the results of this study. Despite the thinness of the muscularis externa, which made it difficult to define the intramural angioarchitecture, the recurrent stellate vessels were a constant feature (6 of 7 specimens), as in the rabbit. The course of the main nutrient vessels again demonstrated the dominance of the left gastric artery.

Although it proved difficult to obtain good injections of the submucosa and mucosa, three fairly complete and three partial specimens were available. The features they displayed were compatible, giving confidence that the characteristics which are described are features of the guinea pig stomach and not anomalies. The key feature is the balance between the submucous and mucosal plexuses. The submucous plexus is sparse, but some larger anastomoses are located within this compartment. However, the great majority are found at the base of the mucosa. Since the simple bilaminar muscularis mucosae is very thin, this feature could very easily be missed, since only careful micromanipulation under stereomicroscopy, and cleared edge sections, will reveal it. The mucosal plexus appears to be less extensive than in some other species. It is also important that many of the inputs to the mucosal plexus have not made any anastomoses in the submucosa.

Most of the mucosal arteries arise from the mucosal plexus; none arise from the submucous plexus. However, some anastomose in neither plane i.e. they are mucosal arteries of extramural origin (MAEO's). These are a clear feature of the guinea pig stomach. Although they were only unequivocally demonstrable in one stomach, in no stomachs were they clearly absent. In all the other specimens, vessels with the same characteristics were present which were probably MAEO's, but partial filling prevented their being confirmed as such. Two alternative explanations could be postulated; that they are mucosal arteries whose proximal anastomoses have been removed, or that they are inputs to the mucosal plexus with unfilled anastomotic vessels. The first is unlikely, because dissection was carried out close to the internal surface of the muscularis externa, which was subsequently examined for retained vessels-none were seen. In addition, the parameters of the MAEO's were significantly different from those of the mucosal arteries. For example, the mean perfusion index of the mucosal arteries was 690+ 787  $\text{um}^4 \text{ mm}^{-2} \times 10^4$  compared with 8003+4703 for the MAEO's. Their diameter is comparable with that of the main inputs to the submucous compartment. The second is unlikely for a number of reasons. The MAEO's have common characteristics, in that they are all stellate vessels located on the distal curvatures, mainly the greater curvature. Unfilled vessels might be expected to be present in all vessels geometries and locations. They are also a part of two continuous spectra of vessels. The first range concerns the level of anastomoses. Most vessels anastomose only in the submucous plexus. A few anastomose in both submucous and mucosal plexuses. A few anastomose in neither. The second range concerns the number and geometry of the anastomoses. There is a spread of degree of anastomotic communication between vessels. This is manifest mainly in a reduction in the number of anastomoses, not size. The spectrum encompasses good anastomotic communication, only a few, but full diameter, anastomoses (low connectivity vessels - LCV's), and no anastomoses (MAEO's). As the number of anastomoses decreases, vessels increasingly show the phenomenon of 'failure to anastomose', seen in both LCV's and MAEO's. Thus, if LCV's and MAEO's were actually normal vessels whose communications with the mucosal plexus had not filled, it would be expected that the picture would be of vessels with only a few branches in the mucosal plane, or with branches that were smaller or 'faded away'. The actual picture is of



<u>Fig. 79</u> 'Failure to anastomose' - two vessels in mucosal plexus approach very closely (central field) then turn down into mucosa before anastomosing. Surface view in GP7GC (51.2x).



<u>Fig. 80</u> The above failure to anastomose as a cleared edge section in GP7GC (12.8x).

vessels with a 'normal' number of full diameter branches in the mucosal plane, which do not 'fade away' but which turn down into the mucosa before they meet a branch from an adjoining vessel. This FTA is not immediately obvious as the gap is only a few microns, and vessel interdigitation means that an unfilled interval is rarely seen. However, both these support the contention that the LCV's and MAEO's are a real feature of the guinea pig angioarchitecture at this level.

# CHAPTER 17 THE FERRET

#### NON - VASCULAR GASTRODUODENAL ANATOMY

# General Appearance (Fig. 8)

The stomachs of all twelve ferret specimens were examined externally in situ and after removal from the abdominal cavity. The ferrets were all male, between 970 g and 1481 g (mean  $1260 \pm 234$  g). The stomach was 'U' shaped with the ascending and descending limbs approximately parallel to one another and to the rostro-caudal axis of the animal. The diameter of the stomach tapered continuously down from rostral to caudal, ranging from approximately 1 to 3.5 cm. The two limbs were separated by 0.8 to 1.0 cm. The rostral third of the rostral limb was covered ventrally by the left lobe of the liver.

The oesophagus entered very obliquely into the medial aspect of the stomach, near to its rostral pole. The incisura angularis was gradual rather than sharp, but was a full  $180^{\circ}$ . The duodenum was clearly differentiated from the pyloric antrum by a distinct narrowing at the gastroduodenal junction. The first part of the duodenum ran in a rostral direction for 1-2 cm before turning to the right abruptly with an angle of  $90^{\circ}$ .

# Omental Attachments

In all the specimens, the omenta had a high adipose content which needed to be removed before the lines of attachment could be clearly defined. The lesser omentum ran along the lesser curvature, from cardia to gastroduodenal junction, effectively bridging the gap between the ascending and descending limbs. The lesser omentum was reflected onto the stomach a few millimetres on either side of the lesser curvature, leaving a narrow intervening gap. It left the first part of the duodenum approximately 0.5 cm distal to the gastroduodenal junction, after moving onto its dorsal aspect. The greater omentum ran along, and was virtually



Fig. 81 Stomach of F12 in situ. Upper paler area normally covered by liver (1.5x).

colinear with, the greater curvature with the two layers almost in apposition. The line of contact on the greater curvature did not run from the rostral pole to the gastroduodenal junction, since the omentum only met the stomach approximately half way down the rostral limb i.e. on the proximal corpus.

#### <u>Muscularis</u> Externa

The tunica muscularis was examined in all the twelve specimens, before, during and after dissection. The tunica was thin and its structure was difficult to define. Gross, microscopic and histological examination (in F11) confirmed the normal mammalian pattern of outer longitudinal and inner circular layers of smooth muscle fibres. Both layers appeared to be complete. No incomplete bundles of fibres could be seen in addition to these. The overall thickness of the tunica did not appear to differ greatly in different regions of the stomach, apart from a moderate increase in the distal antrum. However, there was a distinct sphincteric thickening at the gastroduodenal junction. No such thickening could be defined at the gastro-oesophageal junction. There was a sharp decrease in thickness of the muscularis at the gastroduodenal junction. The duodenal muscle was very thin but still showed the same two layer pattern as the gastric tunica muscularis. This was well seen in F8.

#### **Miscellaneous**

Histological (F12) and cleared edge (F8) sections were prepared from the corpus and antrum. The muscularis mucosae was approximately 20 - 25um thick, comprising 4 - 6 cellular layers. There was no apparent structure and the cell layers did not show any differential orientation. The underlying mucosa was about 400um thick.

Internal examination of the gross specimens showed that the mucosa was very convoluted, especially at the rostral end. Here, the rugae had no particular orientation, whereas, at the less convoluted caudal end, the rugae had a definite longitudinal orientation. An unusual feature was a single longitudinal groove in the mucosa which ran along the complete length of the stomach. This was located on the dorsal side of the lesser curvature. It was evident in 10 of the 12 specimens and was most clearly seen in F7 and F8. In the latter, it extended only as far as the incisura angularis.

The entry point of the bile duct could be seen in five of the specimens (F7;8;9;10;11). This was located between 19 and 28.5 mm from the gastroduodenal junction (24.9  $\pm$  3.5 mm).

#### EXTRAMURAL VASCULATURE

The literature review yielded no research based account of the extramural arterial vasculature. This was therefore examined in detail in this work. The arterial supply to the stomach and first part of the duodenum was dissected and examined from its aortic origin. This was done in seven specimens (F5; 6; 7; 8; 10; 11; 12). In F9, it was only possible to examine the vessels as they approached the stomach, since their more proximal course was excessively fragmented. (Fig 84.90) (Table 20)

In all the specimens, the entire gastric supply was derived from the coeliac artery, a branch of the abdominal aorta. At this point, the aorta had a mean diameter of 2.5 + 0.44mm and the coeliac a mean diameter of 1.8 + 0.1mm. After 8.2 + 1.8mm the first branch of the coeliac was given off which was always the common hepatic artery (mean diameter 1.3 + 0.1mm). In none of the specimens was the classical "tripodal" structure of Von Haller seen, but rather a bifurcation into common hepatic artery and gastrosplenic trunk. In five of seven specimens, the first branch of the latter, after 3.8 + 0.5mm, was the left gastric artery (mean diameter 1.3 + 0.1mm), the trunk continuing for 3.4 + 2.7mm before bifurcating to form two splenic arteries. In F8, this was virtually tripodal, as only 0.5mm separated the left gastric and splenic arteries. However, in F5 and F7, the first branch of the gastrosplenic trunk was a branch of the splenic artery. The trunk then continued to bifurcate into the left gastric artery and a further branch of the splenic artery.

The common hepatic artery had a mean diameter of  $1.3 \pm 0.1$  mm and was the first branch of the coeliac trunk in all the specimens. Its derivatives supplied the liver via the proper hepatic artery and hepatic branches, the caudal stomach via the right gastric and right gastroepiploic arteries, the proximal duodenum via the gastroduodenal and superior pancreaticoduodenal arteries, the pancreas via the superior pancreaticoduodenal artery and pancreatic branches, and miscellaneous branches to the greater omentum and associated adipose tissue. Most of the branches of the common hepatic artery arose dorsal to the stomach or within the pancreas.

In four of the seven specimens, the first significant branch of the common hepatic was the right gastric artery (F5; 6; 8; 12). This had a mean diameter of  $0.4 \pm 0.1$ mm. In the remaining three specimens, it was subsequent to the origin of the gastroduodenal artery. In either case, the right gastric artery supplied the pyloric gastric lesser curvature, the gastroduodenal junction and the rostral duodenum. In addition, it gave rise to hepatic branches. The right gastric anastomosed on the lesser curvature with the left gastric artery in five of the seven specimens (F6; 7; 8; 11; 12) with a mean minimum anastomotic diameter of 0.23  $\pm 0.15$ mm.

The gastroduodenal artery was larger than the right gastric (mean diameter  $0.9 \pm 0.2$ mm). In three specimens(F7; 10; 11) it was the first significant branch of the common hepatic, in the remainder, the second. It supplied, via its derivatives, the proximal duodenum, antral greater curvature, greater omentum and pancreas. The two main branches, usually formed by bifurcation, were the right gastroepiploic and superior pancreaticoduodenal arteries.

The right gastroepiploic artery was present in all the specimens, with a mean diameter of  $0.8 \pm 0.1$ mm. It supplied the proximal duodenum and antral greater curvature. In only

two of the specimens (F10; 12) was it seen to anastomose with the left gastroepiploic artery, with a minimum anastomotic diameter of approximately 0.1mm. Despite a careful dissection, no anastomotic vessel could be found in the remainder. Branches of the artery in F12 could be seen to anastomose with branches derived from the splenic artery within the greater omentum.

In three of the specimens (F8; 10; 11), a discrete vessel supplying the pancreas and duodenum the superior pancreaticoduodenal, could be seen. In the remainder, a number of branches supplying mainly the pancreas were given off from the right gastroepiploic artery. Where present, the superior pancreaticoduodenal had a mean diameter of  $0.9 \pm$ 0.1mm. In F8 alone, it could be seen to anastomose with a vessel derived from the superior mesenteric artery.

Between the first and second main branches of the common hepatic a number of smaller branches were given off, supplying the liver, pancreas and proximal duodenum.

The left gastric artery arose directly from the gastrosplenic trunk in five of the seven specimens. In the remaining two, it arose from the splenic artery. It had a mean diameter of 1.3 + 0.1mm. The topology of its distribution did not appear to be different in either case. The left gastric coursed upwards to pass behind the stomach. It first gave rise to several branches which penetrated the muscularis at the cardia and others which passed onto the distal oesophagus. These branches anastomosed at the base of the oesophagus. The left gastric supplied the whole lesser curvature and adjoining anterior and posterior walls of the stomach by a tree-like branching pattern, rather than a continuous anastomotic vessel giving rise to side branches. This branching occurred entirely on the dorsal aspect of the stomach, so the vessels supplying the ventral aspect were longer than those supplying the dorsal. As a result of the close apposition of the fundus and pylorus, the branches remained fairly short despite the tree-like pattern. In five of the specimens, one of these branches continued to anastomose, as a single vessel, with a branch of the right

gastric artery, with a mean minimum diameter of  $0.23 \pm 0.15$ mm. The left gastric was the dominant vessel on the lesser curvature, supplying the stomach as far as the middle of the pylorus. Hepatic branches, which were small and few in number, were seen only in one specimen (F12). The branches of the left gastric, as they penetrated the muscularis, varied between 0.1 and 0.5mm. They tended to be smaller on the pylorus than the fundus.

In all the specimens, there were two main splenic arteries. These were derived from a gastrosplenic trunk, formed from a bifurcation of the coeliac artery. In five of the seven specimens, the gastrosplenic trunk first gave rise to the left gastric, then split into the two splenic arteries. In F8, the three vessels arose tripodally. In F5 and F7, a splenic artery was the first vessel to arise, the remaining trunk subsequently dividing to give rise to the left gastric and a second splenic artery. The two main splenic arteries (mean diameter 0.9 + 0.1mm) ramified extensively in a tree like pattern within the mesentery and adipose tissue dorsal to the stomach. There was no apparent consistent branching pattern. They gave rise to several pancreatic branches and many branches to the spleen. In addition, many short gastric arteries arose which supplied the greater curvature. These had a mean diameter of 0.5 + 0.1mm and showed extensive arterioarterial anastomoses between themselves. These occurred between vessels derived from the same, and from different, main splenic arteries. In general, one of the splenic arteries tended to supply the rostral spleen and greater curvature, and the other the although this was not a clearly caudal, defined delimitation. In three specimens, F5, F10 & F12, there were additionally leashes of short gastric arteries from the poles of the spleen to the greater curvature. A definable left gastroepiploic artery could only be seen in F10 and F12. This ran adjacent to the greater curvature giving off branches to stomach and omentum and anastomosed with the right gastroepiploic artery, with a minimum diameter of approximately 0.1mm.



Fig. 82 Whole cleared stomach - F7 (1x).



Fig. 83 Branching of coeliac artery in F7. This splits to form common hepatic artery, then left gastric artery, then two splenic arteries (5x).









Fig. 86 to 90 Toplogical diagrams of coeliac artery branching in ferrets.

.

All values are vessel diameters in mm.

-











	F7	F8	F10	F11	F12	MEAN	SD
D (Aorta)	3.1	2.0	2.5	2.6	2.1	2.5	0.4
D (Coeliac art)	1.7	1.7	1.9	1.8	1.7	1.8	0.1
D (LGA)	1.3	1.2	1.3	1.3	1.4	1.3	0.1
D (CHA)	1.2	1.2	1.3	1.4	1.2	1.3	0.1
D (SA <sub>1</sub> )	1.0	1.0	0.9	0.9	0.9	0.9	0.1
$D (SA_2)$	0.9	1.2	0.8	1.0	0.8	0.9	0.2
D (RGĀ)	0.3	0.4	0.4	0.5		0.4	0.1
D (GDA)	0.7	1.2	0.9	0.9	0.8	0.9	0.2
D (RGEA)	0.9	0.9	0.9	0.7	0.7	0.8	0.1
D (SGA)	0.3	0.4	0.4	0.6	0.6	0.5	0.1
L (Pyl to BD)	26	29	19	26	25	25	3.9
Weight (g)	970	971	1421	1279	1437	1260	234
D(min GC anast)			+		0.1		
D(min LC anast)	0.3	0.1		0.1	0.4	0.2	0.15
D(inter SGA	0.1	0.05	+				
anastomoses)							
L(CA to CHA)	8	6	8	11	8	8.2	1.8
L(CHA to 1st br	19	11	17	14	5	13.2	5.5
L(CHA to LGA)		3	4	4	4	3.8	0.5
$L(LGA to SA_{1/2})$		0.5	7	3	3	3.4	2.7

All dimensions in mm.

Table 20 Dimensions of the Ferret Extramural Vasculature

#### Conclusions

In summary, the left gastric artery was the dominant vessel, supplying the distal oesophagus, cardia and lesser curvature from the caudal extreme to the mid pylorus. Its branches supplied well beyond the mid line of the anterior and posterior walls. The lesser curvature aspects of the proximal duodenum and rostral half of the stomach were supplied by the right gastric artery, derived from the common hepatic. In five of the seven specimens, there was a continuous vessel along the lesser curvature, formed by anastomosis of the right and left gastric arteries. The bulk of the greater curvature was supplied by the the short gastric arteries and their parent vessels, the splenic arteries. One of the main splenic branches supplied the rostral stomach and the other the caudal. There were few, if any, arteries reaching the mid third of the greater curvature. The intramural branches of these vessels were short and anastomosed with the lesser curvature branches on the greater curvature half of the anterior and posterior The antral greater curvature was supplied by walls. derivatives of the common hepatic, the right gastroepiploic and the gastroduodenal arteries. In only two specimens was there anastomosis between the left and right gastroepiploic arteries.

# INTRAMURAL VASCULATURE

#### <u>Supply to Muscularis Externa</u>

The muscularis externa was preserved as intact as possible and examined in all the specimens. That from F7 -12 was cleared in the same way as the mucosal specimens and the vasculature examined. However, in general, the vascular filling was incomplete and a composite picture can only be described in overview. The nutrient arterial branches ran subserosally and parallel to the surface of the muscle. Each of the main branches ran away from the curvatures and gave rise to primary tree like branches. These anastomosed with branches derived from the inputs on the converse curvature, the anastomotic line being on the greater curvature side of the mid line of the anterior and posterior walls. Smaller secondary and tertiary branching occurred in the same plane, which formed a scalariform pattern, in contrast to the tree like branching of the primary vessels. From the secondary and tertiary vessels, branches were given off which ran obliquely through the muscularis to the submucosa. In a few areas, some capillary filling occured and the capillary beds could be seen to run parallel to the muscle fibres. In most cases, the larger arteries were paired with an accompanying vein.

The pattern of the vessels and their size did not appear to differ significantly in the different areas of the stomach, although the uneven filling made it difficult to confirm this in any one specimen.

# Submucous Plexus (Fig. 82; 91.92)

The submucous plexus was visible and analysable to some extent in all nine ferret specimens. However, the level of injection varied from incomplete to very dense, so that all parameters could not be examined in every region of every stomach. In addition, the fragile mucosa was sometimes damaged in dissection. The description is, therefore, a composite one, with individual variations noted where possible.

The vessels derived from the extramural trunks left the deep surface of the muscularis externa and passed obliquely through the submucosa. During this course they gave off small vessels which ramified through the submucosa, forming the connective tissue plexus. Towards the base of the submucosa, the vessels formed an extensive submucous plexus of primary, secondary and tertiary arterioarterial anastomoses. All components of this plexus were movable and palpable on stereoscopic microscopic examination and were, therefore, located entirely within the submucous connective tissue. This was confirmed by the histological examination of a number of specimens, prepared as paraffin, frozen and cleared edge specimens. None of the anastomoses were within

•:

or deep to the muscularis mucosae. Twenty measurements were made of the diameter of the plexus vessels in each of three specimens (F8;F10;F11). These varied between 35um and 90um with a mean of 56.4um. Small recurrent vessels were visible, which contributed to the connective tissue plexus.

The mucosa of the ferret stomach was very convoluted with many mucosal folds. Vessels of the submucous plexus were often located within, and followed, these folds. Conversely, however, in a number of cases the vessels bridged the sulci and had to be cut before the mucosa could be flattened. This phenomenon was seen in all specimens.

In F10, many of the anastomotic vessels were seen to narrow as they left the parent vessel within the plexus. The appearance was consistent with a sphincteric structure at this point. This could not be seen in any of the other specimens. F10 also showed sacculations in its filling of some vessels.

The submucous plexus gave rise directly to the mucosal arteries, which passed perpendicularly through the muscularis mucosae to the mucosa.

# Spatial Variations in Submucous Plexus

The branching pattern within the submucous plexus mirrored that of the external vessels on the gastric surface. The inputs from the curvatures branched in a tree like patterns as they course towards the opposite curvature. They anastomosed with branches derived from the opposite curvature inputs a little beyond the mid lines of the anterior and posterior walls. In addition, the vessels give off lateral anastomotic branches which meet the branches derived from adjacent vessels. The intervening space was filled by a plexus of secondary and tertiary vessels. The space between the lines of inputs was wider on the lesser than the greater curvature. In the former, there was an intermediate chain of inputs which branched in a stellate fashion and anastomosed with adjacent vessels. A stellate rather than scalariform pattern was seen on all aspects of the stomach in the antrum.

Although the submucous plexus followed the normal tendency to be less dense on the lesser curvature, and distally, there was no region in any of the specimens where adequate filling permitted the observation that the plexus was absent. It was present in all parts of the stomach and the reduction in density in the above regions was less evident than in some other species. No input vessels were visible which did not have some anastomoses with their neighbours.

The submucous vessels at the gastro-oesophageal junction were well filled in four specimens (6; 7; 8; 11). A distal set of small inputs was found at the base of the oesophagus which gave off anastomotic branches directed mainly distally and, to a lesser extent, circumferentially. A few branches were directed proximally and ran for a few millimetres up the oesophagus. A proximal set of inputs, very close to the first, gave rise to leashes of vessels running up the oesophagus. There were few, if any, circumferential vessels. There were also a few branches which ran distally and anastomosed on the terminal part of the oesophagus, with the branches derived from the distal set of inputs.

A specialised submucous structure was also seen at the gastroduodenal junction in seven specimens, F3; 6; 8; 9; 10; 11; 12. There were two groups of input vessels on either side of the gastroduodenal junction. Those on the gastric side were several millimetres from the junction, whereas the duodenal vessels were much closer. The main branches of the gastric vessels were directed proximally, with smaller distally directed branches. Conversely, the main branches of the duodenal vessels were directed proximally, with smaller distal vessels. The duodenal proximal branches ran in clearly defined leashes across the gastroduodenal junction to anastomose with the smaller gastric vessels near the gastric inputs. The leashes, most clearly seen in F10, were up to 1cm long. Thus, there was continuity at the submucosal level between the gastric and duodenal plexuses, but the gastroduodenal junction area was predominantly supplied by



Fig. 91 Whole cleared lesser curvature half of F10 (1.3x).



Fig. 92 Whole cleared greater curvature half of F10 (0.9x).

the duodenal vessels.

#### <u>Connective</u> <u>Tissue</u> <u>Plexus</u>

The vessels of the connective tissue plexus were very sparsely filled in all the specimens. It was supplied by branches from the main arterioles as they traversed the space between the deep surface of the muscularis externa and the submucous plexus, and also by recurrent vessels from the plexus itself. Its vessels were small (less than 30um) and they ramified throughout the submucous compartment, rather than within a defined plane. It was not possible to observe any significant spatial variations in the structure of the connective tissue plexus.

#### Mucosal Arteries

The mucosal arteries were given off directly by the vessels of the submucous plexus. They penetrated the muscularis mucosae and ramified within the mucosa, where they branched in a tree like manner, to form perpendicular vessels running towards the mucosal surface. This structure was seen in all the cleared specimens where filling at this level occured (ie. all but F7) and confirmed by histological specimens. At the base of the mucosa was a plane of arterioarterial anastomoses between the mucosal arteries which did not constitute a mucosal plexus.

The mean diameter of the mucosal arteries (mean of 30 determinations) was  $19.3\pm8.0$  um with a mean area of supply of  $10.9\pm7.1 \times 10^{-2}$  mm<sup>2</sup>. This gave a mean perfusion index of  $210+216 \times 10^4$  um<sup>4</sup> mm<sup>-2</sup>.

#### Spatial Variations in Mucosal Arteries

The diameter of the mucosal arteries was greatest in the antrum  $(23.4 \pm 9.3 \text{ um})$  and least in the fundus  $(15.0 \pm 7.2 \text{ um})$ . Similarly the area of supply was greatest in the antrum  $(14.1 \pm 9.2 \times 10^{-2} \text{ mm}^2)$  and least in the fundus  $(7.8 \pm 6.1 \times 10^{-2} \text{ mm}^2)$ . The perfusion index was therefore greatest in the antrum and least in the fundus. These differences were significant (2 tail t test. p<0.05). (74b/e 2)

In most of the stomach, the is no mucosal plexus. However, both stereomicroscopy and cleared edge specimens (in F8) indicated that in the antrum, the mucosal anastomoses had the characteristics of a mucosal plexus, as defined by their size and orientation.

#### Arteriovenous Anastomoses

Venous filling occured in several of the specimens, in which parallel arterioles and venules were often seen. However, despite a careful search, no direct arteriovenous connections were seen in any of the specimens.

	FUNDUS			CORPUS (LC)			ANTRUM		
	D	A	PI	D	A	PI	D	A	PI
	13	3	95	19	9	145	19	11	118
	10	3	33	19	8	163	25	6	651
	10	3	33	13	13	22	13	6	48
	10	3	33	25	13	278	25	9	434
	13	5	57	19	9	145	38	34	613
	25	13	300	25	16	244	13	13	22
	25	19	206	25	19	206	25	9	434
	13	13	22	13	3	95	38	25	834
	25	13	300	13	9	32	25	19	206
	6	3	4	25	8	488	13	9	32
MEAN	15.0	7.8	108	19.6	10.7	182	23.4	14.1	339
	±	±	<u>±</u>	±	±	<u>±</u>	<u>±</u>	±	<u>±</u>
SD	7.2	6.1	116	5.3	4.6	136	9.3	9.2	295
WHOLE	19.3	10.9	210						

<u>NOTES</u>

D = Diameter (um) A = Area of supply  $(mm^2 \times 10^{-2})$ PI= Perfusion Index  $(um^4 mm^{-2} \times 10^4)$ The diameter, area of supply and perfusion index were all significantly greater in the antrum than in the fundus (p < 0.05)

Table 21 Diameter, Area of Supply and Perfusion Index for the Mucosal Arteries of the Ferret (F8)

#### Duodenum

The duodenum was well filled in eight of the specimens. The arrangement of the vessels in the submucosa and mucosa was much the same in the duodenum as the stomach. In approximately the first centimetre of the duodenum, the submucous branching pattern is random. Thereafter, it becomes scalariform, with two lines of inputs on the anterior and posterior aspects of the duodenum.

#### DISCUSSION

Examination of the 12 ferret stomachs generally confirmed the descriptions in the literature of the nonvascular anatomy. Poddar & Murgatroyd were unable to find an layer of oblique muscle fibres in the muscularis externa, a result which was confirmed in this study. There was no sign of a sphincteric thickening at the gastro-oesophageal junction, in agreement with Botha, but in contrast to Poddar & Murgatroyd's report. The entry point of the common bile and pancreatic duct at  $25 \pm 3.9$ cm beyond the gastroduodenal junction, was consistent with Poddar & Murgatroyd's and Kainer's values, but greater than Carleton's.

The extramural vasculature was dissected fully in seven the of species-specific specimens, in absence a observational study. The only available comparison is Wiland's description of the mink. The finding that the coeliac artery bifurcated into a common hepatic artery and gastrosplenic trunk is consistent with Wiland's major pattern and parallels the arrangement in cat and dog. However, Wiland was unable to find a right gastric artery, which was present in all seven of the specimens in this study, anastomosing with the left gastric in five cases. Unlike the standard mammalian pattern, a left gastroepiploic artery was present in only two specimens, in each of which it anastomosed with the right gastroepiploic artery. In the remainder, the greater curvature was supplied by branches of the splenic artery and short gastric arteries. Although the left gastric artery showed a considerable degree of dominance over the greater curvature vessels, it was less

dominant over the duodenal vessels than in some other species, as the territory of supply of the right gastric and gastroepiploic vessels extended onto the antrum.

The principal feature of the ferret intramural vasculature is that only one plexus, the submucous, is In most areas of the stomach, all the present. arterioarterial anastomoses are located in this plane, and give rise to the mucosal arteries, which pierce the muscularis mucosae and ramify in the mucosa. Anastomotic vessels are found between the mucosal arteries. In the antrum, however, the characteristics of the mucosal anastomotic vessels change, and become a true plexus. This may be a result of the need for more efficient blood distribution in the area of a thicker muscular coat.In addition, the reduction in submucous plexus on the lesser curvature is less marked than in many other species. No mucosal arteries of extramural origin or low connectivity vessels are found. The picture is, therefore, one of a fairly even plexus structure in one plane, a pattern comparable to that found in the dog.

The supply of the distal stomach by vessels of duodenal origin, the right gastric and gastroepiploic arteries, is mirrored in the submucosa at the gastroduodenal junction, where the leashes of vessels from the duodenum extend onto the distal antrum. It is noteworthy that signs of muscular activity are seen within the submucous vessels in the form of sacculations and possible sphincteric narrowings. This activity was not seen in any other species, and may be a result of the mode of sacrifice in the ferret (carbon dioxide asphyxia) which was not used in any other species. This activity may also have contributed to the relatively poor filling of specimens in this species. Several of the submucous vessels bridged rather than followed the folds in the mucosa. This would suggest that that these folds are never fully flattened as the stomach expands upon filling.

# CHAPTER 18 THE RAT

#### NON - VASCULAR GASTRODUODENAL ANATOMY

#### General Appearance

The external appearance of the stomach was examined in all the twelve rat specimens. The oesophagus had an unusually long abdominal course (1.7 + 0.49 cm) before it joined the stomach at approximately the mid point of its rostral border. In ten specimens, its point of entry was slightly ventral, whilst in the remainder it was on the rostral edge. It formed an incisura cardiaca of approximately 90°. In all the specimens, there was a slight dilatation of the oesophagus as it entered the stomach, due to thickening of the tunica muscularis. The stomach had a mean length of 3.3 + 0.58 cm and the greater curvature was orientated transversely. To the left of the cardia was a distensible sac, the fundus which was small and contracted since all the twelve specimens had been starved for 24 to 48 hours prior to sacrifice. It was perpendicular to the axis of the corpus , pale, thin walled and could be clearly differentiated from the remainder of the stomach. There was a clear dividing line between the two, running from the cardia to the midpoint of the greater curvature (the "grenzfalte"). The corpus and antrum were thicker walled and darker in colour. The antrum formed an incisura angularis of  $90^{\circ}$  -  $110^{\circ}$  and tapered to the gastroduodenal junction. There was an externally visible constriction at the gastroduodenal junction of all the specimens, followed by a slight duodenal dilatation. The duodenum itself formed a C-shaped loop.

#### Omental Attachments

The pattern of the omenta, which contained the main extramural vessels supplying the stomach, was constant in all the 12 specimens. The lesser omentum ran on either side of the lesser curvature, on the anterior and posterior walls of the corpus. It was also reflected onto the fundus to the left of the oesophagus. On the antrum, the omenta on either side of the lesser curvature united to run onto the anterior surface of the duodenum. The greater omentum did not reach the stomach until the middle of the greater curvature, in the region of the corpus-fundus interface. It then ran along the greater curvature to pass across the gastroduodenal junction onto the posterior aspect of the duodenum.

#### <u>Muscularis</u> <u>Externa</u>

The muscularis externa was examined externally in all twelve specimens. In addition, R4 was dissected to examine the internal structure of the gastric wall, and histological sections were made from a number of specimens. Paraffin embedded sections were made from the pyloric greater curvature of Rat 1, the anterior corpus of Rat 1 and the corpus greater curvature of Rat 2.

The oesophageal muscle was thickened in the last 4-5 mm, forming a slight vestibule as it entered the stomach at the cardia. The fundus was pale and thin walled whilst the corpus was significantly thicker with a distinct discontinuity. The gastric muscle became thicker in the antrum, forming a distinct sphincter at the gastroduodenal junction. However, the succeeding duodenal muscle was extremely thin.

Histology confirmed the presence of an outer longitudinal and an inner circular layer of smooth muscle fibres. No oblique layer or sling fibres was seen but the thinness of the muscularis made it difficult to define the structure.

#### <u>Miscellaneous</u>

The gastroduodenal junction was examined externally in all the specimens and internally in R4. The antrum and the duodenum were colinear and they were of approximately equal diameter (0.8 mm). In all twelve specimens there was a constriction at the gastroduodenal junction. Internal examination showed a sphincteric thickening of the

muscularis externa immediately proximal to the gastroduodenal junction. The lumen then widened in the duodenum where the muscle was very thin although there did not appear to be a juxtapyloric gutter.

The muscularis mucosae was examined histologically in the anterior wall, greater curvature, antrum and corpus of R1 and R2. There were no significant difference in the thickness or structure of the lamina. The mean thickness of ten determinations was  $36 \pm 3.2$  um. The layer was three or four cells thick, with no apparent internal structure or orientation of the smooth muscle cells.

## EXTRAMURAL VASCULATURE

The extramural vascularization of the rat stomach was examined at dissection in all twelve specimens. However, this was supplemented by examination of six of them as cleared whole stomachs, with the muscle in situ. The remainder were examined by clearing after removal of the muscularis externa in the normal way.  $(F_{1'g}, g_{3}-g_{4})$ 

In 11 of the twelve animals, the coeliac artery divided tripodally to form the left gastric, common hepatic and splenic arteries. In one (R9) it split into a common hepatic and gastrosplenic trunk, the latter dividing after a few millimetres to form the left gastric and splenic arteries.

The major vessel to the stomach was the left gastric artery. This reached the stomach and divided immediately to the left of the oesophagus at the cardia. It gave rise to a single vessel which passed along the lesser curvature and two vessels which immediately broke up into groups of branches and radiated over the anterior and posterior surfaces. The latter supplied the whole of the fundus and corpus, including the proximal half or two thirds of the greater curvature. Thus, the whole of this part of the greater curvature was supplied by the left gastric artery in none of the specimens was there any indication of a left gastroepiploic artery, derived from the splenic artery. In most of the specimens, the fundus received a few small vessels from the splenic hilum, presumably short gastric arteries, derived from the splenic artery.

The lesser curvature branch of the left gastric artery passed along the lesser curvature all the way to the gastroduodenal junction and often gave a few small twigs to the rostral aspect of the proximal duodenum. It supplied half to two thirds of the anterior and posterior aspects of the distal corpus and the entire antrum.

In addition to these two main branches, the parent trunk of the left gastric artery also gave rise to small branches supplying the base of the oesophagus in ten of the twelve specimens.

The other main artery supplying the stomach was the right gastroepiploic artery, derived from the gastroduodenal artery, a branch of the common hepatic artery. This was formed on the posterior duodenum near the gastroduodenal junction and ran rostrally, supplied one third to a half of the distal greater curvature i.e. the antrum and sometimes the distal corpus. Its branches supply less than half of the anterior and posterior walls in these regions.

The gastroduodenal artery also gave rise to the superior pancreaticoduodenal artery, which supplied the posterior and inferior aspects of the proximal duodenum.

The right gastric artery was very small in these specimens. It arose from the common hepatic artery, and supplied only the superior and anterior aspects of the proximal duodenum. In only one of the animals did any branches pass rostrally to supply a small part of the lesser curvature (R9).

The vessels passed obliquely through the muscularis externa to enter the submucosa. There were very few arterioarterial anastomoses extramurally, either between branches derived from the same parent trunk or from different ones. In particular, there were very few between the left gastric anastomoses and right gastroepiploic arteries. In only one specimen (R9) was there any connection, a single small vessel, between the left and right gastric arteries. A notable exception to this was seen in the proximal corpus of all the specimens, where an



<u>Fig. 93</u> Whole cleared stomach of anterior surface of Rat 4. Note extensive supply by left gastric artery and pale aglandular rumen to right (3x).



<u>Fig. 94</u> Posterior surface of Rat 4. Note anastomotic vessel along distal greater curvature (3x).
arterioarterial anastomotic vessel ran tangentially between branches of the left gastric artery. This was coincident with the 'Grenzfalte', the line of demarcation between the glandular and non-glandular areas of the stomach i.e. the fundus and corpus. In addition, in 9 of the twelve specimens, there was an anastomotic ring around the gastroduodenal junction which was formed from the right gastric and gastroepiploic arteries, anastomosing on the anterior and posterior walls.



Fig. 95 Flattened whole cleared stomach of R13. Note demarcation between glandular and aglandular areas (1.3x).

#### INTRAMURAL VASCULATURE

#### <u>Supply to Muscularis Externa</u>

The extramural arteries pierced the muscularis externa obliquely as they passed towards the submucosa. In seven specimens, the intramural vessels had injected sufficiently well to define the vascular pattern. The penetrating vessels gave rise to smaller branches (25 - 25 um diameter) which tended to run at right angles to the long axis of the stomach. These, in turn, gave rise to capillary loops which ran parallel to the muscle fibres. They were mainly located in two planes, near the serosal and submucosal surfaces of the muscularis. There were also some small recurrent vessels from the submucous compartment contributing to the deep capillary plexus.

#### <u>Spatial Variations in Supply to Muscularis Externa</u>

No spatial variations were apparent, other than a reduced density of vascularization in the fundus where the muscularis was thinnest.

## Submucous Plexus (Fig. 93-95)

The arterial vessels left the deep surface of the muscularis externa and entered the submucosa where they ramified to give rise to a number of branches. Some areas of these submucosal vessels could be seen in all twelve rats, although not all areas were filled in all of them. In particular, it was difficult to obtain satisfactory vascular filling in the antrum, with only four specimens yielding useful results. The branches of the vessels formed a fairly dense plexus of primary and secondary vessels, with mean diameters of  $37.2 \pm 1.6$  um and  $25.1 \pm 1.7$  um respectively (mean of 10 determinations in each of 3 rats in each case).

All these vessels appeared to be located within the submucosa. However, when they were manipulated and examined under stereomicroscopy, most of the anastomoses between the arteries were found to be immobile, although they were usually palpable and compressible. This demonstrated that these anastomoses were not located within the submucosa, but were partially or completely within the muscularis mucosae. There were few anastomoses within the submucosa i.e that were freely mobile. A small proportion of the plexus vessels were seen to lie deep to the muscularis mucosae i.e. within the mucosa.

Histological examination of the corpus greater curvature in R2 supported the interpretation of the cleared specimens. Several sections showed elongated cross sections of vessels within the muscularis mucosae which were apparently running within the muscularis, parallel to its surface.

The arterial plexus gave rise to two sets of vessels. A large number of perpendicular mucosal arteries arose, which passed to the base of the mucosa. A smaller number of slightly smaller recurrent vessels passed obliquely through the submucosa to enter and supply the deep capillary plexus in the muscularis externa.

## Spatial Variations in the Submucous Plexus (Fig. 93-95)

The overall patterns of the submucous plexus could be seen well in the corpus and fundus of all the specimens. The antrum filled less well and could only be clearly defined in four specimens, although parts were filled in most of the specimens.

The branching pattern of the submucous plexus was uniform over most of the stomach. In all the areas, other than the curvatures and the gastroduodenal junction, the vessels branched in a scalariform pattern with secondary branches derived from the same parent trunks meeting mainly in the muscularis mucosae. Anastomoses between branches derived from different parent vessels such as the left gastric and right gastroepiploic arteries tended to meet end to end.

The main line of inputs to the plexus was on either side of the lesser curvature, derived from the left gastric artery. These radiated away from the cardia towards the greater curvature, giving rise to scalariform branches throughout their course. A smaller number of inputs to the plexus were found on the anterior and posterior walls themselves. This pattern was echoed on a smaller scale in the distal corpus, with the inputs from the right gastroepiploic artery. On the lesser curvature itself, there was a line of inputs which branched in a stellate fashion, which were derived from the extramural branch of the left gastric artery which ran along the lesser curvature. In three of the specimens only, a few similar but smaller vessels were seen on the distal third of the lesser curvature. In none of the specimens could any inputs from a left gastroepiploic or short gastric arteries be seen.

The arterioarterial anastomotic pattern in the submucous plexus confirmed the areas of supply described in the section on the extramural vessels. In the distal half to third of the greater curvature, there were extensive anastomoses between the branches of the left gastric and right gastroepiploic arteries, but only a short distance away from the greater curvature. Most of the anterior and posterior walls were, therefore, left gastric territory. Over the rest of the stomach, the anterior and posterior branches of the left gastric anastomosed with one another in the vicinity of the greater curvature.

The density of the plexus was fairly uniform in each specimen across the corpus and fundus. This included the proximal lesser curvature. However, in the four specimens where the antrum was well injected, it could be seen that the distal lesser curvature and the antrum generally had a less dense submucous plexus.

The were two specialised regions of the plexus - the cardia and the gastroduodenal junction. The left gastric artery branched posterior to the gastro-oesophageal junction and some branches ran around the base of the oesophagus on the cardia. These vessels gave rise to a few smaller branches, which ran up both aspects of the oesophagus for up to one centimetre before anastomosing with oesophageal vessels in the submucosa.

A complete anastomotic vascular ring ran around the

duodenum, immediately distal to the gastroduodenal junction. This was formed from the right gastric and left gastroepiploic arteries and anastomosed on the anterior and posterior walls. The major branches from this ran longitudinally distally, into the duodenum. However, smaller branches ran proximally across the gastroduodenal junction, to anastomose on the distal antrum with branches from the gastric submucous plexus. The anastomotic line was several millimetres proximal to the anatomic gastroduodenal junction.

#### Connective Tissue Plexus

In isolated areas of several specimens, small vessels were seen which could have been constituents of a connective tissue plexus. These were located within the submucosa, always in the corpus. They were vessels smaller (10 - 20 um)than the submucous plexus vessels at that point, and located superficial to them.

#### Mucosal Plexus

In most areas of all the specimens available, the vessels had filled with injectate at least as far as the origin of the mucosal arteries. The main exception was the antrum which filled adequately in only four specimens. In all cases, there was only one plexus, the 'submucous plexus', although the actual arterioarterial anastomoses were mostly located within the muscularis mucosae. In some cases, stereomicroscopy showed that the anastomoses were at the base of the mucosa, but these were few in number. There was, however, only one plexus plane although this was diffuse, extending from submucosa, through the muscularis mucosae to the mucosa. There was no definable mucosal plexus, distinguishable from the submucous plexus. However, not all the mucosal arteries passed perpendicularly to the mucosa; some had a short horizontal course, which could be mistaken for a plexus. Within the mucosa there were extensive capillary networks but the vessels were smaller (< 10um) and histologically distinguishable from the mucosal

arteries between which they ran.

#### <u>Mucosal Arteries</u>

The mucosal arteries could be clearly seen in the corpus of all the specimens and in the four well injected antrums. However, they were much less clear in the fundus and on the lesser curvature. In the fundus, contrast between the vessels and the surrounding mucosa was poor, whereas on the lesser curvature, filling was limited and sporadic. The ensuing results are, therefore, based largely upon a assessment of the corpus, and fragmentary evidence from the other regions, particularly the antrum.

The mucosal arteries arose perpendicularly from the vessels of the submucous plexus, and passed towards the mucosa, through the muscularis mucosae. In some cases, they actually arose within the muscularis itself. Most of the arteries formed a number of branches near the base of the mucosa which ramified to form leashes of capillaries which ran towards the mucosal surface. None of the mucosal arteries themselves ran anywhere near the surface. In a few cases, estimated to be less than 5%, the mucosal arteries turned through 90° at the mucosal base and ran for a short distance parallel to the muscularis mucosae. They soon turned again and ramified perpendicularly in the normal However, none of these horizontal vessels pattern. anastomosed with vessels derived from a different mucosal artery. They did, therefore, appear to be true functional mucosal arteries, end arteries supplying only the mucosal capillaries. These capillaries ramified throughout the mucosa. There appeared to be branches parallel to the mucosal surface, but it was not possible to determine whether the capillaries from adjacent parent mucosal arteries anastomosed or merely interdigitated.

## Spatial Variation in Mucosal Arteries

The mean diameter of the the antral mucosal arteries in a typical specimen (R2) was  $10.2 \pm 1.9$  um (mean of ten determinations). This supplied a mean area of  $4.4 \pm 1.5$  mm<sup>2</sup>

۰,

x  $10^{-2}$ , giving a mean perfusion index of  $31 \pm 16$  um<sup>4</sup> mm<sup>-2</sup> x  $10^4$ . The vessels in the fundus could not be quantified, due to poor contrast, but they appeared to have similar diameters, but with areas of supply perhaps twice that on average of the antrum.  $(7ab/e^{2})$ 

The extent of mucosal filling differed dramatically between the fundus and the glandular stomach. The aglandular mucosa was much thinner and therefore less densely filled than the glandular. The interface at the Grenzfalte was very sudden and distinct.

#### Arteriovenous Anastomoses

Mucosal venous filling occured in parts of some specimens, such as R2. However, in neither these nor any other specimens could any direct vascular connections between the arterial and venous circulations be seen, despite a careful search.

6   10   17     5   6   3     2   10   50     5   10   20     6   13   48     3   10   33     6   13   48     3   10   33
5   6   3     2   10   50     5   10   20     6   13   48     3   10   33     6   13   48     3   10   33     10   33   48     3   10   33
2   10   50     5   10   20     6   13   48     3   10   33     6   13   48     3   10   33     10   33   48     3   10   33
5   10   20     6   13   48     3   10   33     6   13   48     3   10   33
6 13 48   3 10 33   6 13 48   3 10 33
3 10 33   6 13 48   3 10 33
6 13 48 3 10 33
3 10 33
5 10 20
3 10 33
MEAN 4.4 10.2 31
SD 1.5 1.9 16

#### <u>Table 22 Area of Supply, Diameter and Perfusion Index of</u> <u>Mucosal Arteries in the Rat (Greater Curvature Antrum of R2)</u>

#### <u>Duodenum</u>

The duodena were fully or partially injected in all twelve specimens. The general arrangement of the laminae was as described for the stomach, although they were thinner, making it more difficult to observe them. The main inputs to the submucosa were on the inferior surface from the superior pancreaticoduodenal artery and / or on the superior surface from the right gastric artery. One seemed dominant in half the samples and vice versa. The vasa recta gave rise to branches running circumferentially around the duodenum to anastomose with those from the other vessel. They gave rise to secondary scalariform branches. This pattern did not appertain in the first 0.8 to 1 cm of the duodenum, where the submucosa contained longitudinal branches, derived from ring immediately distal the the anastomotic to gastroduodenal junction.

#### DISCUSSION

The literature describing the stomach and duodenum of the rat is very extensive but, despite this, several features were observed in this study which have not been previously described or which are at variance with the accounts of some authors. According to Botha, there were well developed sling fibres around the oesophagus, which were not seen in this study. This may have been a result of the thinness of the muscle, which made it difficult to determine the fibre orientation. Botha also described the thickness of the muscularis mucosae varying in different parts of the stomach, whereas this study showed no significant difference. Since Botha does not quantify his assertion, this discrepancy cannot be resolved.

In most of the twelve specimens, the coeliac branched tripodally, as all authors described. However, in one, the coeliac gave rise to only two vessels, a common hepatic artery and a gastrosplenic trunk. This variant does not seem to have been described previously. The characteristic feature of the rat is the exaggerated predominance of the left gastric artery as the prime supply vessel to the stomach, a feature which was fully confirmed here. The immediate area of the distal greater curvature was supplied by the second vessel, the right gastroepiploic artery. However, Guth reports the presence of a left gastroepiploic artery, which was not seen here. The proximal greater curvature was supplied by short gastric arteries, as described by Greene, but in contrast to Guth's and Schnitzlein's reports. The right gastric artery was very small and only sent branches to the distal lesser curvature in one of twelve specimens, in contrast to Schnitzlein who described antral supply as the normal feature of this vessel. Leneman & Burton's hepato-oesophageal artery was not seen as a specific entity in any of the specimens.

As all authors agree, there are very few extramural anastomoses between the arterial vessels. In contrast again to Schnitzlein, an anastomosis between the left and right gastric arteries was seen in only one specimen, as a single vessel. Two specific exceptions are seen to this paucity, which were not specifically described in the literature. A single circumferential vessel united the three branches of the left gastric, coincident with the location of the 'grenzfalte', the interface between the glandular and aglandular parts of the stomach. In all the specimens, a complete anastomotic ring was seen at the gastroduodenal junction, linking the right gastric and gastroepiploic arteries.

The intramural angioarchitecture appeared superficially to be consistent with the descriptions in the literature. Two arterial plexuses were seen in the muscularis externa, as described by Guth, supplied by a few small recurrents from the submucosa, as described by Schnitzlein. However, although the submucous plexus was mentioned by all authors, close examination demonstrated that most of the arterioarterial anastomoses were not located in the submucosa, but were embedded in the muscularis mucosae. A few were found in the submucosa and a few in the mucosa, but there was only one plexus, the plane of which varied between those limits. The variability in plane was more marked than

in other species. The main features of the submucous and mucosal vessels was confirmed, in particular the dominance of the left gastric inputs. However, in contrast to the lack of extramural anastomoses, there are extensive intramural anastomoses, particularly between the left gastric and right gastroepiploic arterial branches. This is consistent with the results of the ligation experiments. Although some degree of plexus paucity was visible on the distal lesser curvature and in the antrum, it was less marked than in many other species.

Several interesting features were evident. Although short gastric arteries were seen externally on the proximal greater curvature, no corresponding inputs were seen in the submucous plexus. This was seen consistently in all the specimens, and appeared to be due to the vessels not penetrating the muscularis, but only contributing to the intramural plexuses. This was not seen so consistently in any other species.

The literature was unclear on the plane of branching of the mucosal arteries. Without exception in this study they gave rise to the capillary bed at the base of the mucosa. The confusion may arise in variability in definition of mucosal artery, arteriole and capillary. The horizontal course of some of the mucosal arterioles, parallel to the mucosal surface, seems to be analogous to those vessels in rabbit and guinea pig which display 'failure to anastomose', but without the failure to anastomose. Similarly, stellate vessels were seen in specific locations on the lesser curvature, as in other species, but no low connectivity vessels or mucosal arteries of extramural origin were seen.

## CHAPTER 19 THE DOG

NON-VASCULAR GASTRODUODENAL ANATOMY

#### General Appearance

The stomachs of eight dogs were examined externally. After a short abdominal course, the oesophagus entered the stomach almost perpendicularly  $(89\pm6^{\circ})$ , with a 1cm vestibule in six of them. The stomach had an unusual piriform shape, tapering continuously from the fundus  $(6.2 \pm 0.5 \text{ cm})$  to the gastroduodenal junction  $(2.0 \pm 0.4 \text{ cm})$ . It was bent sharply back on itself at the midpoint of the corpus, to form an incisura angularis of  $89\pm6^{\circ}$ . As a result of the piriform shape, the adjoining surfaces of the fundus and antrum were closely apposed and parallel. All the specimens showed a marked external constriction at the gastroduodenal junction itself. The first part of the duodenum was short  $(3.9 \pm 0.8$ cm) and colinear with the pyloric antrum. It turned through approximately  $90^{\circ}$  at its end to form a loop, open to the left.

### Omental Attachments

The adipose content of the omenta as they were reflected onto the stomach was relatively sparse in all the specimens but one, so that the lines of attachment could be clearly delineated. The lesser omentum ran on either side of the lesser curvature, although the two lines of attachment were separated by only a few millimetres. As a result of the close apposition of the ascending and descending limbs of the stomach, the lesser omentum bridged the incisura angularis. It then crossed the gastroduodenal junction and moved onto the dorsal aspect of the duodenum. The greater omentum met the stomach at approximately the fundus-corpus interface, then ran precisely along the greater curvature, before crossing the gastroduodenal junction and passing onto the ventral aspect of the duodenum. The greater and lesser omenta were joined by omental rings around the base of the

oesophagus and, less clearly, the gastroduodenal junction.

#### Muscularis Externa

The muscularis externa was examined in all the specimens before, during and after dissection. It varied in thickness from approximately 1mm to 3mm, being thickest at the pylorus and thinnest near the cardia. The orientation of the muscle fibres followed the normal pattern. Longitudinal bundles of fibres were visible on the outer aspect of the stomach, but only along the two curvatures. On the anterior and posterior aspects of the fundus there were bundles of oblique fibres, radiating from the cardia. Deep to these two layers was a complete circular layer. Dissection revealed oblique sling fibres on the deep surface of the muscle, which ran around the oesophageal aperture. Histological examination of the muscularis in two specimens (D2 & D4) showed the fibres to be uniformly orientated within the layers, with comparatively little interfibre connective tissue.

The circular layer of fibres was thickened at the pylorus to form a distinct pyloric sphincter. In two animals (D2 & D6), there was an indication of a second pyloric thickening approximately 0.5cm proximal to the gastroduodenal junction. There was no such structure visible at the gastro-oesophageal junction, although in 4 of the 6 animals which showed terminal oesophageal dilatation, there was a slight degree of circular muscle thickening at the junction.

#### **Miscellaneous**

The interior of the stomach was folded, but generally with no particular orientation. Only along, and to either side of, the greater curvature was a consistent longitudinal orientation seen. The muscularis mucosae was examined histologically in the corpus of Dog 1 and in cleared edge specimens from D1, D4 and D7. It was between 6 and 10 cells thick, but with no obvious orientation of the cell layers. It varied in thickness between 65 and 100 um, being thickest in the pylorus and thinnest in the fundus. The mean thicknesses were pylorus  $(94 \pm 4 \text{ um})$ , corpus  $(78 \pm 4 \text{ um})$  and fundus  $(71 \pm 3 \text{ um})$ . All the muscle cells were contained within the muscularis mucosae, with no visible interdigitation into the submucosa or mucosa.

#### EXTRAMURAL VASCULATURE

The extramural vessels were dissected and examined in all the eight animals, prior to processing of the stomach. In general, the pattern was very similar to the "classical" arrangement seen in man, although there were some minor (Fig.9b) differences. In all the animals, the gastric vessels arose from the coeliac artery, which arose in turn from the abdominal aorta. In seven of the specimens, the coeliac branched tripodally into the hepatic, left gastric and splenic arteries, but in one it bifurcated to form the hepatic artery and a gastrosplenic trunk, which in turn divided to form the left gastric and splenic arteries after 1 cm.

The hepatic artery passed behind the stomach and gave off several proper hepatic arteries, which supplied the hepatic lobules. It then continued as the gastroduodenal artery, which gave rise to the right gastric artery before splitting to form the left gastroepiploic and superior pancreaticoduodenal arteries. The right gastric artery branched on the superior aspect of the first part of the duodenum, to which it provided nutrient branches. It also gave rise to a branch which crossed the gastroduodenal junction and ran rostrally along the lesser curvature, supplying the superior aspects of the antrum. The right gastroepiploic artery supplied the inferior aspect of the proximal duodenum and also ran rostrally along the greater curvature, supplying the inferior aspects of the antrum and distal corpus. The superior pancreaticoduodenal artery supplied the distal duodenum.

Most of the corpus was supplied by the left gastric artery. This branched to the right of the cardia, and sent nutrient branches to the cardia and the superior aspects of

ľ

the corpus, beyond the midline of the anterior and posterior aspects. In all the specimens, the base of the oesophagus was supplied by left gastric branches from a vascular ring running around the oesophageal base. It is notable that the left gastric artery was doubled in six of the specimens.

The left part of the stomach was supplied by vessels derived from the splenic artery. The fundus received a number of short gastric arteries from the spleen. The left gastric artery ran along the left part of the greater curvature, supplying the inferior aspects of the distal fundus and proximal corpus.





Arterioarterial anastomoses were seen at several sites around the stomach. In six of eight, there was a vascular ring around the gastroduodenal junction, contributed to equally by the right gastric and right gastroepiploic arteries. In all the animals, there appeared to be direct anastomotic connections on the greater and lesser curvatures i.e. between the right and left gastroepiploic arteries and between the right and left gastric arteries respectively. The greater curvature anastomosis occurred at mid corpus, whereas the lesser curvature one was around the antrumcorpus interface.

#### INTRAMURAL VASCULATURE

#### <u>Supply to Muscularis Externa</u>

The arteries of the muscularis externa were examined grossly during dissection and in one cleared muscle specimen (D4). A consistent pattern was seen. The extramural vessels ran subserosally, almost parallel to the surface, for a varying distance, before turning and passing through the muscle almost perpendicularly. They left the deep surface and contributed to the submucous plexus. The penetrating vessels gave rise to two intramuscular arterial plexuses. The most superficial was subserosal, and the second was within the body of the muscle, approximately at the interface of the longitudinal and circular muscle layers. The latter plexus tended to be orientated longitudinally. The plexuses gave rise to capillaries which ran between the fibres. However, these were poorly filled, so it was difficult to define their architecture comprehensively.

It was evident during dissection, that many vessels ran into the deep surface of the muscularis from the submucosa. These were shown to be recurrent vessels in the one cleared muscle specimen and all the cleared submucosal specimens, where care was taken to preserve them. They ran into the muscle from the submucous plexus and contributed to the deep intramuscular plexus. It is noteworthy that this contribution was much more extensive than seen in other species and was comparable in degree to the primary supply from the direct branches of the extramural vessels.

The muscularis externa was also examined histologically in 2 specimens, the antral lesser curvature of D4 and the antrum of D2. Penetrating vessels were seen to pass through the muscularis approximately perpendicularly, and were usually found in bundles of several vessels, surrounded by connective tissue. The orientation of fibres was not altered by the location of a vascular bundle. In particular, there was no indication of 'sheaths' of fibres around the bundle or of isolated fibres 'keyed' into the connective tissue, as found in , for example, the pig.

#### <u>Spatial Variations in Supply to Muscularis Externa</u>

The vessels on either side of the lesser curvature had the longest subserosal course before passing through the muscle, those on the greater curvature less, and the vessels actually on the lesser curvature least. The penetrating vessels at the gastroduodenal junction also had virtually no subserosal course. Many areas of the muscle, particularly the pylorus, did not fill well with injectate so the description given is a composite of the material available. However, there was no evidence of any spatial variations in pattern. The muscle was thin in the duodenum, but showed the same arrangement, although the plane of the intramuscular arteriolar plexus could not be defined as a result of the muscle's thinness.

## Submucous Plexus (Fig. 47-48)

The submucosa was examined in all the eight specimens. The filling by injectate was not always uniform, so that some areas were inadequately injected whilst, in others, excessive capillary filling or extravasation masked the submucous plexus. However, all specimens had some usable areas.

The penetrating vessels left the lower surface of the muscularis externa and turned fairly abruptly through  $90^{\circ}$  near the base of the submucous connective tissue to ramify

in a planar plexus, the submucous plexus. The main vessels in the submucosa anastomosed with those derived from other inputs to the system. In addition, they gave rise to secondary and, in some areas, tertiary branches which also anastomosed. The primary anastomoses varied between approximately 100um and 400um in diameter, whereas the smallest tertiary anastomoses had diameters of 20um to 40um. The anastomoses over the entire stomach in all the specimens were carefully examined by micromanipulation under stereomicroscopy. They were all palpable, crushable and manipulable i.e. they were all located within the submucosa. In order to confirm this, conventional histological sections were made from the corpus and antrum of Dog2. All the large vessels were seen to be within the submucosal compartment. There were no vessels larger than 20um within the mucosa. This was confirmed by cleared edge specimens cut from the corpus, lesser curvature and duodenum of Dog1 and Dog4. These supported the evidence for the exclusive location of the plexus within the submucosa.

The submucous plexus gave rise to three sets of output vessels. Perpendicular mucosal arteries were given off which penetrated the muscularis mucosae to supply the mucosa. Relatively large (approximately 100-200um) recurrent branches ran obliquely superficially to supply the intramuscular plexus of the muscularis externa. Finally, small vessels (20-40um) gave rise to the connective tissue plexus within the submucosa.

## Spatial Variations in Submucous Plexus (F'9. 97-98)

Specimens were available of the submucous plexus in all regions of the stomach. The following observations are based upon the examination of 7 lesser curvatures, 8 greater curvatures, 5 funduses, 8 corpuses, 6 pyloruses, 5 gastroduodenal junctions, 3 gastro-oesophageal junctions and 4 duodena.

The plexus was noticeably uniform over the entire surface of the stomach. The three level scalariform pattern of branching described above was seen in most areas. Exceptions were seen in parts of the fundus, lesser curvature and greater curvature. In these areas, a stellate branching pattern was typically seen. In the fundus, the stellate vessels were derived from the short gastric arteries. On the curvatures, they were derived from the vessels which pierced the muscularis precisely on the curvatures, rather than lateral to them. However, these stellates were not seen along the entire length of the curvatures. In 5 of the 7 lesser curvatures, they were not seen before the incisura angularis i.e. they were only found in the distal corpus and pylorus. This pattern was also seen, though less consistently, on the greater curvature.

Anastomoses occurred on the anterior and posterior walls between the vessels derived from the left gastric artery and those from the short gastric and the left and right gastroepiploic arteries. All the anastomoses were within the submucosa. Those between the left gastric and gastroepiploic arteries occurred on the ventral half of the anterior and posterior walls. i.e. the left gastric had the larger area of supply.

Two specialised areas were present, the gastrooesophageal and gastroduodenal junctions. At the former, there was continuity in all the three specimens between the gastric and oesophageal submucosal vessels with anastomosis occuring a few millimetres proximal to the junction. The gastroduodenal junction was observable in 5 specimens. In four, there was an annular anastomosis between the right gastric and right gastroepiploic arteries. In all cases there was continuity between the gastric and duodenal vessels with, in three of the animals, the anastomoses coincident with the junction. In the remaining two, duodenal vessels passed a few millimetres onto the pylorus before anastomosing. Long leashes of vessels across the pyloric canal were not seen.

It was noteworthy that the density of the plexus was fairly uniform in all areas of the stomach. The scalariform anastomotic pattern was found in most areas, and there was little variation in anastomosis size or number. In



Fig. 97 Lesser curvature half of D4-whole cleared stomach. Note the comparative lack of lesser curvature vascular poverty. Oesophagus at top. (0.9x).



Fig. 98 Whole cleared stomach-D4 Greater Curvature. Caudal half (above) and rostral half (below). Note the absence of areas of poor vascularisation. (0.6x).

particular, the lesser curvature plexus was not perceptibly sparser than in the surrounding regions.

#### Connective Tissue Plexus

The connective tissue plexus comprised vessels of 15um to 25um in diameter located throughout the volume of the submucosa. It could only be clearly and extensively discerned in Dog 4, although isolated elements were visible in all the specimens. It was formed from recurrent vessels from the submucous plexus, 20um to 40 um in diameter. No spatial variations were evident in the one specimen in which it could be comprehensively seen.

#### Mucosal Plexus

There was no mucosal plexus in most areas of the dog's stomach. Careful stereomicroscopic examination with manipulation of the specimens showed that all the anastomoses were in the submucosa. This was confirmed by histological and cleared edge specimens. In all the animals, fine anastomotic vessels could be seen at the base of the mucosa, approximately 20um in diameter, but these did not constitute a plexus. However, in the antrum, there were areas where the anastomotic vessels between the mucosal arteries did take on the characteristics of a mucosal plexus in terms of their size and orientation.

## <u>Mucosal</u> <u>Arteries</u>

The mucosal arteries were well injected in six specimens, in which they could be studied by stereomicroscopy, histology and cleared edge sections. These demonstrated a consistent picture. They were given off from the submucosal plexus and passed perpendicularly through the muscularis mucosae to ramify at the base of of the mucosa into three to six branches, which ran towards the mucosal surface. These give rise to a tree like network of capillaries which ramified within the mucosa. In many areas of all the specimens, unfilled intervals were seen between the capillaries derived from adjacent mucosal arteries. In all cases, stereomicroscopic examination, even when no unfilled interval was visible, showed that the capillaries interdigitated but did not appear to anastomose. However, small (20um) anastomotic vessels did run between the bases of the mucosal arteries, parallel to the muscularis mucosae. The mean diameter of the arteries was 67+30um, supplying an area of  $1.13+0.96 \text{ mm}^2$  (mean of 30 determinations in Dog 4). This gave a mean perfusion index of 2727+2334 um<sup>4</sup> mm<sup>-2</sup> x  $10^4$ . A careful search was made for mucosal arteries of extramural origin, which did not communicate with the

	FUNDUS			CORPUS (LC)			ANTRUM		
	D	A	PI	D	A	PI	D	A	PI
	75	175	1808	125	319	7653	38	41	509
	63	69	2283	125	256	9537	50	50	1250
	63	113	1394	75	144	2197	50	56	1116
	50	81	777	100	163	6135	50	19	3289
	38	69	302	88	113	5307	38	50	417
	88	138	4346	75	100	3164	38	19	1097
	13	16	18	88	169	3548	50	25	2500
	100	256	3906	113	281	5802	50	38	1645
	75	106	2985	63	81	1945	50	50	1250
	113	363	4492	38	25	834	25	13	300
MEAN	68	138	2231	89	165	4612	44	36	1337
	<u>±</u>	<u>±</u>	<b>±</b>	<u>±</u>	±	±	<u>+</u>	<u>+</u>	±
SD	30	103	1652	28	94	2752	9	16	942
WHOLE	67	113	2727						

#### NOTES

D = Diameter (um)

A = Area of supply  $(mm^2 \times 10^{-2})$ PI= Perfusion Index  $(um^4 mm^{-2} \times 10^4)$ 

The diameter, area of supply and perfusion index were all significantly greater in the corpus than in the antrum. The diameter and area was also greater in the fundus than the antrum. The perfusion index was significantly greater only in the corpus, compared with the fundus. (p < 0.05)

Table 23 Diameter, Area of Supply and Perfusion Index for the Mucosal Arteries of the Dog (D4)

vascular plexus. None were found, since the submucous plexus, from which all the mucosal arteries arose, was present over the entire stomach.

### Spatial Variations in Mucosal Arteries

The pattern of the arteries was constant in all areas of the stomach. However, the size and area supplied did differ. Ten determinations of area and diameter were made in each of three areas in Dog 4. The arteries of the lesser curvature were largest (89 + 28 um) and supplied the largest area  $(1.65 + 0.94 \text{ mm}^2)$ . Those in the fundus were not significantly different, with a mean diameter of 68 + 30 um and a mean area of  $1.38 + 1.03 \text{ mm}^2$ . However, the antral vessels were significantly smaller than both fundus and lesser curvature, with a mean diameter of 44 + 9 um and area of  $0.36 \pm 0.16 \text{ mm}^2$ . (p<0.05). The lesser curvature had the best perfusion index  $(4612 + 2752 \text{ um}^4 \text{ mm}^{-2} \times 10^4)$ . significantly higher than both fundus and antrum, the latter being the least. It is noteworthy that, in parts of the antrum, which was the area with the poorest perfusion index, the anastomoses between the mucosal arteries took on the characteristics of a mucosal plexus.

#### Arteriovenous Anastomoses

A careful search was made for direct arteriovenous anastomoses, particularly in those areas where venous filling had occurred. Despite this, none were found. All apparent connections were shown on stereomicroscopic examination to be due to vessel overlap.

#### <u>Duodenum</u>

It was difficult to produce satisfactory filling of the duodenal vessels and only 4 specimens were well filled. The input vessels to approximately the first centimetre of duodenum were randomly orientated, but then formed two parallel lines. The submucous plexus was present throughout the first part of the duodenum and continuous with that of the stomach. As in the stomach, all the anastomoses were within the submucosa. In general, only primary and secondary branches were seen in the plexus, which tended to have a scalariform pattern, running between the lines of inputs.

#### DISCUSSION

The eight specimens examined generally confirmed the descriptions in the literature of the gross appearance of the stomach and the structure of the muscularis externa. In these animals, the mean incisura cardiaca was almost 90°, although the stomachs contained little food. Six of the eight animals showed a terminal dilatation of the oesophagus and in four of these, there was a slight thickening of the circular muscle. This is consistent with the findings of El-Ramli(1964) and in contrast to those of Botha (1958a; 1962) and Fatani (1971). Similarly, in two animals, there was a suggestion of a second sphincteric thickening, 0.5cm proximal to the pylorus, as described only by Ellenberger & Baum (1943). The muscularis mucosae is particularly thick and multilayered in this species, although the thickness (65-100um) is comparable with that reported by Mall (1896) (72 - 96 um).

The results of dissection of the extramural vessels did not entirely support Enge's work(1972). The variant to the tripodal coeliac branching, with a hepatic artery and gastrosplenic trunk was found in one of eight specimens. However, a right gastric artery was present in all eight specimens, whereas Enge reported it to be absent in 15 of 24 specimens. Similarly, in this work, all the specimens showed significant anastomosis between left and right gastric and left and right gastroepiploic arteries, on the lesser and greater curvatures respectively. These were absent in 19 of 24 animals in Enge's series. The points of contention relate to smaller vessels and may reflect the resolution advantages of clearing and dissection over a radiographic methodology. The doubling of the left gastric artery reported in all of Piasecki's animals was seen in six of eight specimens in this work.

The examination of the muscularis externa fully confirmed Mall's description of the intramural vessels and particularly the greater importance of the recurrent vessels from the submucous plexus. Microscopic examination of the cleared muscle specimen showed that there was some contribution to the intermuscular plexus from the penetrating arteries, which Mall did not report, perhaps because he did not clear his tissues. The structure of the vascular bundles was also noteworthy, since the specialised orientation of the surrounding muscle fibres was not seen, in comparison with the pig. This may reflect the greater muscular thickness in the latter species. The ability of muscular contraction to compress gastrointestinal blood vessels was demonstrated clearly (in the dog) by Anrep et al. (1933).

The important fact of the location of all the plexus anastomoses within the submucosa, reported by Mall and Piasecki, was generally confirmed in this study. However, these workers did not clearly describe the presence of fine anastomotic branches between the mucosal arteries, at the base of the mucosa. In addition, in the antrum, there was effectively a second, mucosal, plexus in some areas. This may be correlated with the poor perfusion index of the mucosal arteries in the same areas. The mucosal arteries were wider than reported by Mall (67um vs. 25um). This is probably an injection artefact, although other vascular diameters were comparable with Mall's.

Some aspects of the regional variations in the submucous plexus differed from those described by Piasecki. Stellate inputs to the central lesser curvature were seen only distal to the incisura angularis in five of seven specimens. In none of the specimens were there leashes of vessels across the gastroduodenal junction. The plexus in the first centimetre of the duodenum was random rather than 'diamond' shaped, although it subsequently agreed with Piasecki's description. Finally, a significant connective tissue plexus was seen in all the specimens, as opposed to one of Piasecki's, although the filling of these vessels is

4

probably sensitive to degree of filling as the vessels are small and 'beyond' the submucous plexus. However, the most significant of Piasecki's findings, that the submucous plexus is of constant density over the stomach, not poorer on the lesser curvature, and that there are no mucosal arteries of extramural origin or low connectivity vessels, was fully confirmed.

## CHAPTER 20 THE DOLPHIN

#### INTRODUCTION

The Dolphin was included in this study because the marine mammals, particularly cetacea (dolphins and porpoises) and pinnipedia (seals and sea lions) are subject to chronic and acute ulceration both in the wild and in captivity, in contrast to most other species. One of the first to have described this were Schroeder & Wedgeforth (1935) who observed the common occurence of chronic and acute ulcers in pinnipedia (Californian sea lions) in the wild. They suggested that these were due to the presence of foreign bodies in the stomach. Other authors have noted both ulcers and erosions in dolphins and porpoises (Ridgeway 1965; Ridgeway & Johnson 1965; Testi et al. 1971; Harrison personal communication). The lesions have been found in both acute, chronic and healed form. They usually occur in the forestomach (Greenwood - personal communication) but have also been seen in the pyloric stomach and duodenal ampulla (Ridgeway - personal communication). Various aetiologies have been proposed. In addition to the association with foreign bodies observed by Schroeder & Wedgeforth, Young & (1969), suggested that irritation by nematode Lowe parasites, which are commonly found in the cetacean stomach, might be an aetiological factor. Geraci & Gestermann (1966) suggested that high histamine concentrations caused by feeding in captivity with dead fish might be a factor. However, it was observed by Sweeny & Ridgeway (1975) and Greenwood et al. (1976 and personal communication) that whilst foreign bodies, nematodes or histamine could play a part, dolphins clinically displayed many of the symptoms and features of 'spontaneous' ulceration in man. Since chronic peptic ulceration is hardly ever found in most species, it was decided to include the dolphin in the range of species examined in this investigation.

### GASTRODUODENAL ANATOMY-REVIEW OF THE LITERATURE

Observations on the anatomy of marine mammals have been made in the literature for many centuries and have been reviewed by Harrison (1973). More detailed accounts of the gastrointestinal viscera have been given by a number of authors, such as Turner (1889), Fraser (1952), Slijper (1962), Ridgeway (1968) and Green (1972). Detailed dissection studies of the marine dolphins and porpoises have been described by Harrison (1970) and Smith (1972) and of the fresh water porpoises by Yamasaki & Takahashi (1971; 1974; 1975). Botha (1958a) described the gastro-oesophageal junction.

The oesophagus was described by Harrison from an examination of 57 marine cetaceans of various species (Tursiops spp., Delphinus spp., Stenella spp. and Globicephala spp.). It was lined by a thick stratified squamous epithelium, which was not keratinized. No glands were visible. An outer longitudinal and an inner circular muscular layers were present, with a transition from striated muscle proximally to smooth muscle distally. The oesophagus was particularly distensible, with extensive longitudinal folding, a thick submucosa and a well developed muscularis mucosae. Yamasaki & Takahashi (1974) confirmed this description in the La Plata river dolphin (Pontoporia blainvillei) but found several differences in the Ganges fresh water dolphin (Platanista gangetica) (1971). These were principally the presence of some keratinization of the oesophageal epithelium, a thin muscularis mucosae and an outer muscular layer of variable orientation.

In the marine cetacea, the stomach is divided into three definable compartments, the forestomach, the main stomach and the pyloric stomach, with a connecting channel between the second and third (Harrison et al. 1970). This is also true in the Ganges dolphin (Yamasaki & Takahashi 1971), but not in the La Plata dolphin, where the forestomach is absent (Yamasaki & Takahashi 1974).

The forestomach in the marine cetacea is a pear shaped structure, lying dorsal to the remainder of the stomach and

continuous with the oesophagus. The mucosa is thrown into many longitudinal and a few transverse folds. It is oesophageal in type i.e. a thick aglandular stratified squamous epithelium, but, unlike the oesophagus, is heavily keratinized. Botha (1958a) described a rosette of folds which penetrated over an inch into the stomach. The tunica muscularis is thick and Green observed that the muscularis mucosae was also well developed. There is an opening on the anterior aspect of the forestomach, a few centimetres from the oesophagus, which leads into the second, main stomach. In the La Plata dolphin, the oesophagus leads directly into main stomach as there is no forestomach. In the Ganges dolphin, the forestomach is present, but shares a common oesophageal aperture with the main stomach i.e. the oesophagus opens into both compartments equally. In addition, the mucosal folding is largely reticular.

Harrison described the main stomach to be a globular sac, ventral to and to the right of the forestomach, effectively suspended from the base of the oesophagus. There is a small aperture, about 2 cm in diameter, on the left ventral aspect, opening into the forestomach, into which a sphincter protrudes. In the Ganges dolphin, the main stomach was divided into two by a partial internal septum (Yamasaki & Takahashi 1971; 1974). The convoluted mucosa in the marine cetacea contains simple tubular glands, in which are found mucous, chief and parietal cells, the latter being particularly numerous. No argentaffin cells have been detected. In the freshwater dolphins, Yamasaki & Takahashi also described a transitional zone of cardiac glands around the oesophageal aperture.

On the right anterior aspect of the main stomach is a 0.5 - 1.0 cm aperture leading to the connecting channel. This is only a few centimetres from the oesophageal opening and also has a sphincter. The connecting channel is a passage within the right ventral wall of the main stomach which leads to the pyloric stomach. It is 4 - 9 cm long and 1.0 - 1.5 cm wide and has a  $90^{\circ}$  bend about half way along its length, with a short blind recess. There is a sharp

transition at is proximal end, from fundic to pyloric gland types. Where it enters the pyloric stomach there is a sphincter which protrudes into the lumen. Essentially the same pattern is seen in the fresh water dolphins (Yamasaki & Takahashi 1971; 1974) as is found in the marine cetacea (Harrison et al. 1970; Smith 1972), although in the La Plata dolphin the opening is at the base of the main stomach.

The pyloric stomach is an elongated tubular structure in the marine dolphins, such as Tursiops and Delphinus (Harrison 1970), 20 - 25 cm long and 3 - 4 cm in diameter. On the left, it is in contact with the main stomach and receives the connecting channel via a 0.5 - 1 cm aperture on its posterior or posteromedial aspect. On the right is the pyloric sphincter which leads to the duodenal ampulla. The muscularis externa is thinner than that of the main stomach, and the smooth epithelium contains only mucous cells and, according to Harrison (1970), argentaffin cells. The main difference in the freshwater dolphins lies in the shape of the pyloric stomach, which is globular in the Ganges river dolphin and 'J' shaped in the La Plata river dolphin (Yamasaki & Takahashi 1971; 1974).

Descriptions of the duodenum in the cetacea are few, the most detailed appearing to be by Yamasaki & Takahashi (1975) who studied the La Plata river dolphin. The small intestine was approximately 4.5 m long in the adult animal, but the 'C' shaped duodenum was only about 30 cm long. It was divided into two parts, an initial duodenal ampulla and the duodenum proper. This pattern was also seen in the marine dolphins (Harrison 1970; Smith 1972). The duodenal ampulla was approximately 3 cm long and 5 cm wide at the maximum. It was demarcated externally from the pyloric stomach by only a slight groove, so that it could be interpreted as a fourth gastric compartment (Burmeister 1869). However it was clearly demarcated internally by a narrow and well developed pyloric sphincter. It was followed by the duodenum proper, with no sphincter between them. The epithelium was smooth, and contained only mucous and absorptive cells. Simpson & Gardiner (1972) were unable to

find Brunner's glands or Paneth cells, and only occasional Peyer's patches. Villi were only seen at the end of the duodenum, after 30cm. The common bile and pancreatic duct entered 1.5 to 2.9 cm after the beginning of the duodenum proper (Yamasaki & Takahashi 1975).

It was not possible to find any references in the literature to the extramural or intramural vasculature of the cetacean stomach. The only information was the occasional comment on the richness of the intramural arterial supply, especially in the lamina submucosa.

#### ORIGINAL OBSERVATIONS

One specimen of the common dolphin, Delphinus delphis, was examined. It was not possible within the time frame of this investigation to obtain further specimens. This was a male, 2.06 m in length, beached and sacrificed by a bullet to the heart.

#### Gross Anatomy

#### ð

The external appearance of the stomach and duodenum was generally consistent with the consensus in the literature. The oesophagus clearly entered only the forestomach. The mucosa was very heavily and deeply folded in this compartment, mainly with a longitudinal orientation, although some transverse folds were present. A rosette of mucosa was not visible around the oesophageal aperture. An aperture in the vicinity of the cardia, 0.9cm in diameter, led into the main stomach. This compartment did not agree with the descriptions given in the literature, in that it comprised two large subcompartments (6.6 and 4.6 cm in diameter) separated by a narrow non-sphincteric channel (0.8 cm in diameter, 5.2 cm long). The mucosa in the main stomach had comparatively few folds. The opening to the connecting channel was located 1.6 cm from the entry to the main stomach. This narrow muscular channel, located in the wall of the main stomach was 7.1 cm long and had a 3.1 cm blind sac in its course. It led into the pyloric stomach, a tubular compartment with relatively thinner walls and smooth

mucosa. Externally, the duodenal ampulla was only slightly demarcated by a groove. However, internally it was sharply separated from the pyloric stomach by a well formed sphincter, with a lumen 0.1 x 0.4 cm. The ampulla blended without clear demarcation into a 'C' shaped duodenum. However, 6.5 cm from the pylorus was an opening to a large blind appendage, which had thick walls.  $(F_{10})$ 

The spleen and the pancreas were located within the loop of the stomach and duodenum. The spleen was a globular structure, with no clearly defined hilum which was closely adherent to the stomach. It was accompanied ventrally and to the left by a smaller accessory spleen.

When the stomach was opened, five mucosal lesions were found, four in the forestomach and one in the main stomach. These had mean dimensions of 0.6 x 0.3 cm and a mean depth of 0.8 cm. However, the lesion in the main stomach had depth of 1.3 cm and penetrated well into the muscularis externa i.e. it was a true ulcer. The remainder were erosions, as they did not penetrate beyond the mucosa. This was confirmed by histological sections of the ulcer which showed penetration into the muscularis and lymphocytic infiltration at the base, characteristic of a chronic ulcer. Although the stomach contained quantities of nematode parasites, none seemed to be specifically associated with the lesions.

The forestomach also contained quantities of parasites and fish remnants, which were found mainly near the aboral pole. Smaller amounts were seen in the main stomach and the remainder of the stomach. the parasites were identified by the Natural History division of the British Museum as nematodes - Anisakis simplex (Rud 1809). The fish remnants included otoliths from the whiting (Merlangius merlangus) and the saithe (Pollachius vireus) (Sheldrick - personal communication).

1



<u>Fig. 99</u> Whole stomach and duodenum. Anterior aspect of Dn1 (0.3x)



Fig. 100 Dn1-Muscularis externa removed to reveal submucosa (0.3x)



<u>Fig. 101</u> Pyloric stomach and first part of duodenum to show appendage to ampulla. Muscularis externa removed (0.5x).



Fig. 102 Apparent ulcer in area II of stomach (12.8x).

# Extramural Vasculature (Fig. 103-104)

The vessels supplying the stomach and proximal duodenum all arose from the abdominal aorta, which was 2.0 cm in diameter, via the coeliac or superior mesenteric arteries. The coeliac was long (7.2 cm) and trifurcated to form three vessels. The largest of these (0.7 cm) supplied the spleen, proximal main stomach and distal forestomach. After its origin, it ran cranially and to the left and almost immediately entered the spleen, within which it branched and supplied nutrient vessels. Two main branches arose which left the body of the spleen and passed to the contiguous stomach. One ran to the dorso-caudal edge of the stomach, between the main and forestomachs and supplied branches to the dorsal aspects of both. It then passed cranially to the right of the cardia into the cleft ventral to the oesophagus and supplied branches to the base of the oesophagus and the ventral aspect of the main and forestomachs.

The next largest branch of the coeliac artery (0.6 cm) supplied the pancreas, liver, distal main and pyloric stomachs and duodenum. It had two principal divisions. The larger (0.5 cm) supplied the pancreas, stomach and duodenum. It ran rostro-ventrally to the ventral edge of the stomach where it gave rise to branches which coursed along the stomach both rostrally (onto the distal main stomach) and caudally (onto the pyloric stomach) and supplied these regions. The rostral division ran as far as the ventral cleft between the fore and main stomachs where it anastomosed with a branch derived from the first of the coeliac branches. The larger of the two divisions also gave rise to numerous branches to the duodenal ampulla and duodenum proper. The smaller of the two divisions (0.4 cm) ran rostrally to enter the pancreas, to which it supplied nutrient vessels. A single branch was given off which ran ventro-medially to ramify on the duodenal ampulla. The main vessels continued through the pancreas to supply the liver.

The smallest of the three branches of the coeliac artery supplied the spleen and forestomach. It ran ventrally and to the left between two lobes of the spleen to which it supplied nutrient vessels but did not enter. It also supplied the accessory spleen, between the left ventral aspect of the main organ and the forestomach. A branch was given off which ran in the caudal cleft between the fore and main stomachs, supplying branches to both. In addition, the main vessels continued to ramify over the ventral and dorsal aspects of the forestomach, which were also supplied by branches direct from the main and accessory spleens.

Thus, the main areas of supply of the derivatives of (F, y, i o j) the coeliac artery were clear. The largest vessel supplied the distal forestomach, proximal main stomach and spleen. The next supplied the distal main stomach, pyloric stomach, duodenum, pancreas and liver. The smallest supplied the forestomach and spleen. Two main arterioarterial anastomoses were seen. The main one was between the first and second coeliac branches, around the ventral cleft between the fore and main stomachs. The second was between the duodenal and caudal gastric branches of the second coeliac derivative, on the distal pyloric stomach.

The other branch of the aorta in this region was the superior mesenteric artery. This supplied the tail of the pancreas, then ramified extensively within the omentum to supply branches to the distal duodenum and remainder of the small intestine.

#### Intramural Vasculature

In general, the specimen did not fill well with injectate. Only in the distal main stomach and pyloric stomach were patterns visible and even then only to the (Fig.100,100,100)first level of submucous branching. The oesophagus and forestomach did not fill at all, and the proximal main stomach, connecting channel and duodenum only poorly. It could be seen that inputs were scattered fairly evenly over the forestomach and proximal main stomach. However, inputs to the distal main stomach tended to be concentrated on the two 'edges' of the viscus. In the pyloric stomach, they were entirely restricted to these lines. The submucous plexus


<u>Fig. 103</u> Extramural arterial areas of supply in Dn 1 stomach.

.

.



<u>Fig. 104</u> Topological diagram of branches of Coeliac Artery analogue in Dn 1.

All values are vessel diameters in mm.

n = area 'n' of stomach supplied as defined in previous figure.



seemed to be fairly uniform over the distal main and pyloric stomachs and had a scalariform pattern between the lines of input. There appeared to be a continous submucous plexus between the different gastric compartments and between the pyloric stomach and the duodenal ampulla although in the latter case, the interface was at the anatomical junction and no 'leashes' of vessels were seen. No vessels could be seen which were located within a filled plexus yet did not communicate with it i.e. low connectivity vessels or mucosal arteries of extramural origin.

#### DISCUSSION

Only one specimen was available for examination, so it is not possible to assess to what extent it was representative of the species as a whole. However, since the angioarchitecture of this animal does not seem to have been previously described, it is of value to do so.

As a marine cetacean, it displayed the three part stomach characteristic of this group, with the oesophagus entering the first stomach. The globular main stomach was different from the descriptions given in the literature of both marine and fresh water species, in that it comprised two chambers, separated by a narrow channel. This was not the connecting channel between the main and pyloric stomachs, as this was clearly and separately seen with its characteristic diverticulum. Neither was it the septum described in the Ganges dolphin by Yamasaki & Takahashi. It did not appear to be a fixation artefact. In the absence of further specimens, it is not possible to comment on its nature. The other characteristics of the marine cetacean stomach and duodenum described in the literature were seen in this specimen. However, an unusual feature was seen distally, with a large blind sac attached to the duodenal ampulla. This was not patent and was in the wrong position for the bile and pancreatic ducts. It was not reported in any of the series of dissections in the literature and may perhaps be an abnormality.

In view of the rationale for selecting this species for

investigation, the presence of mucosal lesions in the stomach is particularly interesting. Their number in this single specimen suggests they are probably fairly common, as suggested by workers such as Greenwood and Harrison. In particular, the true ulcer in the glandular main stomach, with the histological characteristics of a chronic ulcer was unusual, as Harrison reported that ulcers only occured in the forestomach (personal communication). In this case, there appeared to be no association with parasites or foreign bodies, and Geraci & Gestermann's histamine hypothesis was not relevant.

The extramural vessels could be seen to be related to the conventional mammalian pattern. The coeliac gave rise tripodally to three vessels. The largest is analogous to the left gastric artery, but with a more restricted area of supply. It branches in a tree-like manner in a relatively restricted area and does not give rise to a vessel analogous to the left gastric in its course along the lesser curvature. The second branch appears to be analogous to the common hepatic artery and gives rise to an anastomotic vessel along the edge of the viscus, analogous to the right gastroepiploic and gastric arteries. This is a large and important vessel, supplying the main and pyloric stomachs. The third vessel, analogous to the splenic artery also has a restricted tree-like pattern and is confined mainly to the forestomach. There appears to be no equivalent to the left gastroepiploic, and the hepatic artery supplies a greater proportion of the stomach than the left gastric, although it is slightly narrower at its origin.

Extramural arterial anastomoses were rare. The only one which was found was a small vessel linking the 'right gastric' branch of the hepatic artery' and the 'left gastric'.

Unfortunately, the intramural vessels were poorly filled. However, the primary submucous plexus could be seen in most areas and the secondary vessels in some. No features were seen which could account for the propensity of the species to ulceration. All the visible areas showed a scalariform branching pattern with no sign of stellate or low connectivity vessels or of mucosal arteries of extramural origin. The plexus was continous throughout the stomach, so there were good anastomoses between the branches of the different extramural inputs. Further work should be done on this species to examine the plexus more comprehensively and to particularly look at the ulcer prone areas, which are not the same areas as are susceptible to ulcers in man.

#### CHAPTER 21 COMPARATIVE DISCUSSION

This study was initiated to provide a firm basis for the use of animal models in studies of gastroduodenal pathophysiology, with particular regard to the investigation of vascular factors in peptic ulceration. It is the first comparative qualitative and quantitative analysis of the gastroduodenal vessels in the commonly-used laboratory animals and builds upon the results first reported by Piasecki (1973). In particular, Piasecki described the phenomenon of large end arteries supplying the mucosa which originated outside or within the muscularis externa. These were present in Man and absent in dogs. He termed these "Mucosal Arteries of Extramural Origin" - MAEO's. Piasecki proposed a hypothesis which implicated them in the aetiology of peptic ulceration, suggesting that they would be susceptible to occlusion by local muscle contraction, which would lead to ischaemia in the underlying mucosa. A major objective of this study was to confirm their absence in dog and to ascertain whether they were present in other species. The use of consistent techniques and assessment criteria allows more valid inter-species comparisons than are possible from the cumulation of different investigations. In some species, a greater number of specimens would have been desirable, but in all cases sufficient material was available to draw definitive conclusions. It would also have been advantageous to have been able to standardize the sacrificial methods to a greater degree, but where different techniques were used, no consistent differences in results were observed.

It was possible to group the species on the basis of the pattern of their extramural vasculature, particularly with regard to the left gastric and left / right (Figster) gastroepiploic arteries. When compared with Man, the left gastric artery was more dominant in the rat, rabbit and guinea pig. It supplied the anterior and posterior walls and, in the rat and rabbit, the central greater curvature.





GUINEA PIG

CAT





RABBIT

RAT



MAN DOG SWINE FERRET PRIMATE

<u>Fig. 105</u> Schematic representation of the distribution of the principal arteries supplying the stomach and first part of the duodenum in the species stated and in man.

-

displacing the right gastric artery. This latter feature only was also seen in the cat. On the greater curvature, the left gastroepiploic artery was dominant in the guinea pig and rabbit, supplying the whole greater curvature in the guinea pig. Conversely, it was absent in the rat, the greater curvature being supplied by the right gastroepiploic and left gastric arteries. Thus, the primate, dog, ferret and swine are similar to Man. Rabbit and guinea pig are similar to one another, with the cat and rat being separated from the other species.

In most cases, the degree of filling varied between specimens and within a specific specimen, as has often been reported by other authors (eg Busch 1973). This had some consistent elements, such as the frequent poor antral filling, and some random elements. As a result, it was necessary to select specimens and areas according to the type of observation being made, either qualitative description, quantification or search for a particular feature. In all cases, cross comparisons were made to ensure that no bias was arising from the selection. False positive results were obviated by only accepting the existence of a feature if all relevant vessels were visible.

Α key result of this investigation was the demonstration in some species that a plexus in the submucosa was not the sole or main intramural blood flow distribution mechanism. In many species, there was a second plexus within or deep to the muscularis mucosae, the mucosal plexus. This has not been described before and was only discernible by consistent use of stereomicroscopy and vessel the micromanipulation, calibrated by cleared edge sections. Groups of species could be differentiated by the relative extent of the superficial (submucous) and deep (muscularis mucosae or mucosal) plexuses. In each case, the number and / or diameter of the anastomotic vessels in the plexus could vary from zero to high. The species could therefore be located on a two-dimensional surface, with the extent of the two plexuses defining the axes. This shows that in the dog, (Fiy.106)



Fig. 106 Grouping of species by extent of submucous and mucosal plexus. This clearly differentiates four groups.



Fig. 107 Diagram to illustrate the effect of lack of connection of mucosal arteries of extramural origin. Spasm of muscle at A would not occlude blood flow in the mucosal artery (a) because of the collateral circulation. Spasm at B involving a mucosal artery arising within or outside the muscle might interfere with circulation at (b). The location of many anastomoses in guinea pig, rabbit, and primate within the muscularis mucosae may have have significance in the control of blood flow as regional muscularis contraction may reduce the local mucosal blood (Fig. 107) flow. Similarly, the corollary of the existence of two plexuses is that mucosal arteries may arise from either the submucous or mucosal plexus. Previous authors have not defined mucosal arteries, but this is necessary. If they are defined as the final arterial end supply to the mucosa, they will arise from the final plexus. Thus, in dog, swine and ferret, the mucosal arteries arise from the submucous plexus and have to penetrate the muscularis mucosae before supplying the mucosa. Conversely, in guinea pig, primate, rat, cat and rabbit, blood flow through the final mucosal arteries which arise from the mucosal plexus would be less sensitive to the state of contraction of the muscularis mucosae.

Using the above definition of mucosal arteries, species could be grouped on the basis of mucosal artery diameter, each group differing by an order of magnitude. Rat, cat, primate and ferret,  $0.05-0.1 \text{ mm}^2$ ; swine, rabbit and guinea pig,  $0.3 \text{ mm}^2$ ; dog,  $1.1 \text{ mm}^2$ . This may be compared with man, in which the figure is  $6.8 \text{ mm}^2$  (Piasecki 1975). Thus, the mucosal artery diameter was not related to body size. The same ranking was seen in the species ordered by mucosal artery perfusion index. The arteries varied widely in area, with adjacent vessels in a single specimen often having widely differing values.



Fig. 108 Cleared edge section of corpus of D1. This illustrates one model in which all anastomoses are in the submucous plexus. This plexus gives rise to all mucosal arteries, between which are small anastomotic vessels. This is summarised in the top diagram. (43x) (cf. diagram of cat overleaf).



Fig. 109 Cleared edge section of corpus of C13LC. This illustrates a second model, in which there are equal submucous and mucosal plexuses. The mucosal arteries arise from the mucosal plexus. This is summarised in the diagram above. (55x).



<u>Fig. 110</u> Diagram to illustrate third model (eg. rabbit) in which all anastomoses are in the mucosal plexus and there is no submucous plexus.



Fig. 111 Three dimensional impression of the concept of plexus vessels running within the muscularis mucosae.

	FUNDUS	CORPUS(LC)	ANTRUM	TOTAL
		DIAMETER (um)		
Rat Cat Primate Ferret Pig G'Pig Rabbit <sup>b</sup> Dog	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	10 10 17 19 26 32 40 67
AREA $(mm^2 \times 10^{-2})$				
Rat Cat Primate Ferret Pig G'Pig Rabbit <sup>b</sup> Dog	$\begin{array}{c} 4 \pm 3 \\ 7 \pm 4 \\ 8 \pm 6 \\ 11^{a} \pm 7 \\ 42 \pm 30 \\ 30 \pm 22 \\ 138 \pm 103 \end{array}$	$ \begin{array}{r} 6 \pm 4 \\ 9 \pm 4 \\ 11 \pm 5 \\ 25 \pm 16 \\ 27 \pm 15 \\ 17 \pm 12 \\ 165 \pm 94 \\ \end{array} $	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	4 9 11 29 31 32 113
PERFUSION INDEX (um4 mm-2 x 104)				
Rat Cat Primate Ferret Pig G'Pig Rabbit <sup>b</sup> Dog	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	31 47 197 210 314 690 1531 2727

<u>NOTES</u>

(a) Mucosal arteries measured in diverticulum ventriculi (b) Mean of 50 determinations. All remainder are a mean of 10 determinations.

Table 24 Summary of the Diameter, Area of Supply and	
Perfusion Index Determinations for the Mucosal Arteries	of
the Fundus, Corpus and Antrum of All Species	

•

An important purpose of this investigation was to seek mucosal arteries of extramural origin (MAEO's). The defining criteria were that:

- the artery arose from another vessel outside or within the muscularis externa
- there were no connections with the plexuses in the submucosa, muscularis mucosae or mucosa
- none of the mucosal branches anastomosed with its neighbours.

Many vessels came close to meeting these criteria, but failed as they had some anastomoses with surrounding vessels. The approach to MAEO status took two forms. In one, the anastomotic vessels became narrower, but not particularly fewer. This was seen in primate and swine, particularly on the lesser curvature as part of the general plexus poverty. None of these were definite MAEO's. In the second mode, the anastomotic vessels became less numerous, but not particularly finer. Many vessels with only 1 or 2 anastomoses were seen in guinea pig and rabbit. These were termed Low Connectivity Vessels (LCV's).

These vessels were quite distinct from the first group. A particular feature they usually displayed, not previously reported, was 'failure to anastomose'. Branches often ran in the plane of the plexus and approached a branch from an adjoining mucosal artery. They appeared to anastomose, but high power stereomicroscopy and micromanipulation showed that they approached to within a few microns of one another, then both turned through  $90^{\circ}$  to supply the mucosa, without anastomosis. The absence in the literature of reports of LCV's (and MAEO's) may be because careful examination was necessary to reveal the failure to anastomose.

This phenomenon was also seen in the MAEO's in the rabbit and guinea pig. In these vessels, <u>all</u> the branches failed to anastomose or entered the mucosa in the normal way - there were no anastomoses. These vessels could only be unequivocally identified in the guinea pig and rabbit. Some probable examples were seen in primate and swine, but these did not display 'failure to anastomose'. There was no suggestion of such vessels in any other species. The species limitation, and the difficulty in observing the failure to anastomose, may explain why these vessels have not been reported before by authors other than Piasecki.

Are the VLC's and MAEO's a unique population of vessels or examples of another class of vessels with particular characteristics? The mucosal arteries are obviously a distinct population with low values of diameter (26um), area of supply  $(0.2mm^2)$ , and perfusion index (463)  $um^4 mm^{-2} \times 10^4$ ). These values are pyloric means from all the species examined - see Table 25. Conversely, 'normal' plexus vessels (in the cat pylorus) are much larger (123um,  $22 \text{mm}^2$  & 1581 um<sup>4</sup> mm<sup>-2</sup> x 10<sup>4</sup> respectively). VLC's and MAEO's (in pig, rabbit and guinea pig) are of similar diameter (116um) but significantly smaller areas of supply  $(6mm^2)$ . They therefore have a much larger perfusion index (5199  $um^4$  $mm^{-2} \times 10^4$ ). However, there is a dramatic difference in the connectivity index. The 'normal' cat pyloric plexus vessels had a CI of about 240  $\text{um}^4 \text{ mm}^{-2} \times 10^4$  compared with pig VLC's (22  $\text{um}^4 \text{ mm}^{-2} \times 10^4$ ). The CI:PI ratios therefore differed by more than an order of magnitude (0.26 vs. 0.01). In the case of the MAEO's, of course, the CI and CI:PI ratio was zero. These values support the subjective evidence that the MAEO's and VLC's are a distinct population of vessels, not ordinary plexus inputs with special characteristics or whose branches have been destroyed. They have input vessels of standard diameter supplying a smaller area of mucosa, so the flow capability is good. However, the anastomoses are much less able to compensate for disruptions in flow through the main input, typically capable of supplying only 1% of the flow, as opposed to 26% in the normal vessel. It is worthy of note that although the MAEO's in Man (Piasecki 1973) were somewhat larger and had a larger area of supply (165um & 18  $mm^2$  mean) their mean perfusion index was very similar (4934  $um^4 mm^{-2} \times 10^4$ ).

This study confirmed the conventional view of lesser curvature plexus poverty. This was shown by the variations in connectivity index in the cat. However, it does not necessarily  $(\tau_4 b/ \varphi_5)$ 

	DIAMETER	AREA OF SUPPLY	PI	CI	PI:CI
	(um)	(mm <sup>2</sup> )	(um <sup>4</sup> mm <sup>-</sup>	$^{2} \times 10^{4}$ )	
Mucosal Arteries	26	18	463		
'Normal' Plexus Vessels	123	2200	1581	240	0.26
VLC/MAEO	116	585	5199	22	0.01
Man-MAEO	165	1800	4934		

#### NOTES

1. Values for mucosal arteries are means for all species (except dolphin) studied in this investigation (pylorus only).

2.Values for 'normal' plexus vessels are means for pylorus in Cat 15.

3. Values for Very Low Connectivity vessels and Mucosal Arteries of Extramural Origin are means for all such gastric vessels in pig, rabbit and guinea pig.

4. Values for MAEO's in Man are means of values quoted by Piasecki (1973) in stomach.

<u>Table 25. Comparison of Parameters for Mucosal Arteries,</u> <u>Normal Plexus Vessels and VLC's/MAEO's in Various Species</u> <u>and Man</u> represent insufficiency of blood supply. The perfusion index of the lesser curvature mucosal arteries was not less on average than those in other areas. In addition, since the mucosa is generally thinner and flatter in the lesser curvature (Kirk 1982), the poorer plexus may only be a reflection of a reduced demand.

The results from this study confirmed a number of others in its failure to find any arteriovenous anastomoses (AVA's), despite a careful examination of all areas, including areas with venous as well as arterial filling. The evidence quoted by authors who have claimed to find them is not compelling. Barlow (1951) commented they were "difficult to find". More recent works, using perhaps more rigorous criteria, have all failed to demonstrate and AVA's (Archibald 1975; Zinner 1976; Gannon et al. 1982). As with the LCV's and MAEO's, it is easy to be mislead by overlapping vessels in the absence of stereomicroscopy and micromanipulation. On balance, the evidence for their existence is not strong.

This comprehensive comparative description and quantification of the intramural vasculature is of interest as the basis for further work on a possible vascular factor in peptic ulcer actiology. The measurement of mucosal artery supply may be relatable to the area of size of experimentally induced ulcers and erosions. The suggestion that the muscularis mucosae may play a role in regional blood flow regulation by virtue of the embedded plexus elements is new. The occurence of MAEO's and LCV's in rabbit and guinea pig provides the basis for further work, in view of their hypothesized role in ulcer aetiology (Piasecki 1977). Although ulcers do not occur spontaneously in these species, and so MAEO's can not be the sole factor, their presence provides an animal model for further work. If they can be located in vivo, their reactivity and the effects of their ligation on the blood flow in the underlying mucosa can be investigated.

In more general terms, the comprehensive and quantitative description of the intramural vasculature in

all these species provides a firm foundation for the vascular aspects of subsequent gastroduodenal experimentation. Overall, it supports the hypothesis that the pattern, rather than the density of the intramural plexuses is likely to be the most significant vascular component in the multifactorial aetiology of peptic ulceration. It now remains to use recently available computer-based image and network analysis techniques to quantify the topology and capacity of the various vascular patterns which have been described.

#### PART IV APPENDICES

#### Appendix 1

#### SPALTEHOLZ TECHNIQUE FOR TISSUE CLEARING (Spalteholz 1914)

- 1. Fix in 10% neutral formalin for 5-7 days.
- 2. Wash in running water for 15 hours and until all traces of acid are removed.
- Dehydrate in ethyl alcohol. 24 hours in each of 70%; 95%;
   100%; 100% solutions.
- 4. Immerse in water free benzol until clearing is advanced.
- 5. Transfer to a mixture of benzol and benzyl benzoate (1:1).
- 6. Store in benzyl benzoate + methyl salicylate (3:5).

The entire process takes approximately 14 days for an average specimen.

#### <u>Appendix 2</u>

#### MODIFIED SPALTEHOLZ TECHNIQUE FOR TISSUE CLEARING

- 1. Wash in running tap water for 18 hours.
- 2. Dehydrate in Industrial Methylated Spirits.

24 hours in each of 50%; 75%; 95%; 100%; 100%.

3. Immerse in the following solutions for 24 hours each.

Benzyl benzoate + methyl salicylate (1:1)

11	+	11	(1:3)
	+	u	(1:9)

4. Store in pure methyl salicylate.

#### Appendix 3

#### SORENSON'S PHOSPHATE BUFFER (0.2M)

Solution A : 13.609g potassium di-hydrogen phosphate  $(KH_2PO_4)$  in 500 ml water.

Solution B : 17.800 g di-sodium hydrogen phosphate  $(Na_2HPO_4)$  in 500 ml water.

Take 28.5 ml A + 71.5 ml B.

This gives 0.2M phosphate buffer at pH 8.0.

#### Appendix 4

#### TECHNIQUE TO COUNTERSTAIN CLEARED TISSUE BLOCKS

- 1. Clear specimen by techniques already described.
- 2. Remove block of submucosa and mucosa containing blood vessels of interest. Approximately 5mm x 2mm.
- 3. Pass through the following solutions.

100% ethanol 5 minutes

90% ethanol 5 minutes

- 50% ethanol 5 minutes
- 4. Stain with H & E using conventional method (Appendix 5).
- 5. Counterstain with Van Gieson (Appendix 6).
- 6. Pass through:
  - 50% ethanol 5 minutes
  - 90% ethanol 5 minutes
  - 100% ethanol 5 minutes
  - Methyl salicylate until clear.

#### HAEMATOXYLIN AND EOSIN METHOD

Reagent:

Cole's Haematoxylin	
Haematoxylin	1.5g
1% Iodine in 95% alcohol	50ml.
Saturated aqueous ammonium alum	700ml.
Distilled water	250ml.

Fixation: 10% Formalin or 10% Formal-saline

Sections: Thin paraffin (4 - 7 um)

<u>Method</u>

- 1. Dewax sections in 2 changes of xylene, 1 to 5 minutes in each.
- 2. Bring sections to water through descending grades of alcohols.
- 3. Stain in Cole's haematoxylin for 10 minutes.
- 4. Blue in alkaline tap water for 5 minutes.
- 5. Differentiate in 0.5 1.0% hydrochloric acid in 70% alcohol for a few seconds.
- 6. Blue in alkaline running tap water for 5 minutes.
- 7. Stain in 1% aqueous eosin for 1 3 minutes.

8. Wash in water for 1 - 2 minutes.

9. Dehydrate in ascending grades of alcohol.

10. Clear in xylene and mount in synthetic resin medium.

#### VAN GIESON METHOD

#### <u>Reagents</u>

Weigert's iron haematoxylin:	
Solution A (stain)	
Haematoxylin	1g
Absolute alcohol	100ml

Solution B (mordant)	
30% aqueous ferric chloride	4ml
Concentrated hydrochloric acid	lml
Distilled water	95m]

Mix A and B immediately before use.

Van Gieson's stain: Picric acid + acid fuchsin.

Sections: Paraffin, frozen or cellulose.

#### <u>Method</u>

- Dewax sections in xylene and descending grades of alcohol.Take to water
- Stain with Weigert's iron haematoxylin for 5 15 minutes.
- 3. Blue in tap water.
- 4. Examine microscopically. Differentiate in 0.5% hydrochloric acid in 70% alcohol if required.
- 5. Wash thoroughly in running alkaline tap water. Rinse in distilled water.
- 6. Stain with Van Gieson solution for 1 4 minutes.
- 7. Rinse in distilled water.
- 8. Dehydrate in 95% then absolute alcohol.Clear in xylene and mount in synthetic resin medium.

#### <u>APPENDIX 7</u> <u>Derivation of Approximate Percentage of Stomach</u> <u>Sampled by Oi (1958)</u>

The following calculations are intended to give only an approximate indication of this value. Several assumptions are made to simplify the calculation.

- 1. The stomach is cylindrical.
- 2. The wall of the stomach is smooth.

If a = area of individual sample

- n = number of samples
- d = diameter of gastric cylinder
- 1 = length of gastric cylinder
- p = proportion of stomach from which samples
   removed

Percentage of mucosa sampled = 100 a n %

dlp

i.e. <u>Area of samples x 100</u> Surface area of sampled region

#### RABBIT

Assumed values: l = 9 cm (Botha 1958a) d = 4 cm "  $a = 2 \times 10^{-4} \text{ cm}^2$  (Oi 1958) n = 23 " p = 1.0 "

 $\underline{Proportion} = \underline{0.004}$ 

<u>CAT</u>

Assumed values: Volume = 300 ml (Bourdelle & Bressou 1953) 1 = 8 cm (personal observations) $a = 2 \times 10^{-4} \text{ cm}^2 \text{ (Oi 1958)}$ 

a =  $2 \times 10^{-4} \text{ cm}^2$  (Oi 1958) n = 58 " p = 0.6 "

<u>Proportion = 0.01</u>%

#### APPENDIX 8

#### ABBREVIATIONS USED IN DIAGRAMS AND TEXT

A	Artery
Aa	Arteries
Acc	Accessory
Ant	Anterior
Ao	Aorta
В	Branch
Br	Branches
CA	Cystic artery
CHA	Common hepatic artery
CoA	Coeliac artery
CMT	Coeliacomesenteric Trunk
CTP	Connective tissue plexus
dbr	Dorsal branches (of any vessel)
Desc	Descending
DMP	Deep muscular plexus (of muscularis externa)
Duo	Duodenum
EPA	Epiploic Artery
GC	Greater curvature
GDA	Gastroduodenal artery
GHT	Gastrohepatic trunk
GO	Greater omentum
GSL	Gastrosplenic Ligament
GST	Gastrosplenic trunk
н	Hepatic
HST	Hepatosplenic trunk
IPDA	Inferior pancreaticoduodenal artery
LC	Lesser curvature
LGA	Left gastric artery
LGEA	Left gastroepiploic artery
ro	Lesser omentum
ME	Muscularis externa
Mes	Mesentery
MM	Muscularis mucosae

MP	Mucosal plexus
Muc	Mucosa
OBr	Oesophageal branches
Oes	Oesophagus
OmA	Omental artery
Panc	Pancreas
PBr	Pancreatic branches
PHA	Proper hepatic artery
Post	Posterior
Pyl	Pylorus
RGA	Right gastric artery
RGEA	Right gastroepiploic artery
SA	Splenic artery
SBr	Splenic branches
SGAa	Short gastric arteries
SGBr	Short gastric branches
SM	Submucosa
SMA	Superior mesenteric artery
SMP	Submucous plexus
SPDA	Superior pancreaticoduodenal artery
Spl	Spleen
SSP	Subserous plexus (of muscularis externa)
St	Stomach
vbr	Ventral branches (of any vessel)
1JA	First jejunal artery
	ANASTOMOTIC VESSEI
m	See note 'n'
a	Area 'a' to which blood is supplied

Vessels supplying blood to organs other than foregut

. .

#### APPENDIX 9

#### PROTOCOL FOR KOLMOGOROV-SMIRNOV TWO SAMPLE TEST

1. Arrange each of the two groups of values in a cumulative frequency distribution, using the same intervals (or classifications) for both distributions. Use as many intervals as are feasible.

2. By subtraction, determine the difference between the two sample cumulative distributions at each listed point.

3. By inspection, determine the highest of the differences; this is D.

4. Inspect the appropriate table for the critical value of  $K_d$  (the numerator of D) for the appropriate level of significance and sample size. If the observed value is equal to 9r larger than the that given in the appropriate table for a particular level of significance,  $H_0$  may be rejected at that level of significance.

One table is used for small samples, which must be of equal size and less than 40. A second is used for large (>40) samples, which need not be of equal size.

(Siegal 1956).

#### APPENDIX 10

# **<u>Piasecki, C. & Wyatt, C.A. (1980).</u>** Comparative Study of the Intramural Blood Supply to the Gastric Mucosa with Special Reference to End Artery Systems. J. Anat. <u>130(3)</u>, 650.

# 22. Surgical anatomy of the zygomatico-temporal distribution of the facial nerve. By M.E. ATKINSON and A. AL-KAYAT. Department of Human Biology and Anatomy and Department of Oral Surgery, University of Sheffield

A number of surgical approaches to the temporo-mandibular joint (TMJ) and zygomatic arch have been described in the literature. Some are unsatisfactory for cosmetic reasons or restricted access whereas others endanger important structures of which the facial nerve is the most significant. A detailed study of the zygomatico-temporal distribution of the facial nerve was carried out to enable a safer surgical approach to the TMJ to be found.

The relationship of zygomatic and temporal branches of the seventh cranial nerve to easily identifiable and palpable bony landmarks was measured on 56 hemisections of hard injected cadavers. The relationship of the nerve to fascial planes was also studied.

Measurements revealed that the most posterior branches of the zygomatico-temporal nerves lay only 0.8 cm anterior to the external auditory meatus (mean  $2.0\pm0.5$  cm) and the bifurcation of the main nerve trunk lay as close as 1.5 cm inferior to the meatus (mean  $2.3\pm0.3$  cm). These measurements show that if direct dissection is kept within these limits to ensure the integrity of the facial nerve the resultant access to the TMJ is very poor.

A means was sought whereby the facial nerve could be reflected within a skin-fascia flap obviating the need for nerve dissection. This was achieved by applying both the quantitative and qualitative observations to the pre-auricular method of Rowe (*Proc. R. Soc. Med.* 65, 1972). Dissection was carried out in a plane between superficial and temporal fascia and then in the space between the two layers of temporal fascia which bifurcate to gain attachment to the zygomatic arch. Dissection could be extended inferiorly by periosteal reflection. These planes are easily located and the nerves lie superficial to the most superficial layer of dissection in every case.

A combination of careful anatomical measurement and observation, which preclude certain procedures whilst suggesting others, have led to an improved operation for exposure of the TMJ and zygomatic arch.

#### 23. Comparative study of the intramural blood supply to the gastric mucosa with special reference to end artery systems. By C. PIASECKI and C. A. WYATT. Department of Anatomy, Royal Free Hospital School of Medicine, London

Past studies of intravascular pathways to the human gastro-duodenal mucosa showed that some individuals have patches of mucosa supplied by end arteries. These patches occur only in the ulcer bearing areas.

In current studies attempts were made to see if a similar 'end artery' system exists in the stomach and duodenum of laboratory animals. If so, this would provide an animal model for future dynamic experiments on such vessels. The intramural gastro-duodenal vascular supply was studied by injection and clearing in dogs, cats, pigs, rats, primates, guinea-pigs and rabbits.

Differences in patterns and the possibility of end artery systems will be described.

## 24. Imaging of the pancreas – internal and external anatomy. By R. Dick (introduced by RUTH E. M. BOWDEN). Department of Radiology, Royal Free Hospital, London

Within the past decade, important advances have resulted in successful imaging of the shape and internal structure of the pancreas, an organ which previously defied raidological visualization. The internal duct structure may be demonstrated by endoscopic retrograde pancreatography (ERP). The structure of the pancreas itself may be visualized by either echography or computed tomography (CT). Some examples of ERP and CT of the pancreas are presented.

### 25. Fat accumulation in the rat heart during fasting. By M. G. ADAMS, R. BARER, S. JOSEPH and F. OM'INIABOHS. Department of Human Biology and Anatomy, University of Sheffield

During starvation depot fat is mobilized and lipid droplet accumulation (LDA) may occur in many organs (Barer *et al.*, *J. Anat.* 123, 1977). While LDA in the heart is well known and has been fully studied biochemically, detailed histological studies are few, and nonexistent for the rat.

Groups of 6 male and 6 female rats aged 6, 12, 18 and 24 weeks were fasted for 1, 2 or 3 days (water *ad lib*). After killing, the heart was frozen rapidly and standard cryostat sections were cut longitudinally to expose all four chambers. Neutral fat was demonstrated by Oil Red O staining. LDA was estimated by entering scores on a stencilled diagram of the heart, divided arbitrarily into 14 regions (5 for each ventricle, 2 for each atrium). Mean scores for each area and total scores for each heart were calculated, given a good semi-quantitative impression of fat distribution.

#### **BIBLIOGRAPHY**

Adachi, B. (1928). Das Arteriensystem der Japaner. Kenkyusha Press. Tokyo Adams, P.F. (1965). Catheterization of Arteries in the Rabbit. Radiol. 84, 531ff. Altura, T. D. & Altura, B. M. (1975). Pentobarbital and Contraction of Vascular Smooth Muscle. Am. J. Physiol. <u>229 (6)</u>, 1635-40. Anrep, G.V. et al. (1933). Effect of Muscular Contraction on Blood Flow in Skeletal Muscle, Diaphragm and Small Intestine. Proc. Roy. Soc. B. <u>114</u>, 245-57. Arabehety, J.T. et al. (1959). Sympathetic Influences on Circulation of Gastric Mucosa in Rat. Am. J. Physiol. 197(4), 915-22. Archibald, L. et al. (1975). Measurement of Gastric Blood Flow with Radioactive Microspheres. J. Appl. Physiol. <u>38 (6)</u>, 1051-6. Babkin, B. P. et al. (1943). The Restoration of the Functional Capacity of the Stomach when Deprived of its Main Blood Supply. Canadian Med. Assoc. J. <u>48</u>, pp.1 ff. Babkin, B. P. (1944). In "Secretory Mechanisms of the Digestive Glands". pp. 99-100. Paul B. Hoeber. Baez, S. (1959). The Microcirculation of the Intramural Vessels of the , Small Intestine in the Rat. In "Microcirculation", Reynolds, S.R.M. & Zweifach, B. W. (Eds.) Univ. Illinois Press. Urbana. pp. 114-25. Barclay, A. E. & Bentley, F. H. (1949a). The Vascularization of the Human Stomach. Gastroenterology 12, 177-83. Barclay, A. E. & Bentley, F. H. (1949b). The Vascularisation of the Human Stomach - Shunting Effect of Trauma. Br. J. Radiol. <u>22</u>, 62-7. T. E. et al. (1951). Arteries, Veins and Barlow, Arteriovenous Anastomoses in the Human Stomach. Surg., Gyn., Obst. <u>93</u> (6), 657-71.

Baronofsky, I. D. (1948). An Experimental Evaluation of the Ligature of Stomach Vessels for Peptic Ulcers. Gastroenterology <u>10</u>, 301ff. Basu Mallik, K.C. (1955). Experimental Study of Pathogenesis of Gastric Ulcer Produced by Pilocarpine. J. Path. Bact. 70, 315-24 Baumel, J. J. et al. (1970). Display of Arteriovenous Anastomoses by Double Latex Injection after Vasodilation with Chloroform. Microvasc. Res. 2, 500-3. Bell, J. & Bell, C. (1826). Anatomy of the Human Body. Vol. II. 6th. ed. London. Bellamy, J. E. C. et al. (1973). The Vascular Architecture of the Porcine Small Intestine. Can. J. Comp. Med. 37, 56-62 Bensley, R. R. (1902). The Cardiac Glands of Mammals. Am. J. Anat. 2, 105-56. Bensley, R.R. (1948). See Craigie (1948). Berg, B. N. (1942). Pathological Changes in Nutritional Gastritis in the Rat. Am. J. Path. 18, 49-61. (1946). Vascular Changes in Mucosa Berg, B.N. in Experimental Nutritional Gastritis. Gastroenterology 7, 340-54. Berg, B. N. (1947). Gastric Ulcer Produced by Vascular Ligation. Arch. Surg. 54, 58-66. Berg, R. (1961). Systematische Untersuchungen uber das Verhalten der Aste der Aorta Abdominalis bei Felis domestica. Anat. Anz. <u>110</u>, 224-50. Berlet, K. (1923). Uber die Arterien des Menschlichen Magens und ihre Beziehungen zur Aeticlogie und Pathogenese des Magenges-chwurs. Z.F. Path. 30, 472-89. Bernheim, B. M. (1932). Partial and Total Devascularization of the Stomach. Ann. Surg. 96, 179ff. Bielak, J. et al. (1967). The Celiac Trunk in M. rhesus and M. cynomolgus. Folia Morphol. (Warsz) 26(3), 256-61. Blalock, A. & Levy, S. E. (1939). Gradual Complete Occlusion of the Celiac Axis, the Superior and Inferior Mesenteric Arteries with Survival of Animals: Effect of Ischaemia on Blood Pressure. Surg. 5, 175ff.

Blick, E.F. (1972). A Review of 2nd. Order Effects on Poiseuille's Equation for Application to Blood and Other Viscous Fluids. Med. Res. Eng. 11, 27-31. Bochenek. (1960). Anatomy of Man. Vol. V. Pzwl. Warsaw. (Cited in Bielak) Botha, G. S. M. (1958a). A Note on the Comparative Anatomy of the Cardio-oeosphageal Junction. Acta Anat 34, 52-84. Botha, G. S. M. (1958b). Mucosal Folds at the Cardia as a Component of the Gastro-oesophageal Closing Mechanism. Br. J. Surg. <u>45</u> (194), 569-80. Botha, G. S. M. (1958c). Histological Observations on the Gastro-oesophageal Junction in the Rabbit. J. Anat. 92 (3), 441-6. Botha, G. S. M. (1962). The Gastro-oesophageal Junction. J. & A. Churchill Ltd. London. Boulter, P. S. & Parks A.G., (1963). Submucosal Vascular Patterns of the Alimentary Canal and their Significance. Br. J. Surg. <u>47</u>, 546-50. Bourdelle, E. & Bressou, C. (1920). Anatomie Regionale des Animaux Domestiques. Vol. III. Porc. J. B. Bailliere et fils. Paris. Bourdelle, E. & Bressou, C. (1953). Anatomie Regionale des Animaux Domestiques. Vol IV. Carnivores- Chien et Chat. J. B. Bailliere et fils. Paris. Bradley, O. C. (1912). A Guide to the Dissection of the Dog. Longmans. London. Brash, J. C. (ed.) (1951). Cunningham's Textbook of Anatomy. 9th. edition. O. U. P. London. Breazile, J. E. & Brown, E. M. (1976). Anatomy. In "The Biology of the Guinea Pig". Wagner, J. E. (ed). pp. 53-62. Academic Press. New York. Brenckmann, E. (1929). Effet de l'histamine sur la Topographie des Zones d'elaboration de l'acid Chlorohydrique de la Musqueuse Fondique chez l'animal adulte, jeune et nouveau-ne. C. R. Soc. Biol. (Paris) 102, 686ff. Brenkmann, E. (1932). Etudes Physiologique sur l'Estomac Depourvu de ses Principales Arteres. Archives des Maladies de l'Appareil Digestif. 22, 625-36.

Buchin, R. F. & Edlich, R. F. (1969). Quantification of Gastric Arteriovenous Blood Flow by Microsphere Clearance Method. Arch. Surg. Chicago <u>99</u>, 579ff.

Buckberg, G.D. et al. (1971). Some Sources of Error in Measuring Regional Blood Flow with Radioactive Microspheres. J. Appl. Physiol. <u>37</u>, 598ff.

Bulkley, G. et al. (1970). Gastric Microcirculatory Changes in Haemorrhagic Shock. Surg. Forum <u>21</u>, 27-30.

Burkl, W. (1958). Mikroskopische Anatomie der Grossen Speicheldrusen. Primatologia. <u>3(1)</u>, 41-61.

**Burmeister (1869).** Pontoporia bainvillei Gray. Anal. Mus. Publico Buenos Aires. <u>1</u>, 369-445.

Busch, C. (1973). Gefassversorgung der Magenwand von Schwein. Vet. Med Inaug. Diss. Munich.

**de Busscher, G. (1948).** Les Anastomoses Arterio-veineuses de l'Estomac. Acta Neurol. Morph. <u>6</u>, 87-105.

Carleton, A. (1935). The Distribution of Brunner's Glands in the Duodenum of Mammals. Proc. Zool. Soc. Lond. <u>2(1)</u>,385-90 Chang, M.C. (1965). Implantation of Ferret Ova Fertilized by Mink Sperm. J. Exp. Zool. <u>160</u>, 67-79.

Cheung, L.Y. & Chang, N (1977). Role of Gastric Mucosal Blood Flow and  $H^+$  Back Diffusion in Pathogenesis of Acute Gastric Erosions. J. Surg. Res. <u>22</u>, 357-61.

Clarke, C.G. & Vane, J.R. (1961). The Cardiac Sphincter in the Cat. Gut. 2,252-62.

Craigie, E.A. (1948). Bensley's Practical Anatomy of the Cat. Univ. Toronto Press. Toronto. 8th. Edition.

Craigie, E.A. (1951). A Laboratory Guide to the Anatomy of the Rabbit. Univ. Toronto Press. Toronto.

**Crouch, J.E. (1969).** Text Atlas of Cat Anatomy. Lea & Fiebiger. Philadelphia.

Cunningham's Textbook of Anatomy. (1951). Oxford University Press. London.

**Delaney, J.P. (1953).** Revascularization of the Cat Liver following the Interruption of its Arterial Supply. Surg. Forum. <u>4</u>, 388-92.

**Delaney, J.P. et al. (1972).** Control of Arteriovenous Shunting. Surg. Forum. <u>23</u>, 241-2.

Delaney, J.P. (1975). Paucity of Arteriovenous Anastomoses in the Stomach. Surg. <u>78(4)</u>, 411-3.

Delaney, J.P. & Grim, E. (1964). Canine Gastric Blood Flow and its Distribution. Am. J. Physiol. <u>207(6)</u>, 1195-1202.

Dieulafe, R. (1936). Contribution a l'etude de l'irrigation de l'intestine grele - consequences de la ligature experimentale des vaisseaux mesenteriques. Arch. d'anat. d'histol. et d'embryol. <u>21</u>, 97-108.

Disse, H. (1903). Uber die Blutgefasse der Menschlichen Magenschleimhaut, Besonders uber die Arterien Derselben. Arch. Mikroscop. Anat. <u>63</u>, 512-30.

**Djorup, F. (1922).** Untersuchungen uber die Feinere Topographische Verteilung der Arterien in den Verschiedenen Magens. Zschr. Anat'. Entw. <u>64</u>, 279-347.

Donaho, P.L. et al. (1972). Holography of the Microcirculation. Bibl. Anat. <u>11</u>, 6-12.

Dunne, H.W. (ed.) (1970). Diseases of Swine. Iowa Univ. Press.

Ellenberger, W. & Baum, H. (1943). Handbuch der Vergleichenden Anatomie der Haustiere. J. Springer. Berlin. 18th. ed.

El-Eishi, H.I. et al. (1973). Arterial Supply of the Human Stomach. Acta Anat. <u>86</u>, 565-80.

El-Ramli, H.A. (1964). Functional Anatomy of the Diaphragm. Ph.D. thesis. Univ. London.

Enge, I. et al. (1972). Selective Coeliac and Hepatic Artery Angiography in the Normal Dog. Scand. J. Gastroenterol. 7,361-8.

Fatani, J. (1971). Neuromuscular Studies of the Gastrooesophageal Junction. Ph.D. thesis. Univ. London.

Favre, P. (1967). Contribution a l'etude du systeme arteriel du cobaye. Abdomen, bassin, membre pelvien. These de Doctorat Veterinaire. Alfort.

Flower, W.H. (1872). Lectures on the Comparative Anatomy of Mammalia. Med. Times & Gazette. 1 & 2.

Ford, L.R. & Fulkerson, P.R. (1962). Flows in Networks. Princeton University Press. Princeton.
Gannon, B. et al. (1982). Microvascular Architecture of Glandular Mucosa of Rat Stomach. J. Anat. <u>135(4)</u>, 667-83. Geraci, J.R. & Gestermann, B.A. (1966). Relationshp of Dietary Histamine to Gastric Ulcer in Dolphin. J. Am. Vet. Med. Assoc. <u>149(7)</u>, 854-90.

Gerhardt, U. (1909). Das Kaninchen Zugleich eine Einfahrung in die Organisation der Saugetiere. Leipzig.

Gilbert, S.G. (1971). Pictorial Anatomy of the Cat. Univ. Washington Press. Seattle.

Gomercic, H. & Babic, K. (1972a). A Contribution to the Knowledge of Variations in the Arterial Supply to the Duodenum and Pancreas in the Cat. Zentrabl. Veterinaermed. <u>1</u>, 269.

Gomercic, H. & Babic, K. (1972b). A Contribution to the Knowledge of the Variations in the Arterial Supply to the Duodenum and Pancreas in the Dog. Anat. Anz. <u>132</u>, 281-8. Gomercic, H. & Babic, C. (1975). Variations in the Arterial Supply of the Duodenum and Pancreas in the Domestic Cat. Acta Anat. <u>91</u>, 213-21.

**Gray, H. (1858).** Anatomy - Descriptive and Surgical. Porter & Sons. London. 1st. edition.

**Grayson, J. (1974).** The Gastrointestinal Circulation. In "Gastrointestinal Physiology" pp. 105-38. MTP International review of science. Physiology-series I vol. 4. Butterworths.University Park Press. London & Baltimore.

Green, R.F. (1972). in Mammals of the Sea. S. Greenwood (ed). 247-97. C.C.Thomas. Springfield.

**Greene, E.C. (1935).** Anatomy of the Rat. Trans. Am. Phil. Soc. <u>27</u>. Reprinted 1955-Hafner, Philadelphia.

**Greenwood, A.G. et al. (1976).** Diseases of Upper Gastrointestinal Tract of Dolphins. Proc. Am. Assoc. Zoo Vet. Conf.

**Greenwood, M. (1884).** Observations of Gastric Glands of Pig. J. Physiol. <u>5</u>, 195-208.

**Grzybowski, J. (1926).** La phylogenese de l'artere coeliaque chez les primates. Extract de C.R. des seances de la soc. des sciences et des lettres de Varrone.

Gupta, C.D. et al. (1978) Area Distribution of Gastric Arteries in Dog's Stomach. Anat. Anz. 143, 490-4. Guth, P.H. et al. (1975). Experimental Chronic Gastric Ulcer due to Ischaemia in Rats. Am. J. Dig. Dis. 20(9), 824-34. Guth, P.H. & Rosenberg, A. (1970). A Method for In Vivo Study of Gastric Microcirculation. Microvasc. Res. 2, 111-2. Guth, P.H. & Rosenberg, A. (1972). In Vivo Study of Gastric Microcirculation. Am. J. Dig. Dis. 17(5), 391ff. Guth, P.H. & Smith, E. (1974). Vasoactive Agents and Gastric Microcirculation. Microvasc. Res. 8(2), 125-31. Guth, P.H. & Smith, E. (1975). Escape from Vasoconstriction in the Gastric Microcirculation. Am. J. Physiol. 228(6), 1893-5. Hahn, E.W. & Wester, R.C. (1969). The Biomedical Use of Ferrets in Research. Marshall Research Animals Inc. North Rose. New York. von Haller, A. (1745). Iconum Anatomicarum Partum Corporis Humani. A. Vandenhoeck. Gottingae. Harjola, P.T. & Sivula, A. (1966). Gastric Ulceration Following Experimentally Induced Hypoxia and Haemorrhagic Shock. Ann. Surg. <u>163</u>, 21-8. Harrison, B.M. (1952). Dissection of the Cat. H. Kimpton. London. 2nd. ed. Harrison, R.J. et al. (1970). The Oesophagus and Stomach of Dolphins (Tursiops, Delphinus, Stenella. J. Zool. 160, 377-90. Harrison, R.J. (1973). Preface in Anatomy of Marine Mammals. Vol. I. Academic Press. Hase, T. & Moss, B.J. (1973). Microvascular Changes in Gastric Mucosa in Development of Stress Ulcer in Rats. Gastroenterology. <u>65</u>, 224-34. Havlicek, H. (1934). Anatomische und Physiologische Grundlagen der Thromboseentstehung und deren Verhutung.Arch. Klin. Chir. <u>180</u>, 74ff. Hoffman, L. & Nather, K. (1921). Zur Anatomie der Magenarterien. Arch. fur Klin. Chir. 115, 650-71.

Holm, B. et al. (1949). The Effect of Experimental Devascularization on Gastric Mucosae. Gastroenterol. 12, 116-21. Holm, B. & MacKay, A.G. (1949). Effect of Surgical Devascularization on the Production of Mann-Williamson Ulcers. Surgery 25, 446-50. Howell, A.B. (1926). Anatomy of the Wood Rat. In "Monographs of the American Society of Mammalologists." No.1. Williams & Wilkins. Baltimore. Hubens, A. (1971). Etude de Differences Morphologiques dans la Vascularization de la Muqueuse Antrale et de la Muqueuse Corporeale de l'estomac du Rat. C.R.Soc Biol. (Paris). <u>165(2)</u>. 2230-1. Hunt, H.R. (1924). Laboratory Manual of the Anatomy of the Rat. Macmillan. New York. Ivy, A.C. (1919). Studies on Experimental Gastric and Duodenal Ulcer. Am. J. Physiol. 49, 143-4. Ivy, A.C., Grossman, M.I. & Bachrach, W.H. (1950). Peptic Ulcer. J. & A. Churchill. London. Jacobson, E.D. et al (1966a). Effect of Histamine on Gastric Blood Flow in Conscious Dogs. Gastroenterology. 51. 466. Jacobson, E.D. et al (1966b). Gastric Secretion in Relation to Mucosal Blood Flow Studied by a Clearance Technique. J. Clin. Invest. <u>45</u>. 1ff. Jacobson L.F. & Noer, R.J. (1952). Vascular Patterns of Intestinal Villi in Various Laboratory Animals and Man. Anat. Rec. <u>114(1)</u>. 85-102. Jamieson, E.B. (1916). Basle Nomina Anatomica. Green & Sons. London & Edinburgh. Jatrou, S. (1920). Uber die Arterielle Versorgung des Magens und ihre Beziehung zum Ulcus Ventriculi. Dtsh. Z. F. Chir. 159, 196-223. Jervis, H.R. et al. (1973). Acute Duodenal Ulceration in the Guinea Pig Due to Fasting. Lab. Invest. <u>28(4)</u>. 501-13. Kairaluoma, M.I. et al. (1972). Experimental Gastric Ulcer in the Shay Rat - II) Healing of gastric Ulcer, Development of Stomach Supply and Effect of Vagotomy on Healing. Acta Chir. Scand. Suppl. 428.

395

1.

Kainer, R.A. (1954a). Gross Anatomy of the Digestive System of the Mink - I) Headgut and Foregut. Am. J. Vet. Res. 15. 82-90. Kainer, R.A. (1954b). Gross Anatomy of the Digestive System of the Mink - II) Midgut and Hindgut. Am. J. Vet. Res. 15. 91-7. Kawarda, M.D. et al (1975a). The Pathophysiology of Stress Ulceration and its Prevention (i) Pharmacologic Doses of Steroid. Am. J. Surg. <u>129(3)</u>. 249-54. Kawarda, M.D. et al. (1975b). Pathophysiology of Stress Ulcer and its Prevention (ii) PGE1 and Microcirculatory Response. Am. J. Surg. <u>129</u>. 217-22. Kirk, R.M. (1968). Site and Localisation of Duodenal Ulcers. A Study at Operation. Gut. 9, 414ff. Kirk, R.M. (1982). Factors Deermining the Site of Chronic Gastroduodenal Ulcers. Hepatogastroenterology. 29, 75-85. Kivilaasko, E. et al. (1974). Gastric Ulcer in Pigs Subjected to Haemorrhagic Shock. Scand. J. Gastroenterol. 9. 685-90. Kobayashi, K. (1973). An Experimental Study of Arteriovenous Anastomoses in the Stomach. Jap. J. Gastroenterology. 70. 442-55. Kondrashov, K.E. (1964). The Arterial Supply of the Duodenum in the Dog. Sbornik: funktsionalnaya Anatomiya sosudistoi Sistemy. 2. 74-82. (Cited in Gomercic & Babic 1972). Kowalczyk, T. (1970). "Gastric Ulcers" in "Diseases of Swine". 3rd. ed. Kuczynski, A. (1890). Month. Internat. J. Anat. Physiol. <u>vii</u>. p.419. (Cited in Carleton 1935). Lakhtina, V.P. & Kozlov, V. (1975). Investigation of Microcirculation in Gastric Wall of Albino Rat by Method of Vital Microscopy. Archiv. Anatomii Gistologii i Embriologii. <u>68</u>. 61-6. Lambert, R. (1965). Surgery of the Digestive System in the Rat. Charles C. Thomas. Springfield.

Layne, J.A. & Bergh, G.S. (1943). The Effect of Ligation of the Arteries of the Stomach upon Acid Gastric Secretion and upon the Endoscopic Appearance of the Gastric Mucosae in the Dog. Surgery. <u>13</u>. 136ff.

Leneman, F. & Burton, S. (1967). The Hepato-oesophageal Artery in the Rat. Acta Anat. <u>68</u>. 334-43.

Leone, V. (1948). Observazione sulla Vascolarizzazione Sanguigna dello Stomaco. Arch. Ital. Anat. Embriol. <u>53</u>,264-86.

Lineback, P. (1961). "The Vascular System" in "Anatomy of the Rhesus Monkey (M. mulatta)". Harleman, C.G. & Straus, W.B. (Eds.). Hafner Pub. Co. New York.

Lundgren, O. (1967). Blood Flow Distribution and Countercurrent Exchange in the Small Intestine. Acta Physiol. Scand. Suppl. 303.

Luther, E. (1923). Vergleichende Anatomische Untersuchungen uber die Aorta Abdominalis und ihre Verzweigungen beim Meerschweinchen und Kaninchen. These de Doctorat Veterinaire. Hannover. Cited in Perneczky (1969).

McClure. R.C. et al. (1973). Cat Anatomy - an Atlas, Text and Dissection Guide. Lea & Febiger. Philadelphia.

McMaster, P.D. (1922). Do Species Lacking a Gall Bladder Possess its Functional Equivalent? J. Exp. Med. <u>35</u>. 127-40. Mall, F. (1896). The Vessels and Walls of the Dog's Stomach. John Hopkins Hosp. Rep. <u>1</u>. 1-36.

Mann, F.C. (1916). A Study of the Gastric Ulcers Following Removal of Adrenals. J. Exp. Med. <u>23</u>, 203-10.

Mann, F.C. (1920). A Comparative Study of the Anatomy of the Sphincter at the Duodenal End of the Common Bile Duct, with Special Reference to Species of Animals Without a Gall Bladder. Anat. Rec. <u>18</u>. 355-60

Martin, H.N. & Moales, W.A. (1884). Vertebrate Dissection -Part III - How to Dissect a Rodent. Macmillan. New York.

Mayo, W.J. (1908). Anaemic Spot on the Duodenum. Surg., Gyn. &. Obst. <u>6</u>, 600ff.

Meiselman, H.J. (1975). Formation of Hollow Vascular Replicas using a Gallium Injection Technique. Microvasc. Res. <u>9</u>. 182-9.

4

Michels, N.A. (1955). Blood Supply and Anatomy of the Upper Abdominal Organs. J.B. Lippincott Co. Philadelphia. Mignot, J. (1956). Techniques d'etude de la Vascularization Sanguine Applique a l'anatomie Pathologique. Ann. d'Anat. Path. (Paris). 385-96. Miller, D.S. et al. (1969). Vascular Architecture of Small Intestine Villi in the Rat. Different Forms of Scand. J. Gastroenterol. 4. 477-82. Miller, E,B. (1954). A Study of the Capillaries of the Gastric Mucosa. Surgery. 36. 898-902. Miller, M.E. et al. (1967). Anatomy of the Dog. W.B. Saunders. Philadelphia and London. Mincev, M. et al. (1971). On Variability of Truncus Coeliacus and its Ramifications According to our 107 Selective Angiographs. Folia Med. 13, 114-21. Minne, M.M. et al. (1952). Etude Artergraphique du Plexus Sousmuqueuse de l'Estomac. Lille Chir. 728-37. Mohuiddin, A. (1966). Blood and Lymph Vessels and Jejunal Villi of the White Rat. Anat. Rec. 156. 83-90. Morell, G.H. (1872). Comparative Anatomy and Dissection of Mammalia. Part I. 180-208. Longman & Co. London. Morozova, T.D. (1965) Ileocecal Area of the Intestine of the Guinea Pig and its Vascularization. Tr.Khar'kov Med. Inst. 65. 342-8. (Cited by Shively & Stump 1975). Nian-Chia Chang (1958). Acta Anat. Sinica. 3(3), 197ff. Nims, J.C. et al. (1973). Chamber Techniques to Study the Microvasculature. Microvasc. Res. 5. 105-18. Noer, R.J. (1943). Blood Vessels of the Jejunum and Ileum. A Comparative Study of Man and Certain Laboratory Animals. Am. J. Anat. <u>73(3)</u>. 293-321. Nomina Anatomica (1963). Excerpta Medica Foundation. 3rd. Ed. Nomina Anatomica Veterinaria (1973). International Committee for Veterinary Anatomical Nomenclature. 2nd. Ed. Vienna. Nylander, G. & Olerud, S. (1960). A Simple Microangiographic Procedure for the Study of Vascular Patterns in the Alimentary Canal. Acta Soc. Med. Upsal. <u>65</u>, 374-9.

Nylander, G. & Olerud, S. (1961). Vascular Patterns of the Gastric Mucosa of the Rat Following Vagotomy. Surg. Gynecol. Obstet. <u>112</u>. 475ff. Obel, W. et al. (1967) The Common Hepatic Artery in M. rhesus and M. cynomolgus. Folia Morphol (Warsz.) 26(4). ∧ Oi, M. et al (1958). Distribution of Parietal Cells of Stomach in Animals - Dog, Cat, Rabbit, Guinea Pig and Rat. Jikei Med. J. <u>5</u>. 67-95. Oi, M. et al. (1959). The Location of Gastric Ulcers. Gastroenterology. <u>36</u>, 45-56. Oi, M. et al. (1969). Possible Dual Control Mechanism in Origin of Peptic Ulcer. Gastroenterology. <u>57(3)</u>, 280-93. Oka, S. (1970). The Microcirculation of the Gastrointestinal Mucosae and the Blood Vessel System. Saishin Igaku. 25(8). 1705-13. Ota, K. & Okudaira, G. (1965). J. Jap. Med. Assoc. (Nikon Ishikai Zasshi). <u>54</u>. 146ff. Osemlak, J. et al. (1968a). The Left Gastric Artery in M. rhesus and M. cynomolgus. Folia Morphol. (Warsz). 27(2). 195~204. Osemlak, J. et al. (1968b). The Lienal Artery in M. rhesus amd M. cynomolgus. Folia Morphol. (Warsz). 27(2). 205-14. Osman Hill (1974). Primates - Comparative Anatomy and Taxonomy. Vol. VII. Cynopithecinae. Edinburgh Univ. Press. Persson, B.H. (1952). A Method for Freeze Dehydration of Tissues in a Liquid Medium. Acta Soc. Med. Ubsal. <u>57</u>. 155-60. Peters, R.M. & Womack, N.A. (1958). Haemodynamics of Gastric Secretion. Ann. Surg. <u>148</u>. 537-50. von Perneczky, A. (1969). Die Aste der Aorta Abdominalis beim Meerschweinchen. Anat. Anz. 125. 443-53. Pfeiffer, C.J. (1970). Surface Topology of the Stomach in Man and the Laboratory Ferret. J. Ultrastruct. Res. 33. 252-62. Pfeiffer, C.J. (1974). Unusual Ultrastructural Variants in the Ferret Parietal Cell. Experentia 26(4). 295-6. Piasecki, C. (1971). A Possible Vascular Factor in the Actiology of Peptic Ulceration. Br. J. Surg. <u>58</u>. 660-2.

**Piasecki, C. (1973).** The Intramural Blood Vessels of the Lesser Curvature of the Stomach and First Part of the Duodenum in Man and Dog. Ph.D. Thesis. University of London. **Piasecki, C. (1974).** The Blood Supply to the Human Gastrointestinal Mucosa, with Special Reference to the Ulcer Bearing Areas. J.Anat. <u>118</u>. 295-335.

**Piasecki, C. (1975).** Observations on the Submucous Plexus and Mucosal Arteries of the Dog's Stomach and First Part of the Duodenum. J. Anat. <u>119</u>. 133-48.

**Piasecki, C. (1977).** Role of Ischaemia in the Initiation of Peptic Ulceration. Ann. Roy. Coll. Surg. England. <u>59</u>. 476-8. **Piasecki, C. & Wyatt, C.A. (1980).** Comparative Study of the Intramural Blood Supply to the Gastric Mucosa with Special Reference to End Artery Systems. J.Anat. <u>130(3)</u>. p.650.

**Piasecki, C. & Wyatt, C.A. (1986).** Patterns of Blood Supply to the Gastric Mucosa. A Comparative Study Revealing an End Artery Model. J. Anat. <u>149</u>. 21-39.

**Poddar, S. & Murgatroyd, L. (1976).** Morphological and Histological Study of the Gastrointestinal Tract of the Ferret. Acta Anat. <u>96</u>. 321-4.

**Potter, G.E. et al. (1958).** The Circulatory System of the Guinea Pig. Bios. <u>29</u>. 3-13.

Puget, A. & Voisin, M.C. (1973). Microscopic Anatomy of the Digestive System of Orchotonia rufescens rufescens.

Acta Anat. <u>84</u>. 267-81.

Rau, W. (1981). Haemodynamics of Gastroduodenal Circulation. Langenbecks Arch. Chir. <u>354</u>, 281-91.

Rau, W. (1983). On the Formaal Cause of Gastric Ulcer. Langenbecks Arch. Chir. <u>360</u>, 43-57.

Rau, W. (1986a). Funktionelle Anatomie der Magengtrombahn: Lokalisierende Factoren in der Pathogenese des Magenesschwurs. Langenbecks Arch. Chir. <u>367</u>, 129-38.

Rau, W. (1986b). Experimentelle Bedingungen zur Reproduktion eines Submucosen Steal-Phanomens am Hundemagen. Langenbecks Arch. Chir. <u>367</u>, 139-45. Rau, W. (1987). Gastric Ulcer as a Problem of Energy Balance. Design of a Cellular Automaton for Simulation of Disorders of Flow Distribution to Stomach Wall. Langenbecks Arch. Chir. <u>370</u>, 91-110. Rauch, R. (1962). Beitrag zur Arteriellen Versorgung der Beckenhotele bei atze und Hund. Bauch - und Dissertation. Berlin. (Cited in Gomercic & Babic 1972). Reeves, T.D. (1920). Study of Arteries Supplying Stomach and Duodenum and Their Relation to Ulcer. Surg., Gynaecol. & Obstet. <u>30</u>, 374-85. Reighard, J. & Jennings, H.S. (1929). Anatomy of the Cat. H.Holt & Co. New York. Reynolds. D.G. et al. (1967). Vascular Architecture of the Small Intestinal Mucosa of the Monkey (M. mulatta). Anat. Rec. <u>159</u>. 211-8 Ridgeway, S. (1965). Medical Care of Marine Mammals. J. Am. Vet. Med. Assoc. <u>147(10)</u>, 1077-85. Ridgeway, S. (1965). Medical Care of Marine Mammals. J.Am. Vet. Med. Assoc. <u>147(10)</u>, 1077-85. Ridgeway, S. & Johnston, D.G. (1965). Two Interesting Disease Cases in Wild Cetaceans. Am. J. Vet. Res. 26, 771-5. Rojecki, F. (1889). J. Anat. Physiol. Paris. 25. 343-86 & 513-61. Saito, I. et al. (1974). Studies on Oesophogastric Ulcers in Swine. II - Blood Supply to Normal Gastric Wall of Swine. Bull. fac. Ag. Migazaki Univ. <u>31(1)</u>, 201-9. Sales, J. et al. (1975). Gastric Mucosal Ischaemia in Experimental Stress Ulcer. J. Surg. Res. 18. 65-74. Salomon (1893). Arch. Mikr. Anat. <u>41</u>, 19-27. Schnitzlein, H.M. (1957). Regulation of Blood Flow Through Stomach of Rat. Anat. Rec. <u>127</u>, 735ff. Schroeder, C.R. & Wedgeforth, H.M. (1935). Occurence of Gastric Ulcer in Sea Mammals of the California Coast -Aetiology and Pathology. J. Am. Vet. Med. Assoc. 87, 333-42. Schwalbe, G. (1872). Arch. f. Mikros. Anat. viii. (Cited in Carleton 1935). Schwartz, G. (1910). Uber Penetrirende Magen - und Jejunal Geschwure. Beitr. z. Klin - Chir. 67. 96ff.

Sehgal (1966). Nature. 210, 972-3. Seirafi, R. (1962). The Vascular Component in the Causal Genesis of Peptic Ulcer. Surg. 51, 233-40. Sekine, T. & Shiratori, T. (1974). Significance of Local Circulatory Change in the Experimental Production of Gastric Ulcer. Tokyo J. Exptl. Med. <u>114</u>. 121-30. Shay, H. et al. (1945). A Simple method for the Uniform Gastric Ulceration in the Rat. production of Gastroenterology. 5. 43-61. Sherman, J.L. (1963). Normal Arteriovenous Anastomoses. Med. (Baltimore). <u>42</u>. 247-67. Sherman, J.L. & Newman, S. (1954). Functioning Arteriovenous Anastomoses in the Stomach and Duodenum. Am. J. Physiol. 179. 279-81. Shively, M.J. & Stump, J.E. (1975). The Systemic Arterial Pattern of the Guinea Pig: The Abdomen. Anat. Rec. 182. 355-66. Shoemaker, C.P. & Powers, S.R. (1966). Absence of Large Functional Arteriovenous Shunts in Stomach of Anaesthetised Dogs. Surg. <u>60(1)</u>, 118-26. <u>Siegal, S. (1956).</u> Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company. New York. Simpson, D.G. & Gardiner, M.B. (1972). Comparative Microanatomy of Selected Marine Mammals. Ch. 5 in Mammals of the Sea - Biology and Medicine. Ridgeway, S. (ed). C.C.Thomas. Springfield. 298-418. Sisson, S. et al. (1945 & 1953). Anatomy of Domestic Animals. W.B. Saunders. Philadelphia. Slijper, E.J. (1962). Whales. Butterworth. London. Sloss, M.W. (1954). Microscopic Anatomy of Digestive Tract of Sus scrofa domestica. Am. J. Vet. Res. 15, 578-93. smith (1972). Stomach of Harbour Porpoise Phocoena phocoena (L). Canad. J. Zool. <u>50</u>, 1611-6. Solcia, E. et al. (1969). Studies on G Cells of Pyloric Mucosa, the Probable Site of Gastrin Secretion. Gut. 10, 379-88. Spalteholz, W. (1914). Uber das Dusch Sichtigmachen von Menslichen und Tierischen Preparten. S. Hirtzal. Leipzig.

spanner, R. (1932). Neue Befunde uber die Blutwege der Darmwand und ihre funktionelle Bedeutung. Morph. Jahrb. 69. 394-454. spira, J. (1956). Gastro-Duodenal Ulcer. Butterworth. London. Stromsten, F.H. (1952). Davison's Mammmalian Anatomy, with Special Reference to the Cat. Blakiston & Co. 7th. Ed. Svanes, K. & Skarstein, A. (1975a). Microcirculation of the Cat Stomach. Eur. J. Surg. Res. 7. 53-64. Svanes, K. & Skarstein, A. (1975b). Blood Flow Distribution in the Stomach of Cats with Gastric Ulcer. Scand. J. Gastroenterol. <u>10(4)</u>. 339-40. Sweeney, J.C. & Ridgeway, S.H. (1975a). Common Diseases of Small Cetaceans. J. Am. Vet. Med. Ass. 167. 533-40. Sweeney, J.C. & Ridgeway, S.H. (1975b). Procedures for the Clinical Management of Small Cetaceans. J. Am. Vet. Med. Ass. <u>167</u>. 540-5. Taylor, T.V. et al. (1975) Observations of the Gastric Mucosal Blood Flow Using <sup>99</sup>Tc in Rat and Man. Br. J. Surg. 62(2). 157. Taylor, T.V. & Torrance, B. (1974). Is There an Antral -Body Portal System in the Stomach? Gut. 15(10). 836. Taylor, T.V. & Torrance, B. (1975). Is There an Antral -Body Portal System in the Stomach? Gut. 16. 781-4. Taylor, T.V. & Torrance, B. (1976). The Effect of Fasting on Mucosal Blood Flow in the Antrum and Corpus of the Stomach. Eur. J. Surg. Res. 8. 227-35. Taylor, W.T. & Weber, R.J. (1951). Functional Mammalian Anatomy (with Special Reference to the Cat). Van Nostrand. New York. Testi, F. et al. (1971). Gastrite Ulcerative da Nematodi in un Delfino del Mare Adriatico. Parassitologie. 13, 355-60. Testut, L. (1912). Traite d'Anatomie Humaine. 4. Doin. Paris. Thamm, H. (1941). Die Arterielle Blutversorgung des Magendarmkanals, Seiner Anhangsdrusen (Leber, Pankreas) und der Milz beim Hunde. Morph. Jb. <u>85</u> 417-46. (Cited in Gomercic & Babic 1972).

Theile, F.W. (1852). Arch. Anat. Physiol. Wiss. Med. 419-49. Thompsett, D.H. (1970). Anatomical Techniques. Livingstone. Edinburgh. 2nd. ed. Trautman, A. & Fiebiger, J. (1957). Fundamentals of Histology of Domestic Animals. Comstock Pub. Assoc. New York Trixl, H. (1973). Angioarchitecture of Stomach of Gottingen Minature Pig. Inaug. Diss. Tierartzliche Fakultat. Univ. Munchen. Turner, W. (1889). Additional Observations on the Stomach in the Ziphoid and Delphinoid Whales. J. Anat. Physiol. Lond. 23, 466-92. Varhaug, J.E. et al. (1977). Effect of Partial Devascularization on the Blood Flow Distribution in Cat Stomach. Eur. Surg. Res. 9. 432-43. Varhaug, J.E. et al. (1979a). Effect of Partial Gastric Devascularization on Mucosal Blood Flow and Acid Secretion in Cats. Eur. Surg. Res. 11. 15-26. Varhaug, J.E. et al. (1979b). Gastric Ulceration and Changes in Acid Secretion and Mucosal Blood Flow after Partial Gastric Devascularization in Cats. Acta Chir. Scand. 145. 313-9. Varhaug, J.E. et al. (1980). The Effect of Intragastric Gastric Blood Flow after Partial Pressure on Devascularization of the Stomach in Cats. Eur. Surg. Res. 12. 415-27. Varhaug, J.E. et al. (1984). Gastric Ulceration related to Regional Ischaemia, Acid Secretion and Bile Reflux in "Mechanisms of Mucosal Protection in Cats. pp.273-7 in the Upper Gastrointestinal Tract". Allen, A. et al (eds.). Raven Press. New York. Vesalius, A. (1555). De Humani Corporis Fabrica. Joannes Basel. Facsimile. Liber II. "Culture and Oporinus. Civilisation".Bruxelles. 1964. Villemin, F. (1922). Arch. de Morph. Gen. et Exp. Fasc iii. (Cited in Carleton (1935). Virchow, R. (1853). Historiches, Kritisches und Positives zur Lehre der Unterleibzaffektionen. Virchow's Arch. 5. 362. (Cited in Jacobsen 1965).

**Volini, I.P. (1938).** Pseudo Ulcers of the Duodenum of the Normal Dog. Including a Study of the Intestinal Ulcers in the Normal Dog. Arch. Surg. <u>37</u>, 259-67.

Walder, D.N. (1951). Arteries, Veins and Arteriovenous Anastomoses in Human Stomach. Surg., Gynaecol. & Obstet. <u>93(6)</u>, 657ff.

Walder, D.N. (1952). Arteriovenous Anastomoses of the Human Stomach. Clin. Sci. <u>11</u>. 59-71.

Walder, D.N. (1953). Some Observations on the Blood Flow in the Human Stomach. pp.210ff. in "Visceral Circulation". CIBA Foundation. Littlebrown & Co. Boston.

Wanke, M. (1962). Die Begleitmuskelfasern der Magengefasse und ihre Bedeutung fur die Pathogenese des Ulcus Ventriculi. Arch. Klin. Chir. <u>300</u>. 166-86.

Ware, R.W. (1975). 3-D Reconstruction from Serial Sections. Int. Rev. Cytol. <u>40</u>, 325-34.

Watt, J. (1959). The Mechanism of Histamine Ulceration in the Guinea Pig.Gastroenterology. <u>37(6)</u>. 741-59.

Weigman, D.L. et al. (1975). Microvascular Response to Hypoxia, Hyperoxia, Hypercarbia and Localised Acidosis. Bibl. Anat. <u>13</u>, 159-60.

Weissflog, W. (1903). Faserverlauf der Muskulatur des Magens von Pferd, Schwein, Hund und Katze. Archiv. fur Wissenschlaftiche und Praktische Thlerheil Kunde. 286-331.

Whitehouse, R.H. & Grove, A.J. (1937). Dissection of the Rabbit. University Tutorial press. London.

Whittle, B.J.R. (1976). Study of Vascular and Acid Secretory Responses of Rat Gastric Mucosa to Histamine. J. Physiol. 257, 407-18.

Wiland, C. (1970). The Celiac Trunk in Minks. Folia Morph.(Warsz). <u>29</u>. 160-5.

Wingate, D. (1976). The Eupeptide Theory. A General theory of Gastrointestinal Hormones. Lancet. <u>1</u> (7958). 529-32.

Yamasaki, F. & Takahashi, K. (1971). Digestive Tract of the Ganges Dolphin, Platanista gangetica. Okajimas. Fol. Anat. Jap. <u>48</u>, 271-93

Yamasaki, F. & Takahashi, K. (1974). Digestive Tract of the La Plata Dolphin, Pontoporia bainvillei - Oesophagus & Stomach. Okajimas. Fol. Anat. Jap. <u>51</u>, 29-52 Yamasaki, F. & Takahashi, K. (1975). Digestive Tract of the La Plata Dolphin, Pontoporia bainvillei - Small & Large Intestine. Okajimas. Fol. Anat. Jap. <u>52</u>, 1-26 Young, P.C. & Lowe, D. (1969). Larval Nematodes from Fish of Family Acisakinae and Gastrointestinal Lesions in Mammals. J. Comp. Path. <u>79</u>, 301-13. Zinner, M.J. et al. (1975). Adrenergic Mechanisms in Canine Gastric Circulation. Am. J. Physiol. 229(4). 977-82. Zweifach, B.W. (1957). General Principles Governing the Behaviour of the Microcirculation. Am. J. Med. 23. 684ff. Zweifach, B.W. & Chambers, R. (1946). The Functional Activity of the Capillary Bed with Special Reference to Visceral Tissue. Ann. N. Y. Acad. Sci. 46. 883ff.

J. Anat. (1986), 149, pp. 21–39 With 13 figures Printed in Great Britain

× ?

.

A Restau

# Patterns of blood supply to the gastric mucosa. A comparative study revealing an end-artery model

#### C. PIASECKI AND C. WYATT

Department of Anatomy, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF

#### (Accepted 19 December 1985)

#### INTRODUCTION '

The mechanism that induces chronic peptic ulceration continues to elude us. The factors which are still attracting most research into the cause of peptic ulceration – namely acidity and the mucus barrier – do not explain what are perhaps the most outstanding features of the disease. These are (1) the fact that peptic ulcers usually occur singly, and (2) the remarkable localisation of gastric ulcers to the lower parts of the lesser curvature and of duodenal ulcers to the anterior and posterior walls of the first two cm of the pylorus. This suggests the operation of strong localising factors.

Many local mechanisms have been suggested, but all lack anatomical and clinical evidence of their real involvement. These include local pressure (Ivy, Grossman & Bachrach, 1950), effect of a 'pyloric jet' (Kirk, 1968), islets of ectopic mucosa (Ivy *et al.* 1950; Spira, 1956), and the association of ulcers with the fundopyloric mucosal boundary and well defined muscle bundles (Oi, Ito & Kumagai, 1969).

The existence of a local vascular factor – always to be suspected when localisation is prominent – is supported by three pieces of evidence. Clinically, it is well known that pulling the stomach downwards and to the left at operation results in a patch of pallor on the anterior wall of the first 2.5 cm of the duodenum (known as 'Mayo's anaemic spot'). This pallor disappears on relaxing the tension (Kirk, 1968; Mayo, 1908). Anatomically, postmortem injection studies in man reveal a poor vascularity in the lesser curvature (Disse, 1903; Reeves, 1920; Jatrou, 1920; Hoffman & Nather, 1921; Berlet, 1923; Barlow, Bentley & Walder, 1951; Piasecki, 1974). Finally, it has been shown that in the human stomach there occur occasional patches of mucosa supplied by arteries of extramural origin which, after piercing the muscle, do not communicate with the submucous plexus, i.e. they are end-arteries. They are either single or in groups of two and three and collectively have a distribution which corresponds closely with the regions prone to chronic ulceration (Piasecki, 1974).

In animals, regional differences have hardly been studied. In dogs, there is a poor plexus in the lesser curvature (Piasecki, 1975) and in rats the antral region has finer vessels (Berg, 1946), but in other animals there is virtually no detailed study of the subject, the only information being available as a homogeneous description from textbooks. A study was therefore undertaken to fill this gap and specifically to seek an animal model of end-arteries.

#### MATERIALS AND METHODS

#### Materials

Adults of eight genera were used, and obtained under the MRC accreditation scheme. They comprised 8 ferrets (*Mustela putorius*, all male); 9 cats (*Felis domesticus*, 6 female, 3 male); 8 dogs (*Canis familiaris*, 6 male, 2 female); 15 swine (*Sus scrofa*, 8 female, 7 male); 11 primates (*Macacus rhesus*, 10 male, 1 female); 12 rats (*Rattus norvegicus*, 6 male, 6 female); 12 guinea-pigs (*Cavia porcellus*, 10 male, 2 female) and 14 rabbits (*Oryctolagus cuniculus*, 9 male, 5 female).

They were killed according to availability and as appropriate to the species. Cats, dogs and rabbits were killed by an overdose of barbiturate. Swine were killed by electric shock in an abattoir and exsanguinated; primates were exsanguinated under barbiturate anaesthesia; rats and guinea-pigs were killed by cervical dislocation and ferrets with carbon dioxide.

### Preparation and injection

- 1

د

The aorta or both coeliac and superior mesenteric arteries were cannulated and the vascular system washed out with normal heparinised saline until surface vessels appeared bloodless. In the smaller species this was done *in situ*, with the portal vein cut, but in swine and primates the abdominal organs had to be removed prior to perfusion.

Most specimens were then stored at 4 °C for 12–24 hours to allow postmortem spasm to disappear. However, quarantine regulations necessitated the immediate injection and fixation of the primates. This was also done to four of the seven cats in order to assess any major differences in results due to this time lapse. After refrigeration and thawing, the cannulated vessels were injected with contrast medium using a hand syringe with a sidearm connected to a manometer. Injection pressures were not allowed to exceed 100 mmHg and injection was stopped when arteriolar vessels on the surface of the stomach appeared filled. The field of injection was limited by clamping the superior mesenteric artery and the aorta. The contrast media used were either (1) 25 % Pelikan india ink in 5 % gelatin in distilled water, or (2) 50 % latex rubber (Hopkins & Williams) coloured with the manufacturers' dye, together with  $0.2 \, \text{m}$  sodium hydroxide or Sorensen's phosphate buffer.

Following injection, the stomach was removed together with its omenta and the organ fixed for 24 hours in 10% formalin. After fixation, the named arteries in the omenta were dissected out and their pattern and distribution recorded with drawings. The muscle layer was then dissected away leaving the submucosa and mucosa. During this dissection, the arteries piercing the muscularis externa were cut as they emerged from the deep surface of this layer. Great importance was paid to this feature because it was realised that some of the perforating vessels divided within the muscle, others divided later after reaching the submucosa. Some vessels that divided as they emerged from the muscle were teased away from the muscle, the parent trunk was cut and the division preserved intact with the submucosa.

The stomach was then opened along the anterior and posterior walls, the interior washed out and the pieces secured to card by pins. Specimens were then washed in running tap water for 18 hours and fixed again for a few days. They were then dehydrated and cleared by the method of Spalteholz, except that benzyl benzoate was used instead of benzene.

# Blood supply of the gastric mucosa

# Methods of observation

Transilluminated specimens floating in methyl salicylate were observed by stereomicroscopy and internal diameters of vessels were measured with an eyepiece micrometer. Vessels were frequently manipulated with fine forceps under direct vision in order to decide between junctions and overlap, and to determine their mobility. Mobility indicated that a vessel was in the loose submucosa, whilst immobile vessels were embedded in or were deep to the muscularis mucosae. The latter was found to resist being pierced by forceps.

The passage of vessels within the muscularis mucosae was confirmed histologically. For each species, ten blocks were cut out under direct microscopic observation. Each block contained a segment 1 cm long of a single anastomotic artery and included the part of its course which was immobile. Serial paraffin wax sections were cut at a thickness of 15  $\mu$ m. This thickness was necessary to prevent the gelatine-ink mixture from falling out of the section, and hence to preserve the vessel's identity. Sections were stained with haematoxylin and eosin and Masson's trichrome.

#### RESULTS

#### Gross observations

The opportunity was taken to dissect the named gastric arteries and assess their area of supply. This was done in eleven primates, twelve swine, seven ferrets, ten rabbits, nine cats, twelve guinea-pigs and twelve rats. The overall pattern was similar to that in man, but relative sizes of areas of distribution varied between species, as shown in Figure 1. The following were the most noteworthy features.

#### Relative dominance of the left gastric artery in guinea-pig, rat and rabbit

÷

2

Dominance was manifest at two sites, the greater curvature and the pylorus and the first part of the duodenum. In guinea-pigs, rats and rabbits the left gastric artery supplied a very much larger part of the stomach than in other species. The area was largest in rats and rabbits. In these species the left gastric artery was the exclusive supply to the central portion of the greater curvature (Fig. 1). In guinea-pigs, the artery did not extend to the greater curvature, but supplied most of the anterior and posterior walls, leaving only a narrow band to be supplied by the rami gastrici of the right gastroepiploic artery. In four species, i.e. guinea-pig, rat, rabbit and cat, the left gastric artery supplied the whole of the lesser curvature as far as the pylorus and its terminal branches continued across the pylorus into the first part of the duodenum.

# Absence of right gastric artery in the lesser curvature of guinea-pig, rat, rabbit and cat

In consequence of the extension of the left gastric artery into the duodenum in these species, the right gastric artery was very small and terminated by supplying the duodenum. It gave small branches to the lesser curvature in only 2 out of 10 rabbits, 2 out of 9 cats, 1 out of 12 rats and 2 out of 12 guinea-pigs (and 4 out of 12 swine).

# Dominance of right gastroepiploic artery in rat, and of the left in guinea-pig and rabbit

In the rat, the second largest artery after the left gastric was the right gastroepiploic which supplied half or more of the greater curvature, the left half of the





Fig. 1. Schematic representation of the distribution of the principal arteries supplying the stomach and first part of the duodenum in some common animals and in man. Named arteries: RG, right gastric; LG, left gastric; RG-E and LG-E, right and left gastroepiploic.

curvature belonging to left gastric territory. The fundus received a few small twigs from the splenic artery, which appeared to supplement the left gastric. In the rabbit, the left gastroepiploic was a large artery supplying the left half or more of the greater curvature. The right artery was quite small. In guinea-pigs, the left gastroepiploic dominated the whole of the curvature extending from spleen to pylorus in all specimens, into the duodenum in 2 out of 12 specimens, and anastomosed with a very small right gastroepiploic.

In summary, species showing a distribution almost identical to that in man were the rhesus monkey, dog, swine and ferret. The cat would have fitted into this category, but for the absence of a right gastric artery in the lesser curvature.

#### Submucous plexus

#### Gastroduodenal junction

In all species, there was an incomplete arterial ring around the circumference of the duodenum immediately distal to the pylorus. It was formed from branches of the right gastric artery above and from the right gastroepiploic artery below, which anastomosed with each other on the ventral and dorsal walls or on both. Branches from this anastomosis were given off distally along the duodenum, but also proximally into the pyloric muscle and along the submucosa of the canal. The latter branches characteristically streamed in parallel along the pyloric canal from the duodenum



Fig. 2. Gastroduodenal junction in the rabbit, showing pyloric arteries (PA) entering the duodenum and giving branches distally to the duodenum and proximally to the pyloric canal, the latter ending in the antrum. Note the continuity of the plexus.

medially into the gastric antrum, giving off mucosal arteries to the canal and antral mucosa. They anastomosed freely near their termination with gastric vessels (Fig. 2).

#### Poverty of submucous plexus in lesser curvature and antrum

For purposes of description, the lesser curvature was delineated as the area bounded by the attachments of the lesser omentum to the anterior and posterior walls of the stomach. In all species, this area was narrow at its middle portion and very wide at the antral region where it embraced more than half the circumference. Whilst the main branches of supply (vasa recta) followed the borders of the curvature at the peritoneal attachments, the central area received smaller branches which were embedded in omental fat and invisible on external inspection. After piercing the main muscle layer, these vasa recta gave radiating branches which formed the submucous plexus. Owing to considerable difficulty in obtaining complete injections of the antral area, observations on this part were based on five monkeys, seven cats, five ferrets, seven guinea-pigs, six rabbits, four rats and ten swine.

The existence of the well known submucous plexus lying between muscle and mucosa was confirmed. However, in the distal third of the lesser curvature and in the antrum, the plexus was composed of much finer vessels than in the fundus and body. Relative to the rest of the stomach, the reduction of the plexus varied between

2

26



Fig. 3(a-c). (a) Greater curvature, anterior and posterior walls of the rabbit stomach from body to antrum, showing the gradually diminishing plexus. (b) Detail of the plexus at the cardiac end of the lesser curvature. (c) Detail of the plexus at the antral end of the lesser curvature showing narrower vessels.

0.0

### Components of the submucous plexus embedded within the muscularis mucosae

The usual method of stereomicroscopic observation did not allow appreciation of the tissue in which the vessels lay. Only when individual arteries were moved with a fine needle under direct vision did it become clear that some parts of the vessels were mobile, whilst other parts of the same vessel were tethered because they were embedded in a firm layer whose surface could be 'palpated' with an exploratory needle. The immobile segments of vessels could not be moved without disrupting the firm layer. In order to demonstrate which, and how many vessels were fixed in this way, all the tissue superficial to the muscularis mucosae was removed by dissection, i.e. the loose submucosa containing mobile vessels was separated from the firm layer as a single sheet. Figure 4 shows a part of the stomach in a rabbit before and after removal of this layer, demonstrating that all the main anastomotic vessels were immobile; whilst Figure 5 shows that in a cat they were all mobile and were removed with the submucosa.

۰ ،

Selected vessels containing both mobile and fixed segments were excised together with the underlying mucosa; serial transverse sections showed that the immobility was due to the vessel being embedded within the muscularis mucosae (Fig. 6). Figure 7 gives a three dimensional impression of the arrangement in the rabbit. To avoid disrupting the vascular patterns, the majority of specimens were studied by moving the vessels with fine forceps. The inclusion of the plexus within the muscularis mucosae was approximately uniform in the various areas of each stomach, but varied considerably between species. In the dog, swine and ferret, the entire plexus was within the submucosa. However, in the rat, monkey and rabbit, the whole plexus was within or deep to the muscularis mucosae. In the guinea-pig and cat an intermediate pattern existed. In the guinea-pig, the majority of large and small anastomoses were in the muscularis mucosae, but there was also a scanty anastomosis of large vessels in the submucosa. In the cat all large anastomoses,  $30-80 \ \mu m$  in diameter, were in the submucosa, and there was a well defined rich plexus of smaller vessels, 20–70  $\mu$ m in diameter, deep to the muscularis mucosae. This was clearly visible in the separated layers depicted in Figure 5. In the other species, most plexiform arrangements were in one or other layer. No difference was seen between vessels in cats injected immediately after death and those injected after fixation in formalin.

# Mucosal arteries

Arising from the intramural plexus, mucosal arteries ramified in the mucous membrane (Fig. 8). Since in dog, swine, ferret and cat the main plexus lay in the submucosa, mucosal arteries had to pierce the whole thickness of the muscularis mucosae on their way to the mucosa. However, in guinea-pig, rhesus monkey, rat and rabbit, in which the main plexus lay within or deep to the muscularis, mucosal arteries passed through little or no muscularis. In the cat, the main plexus was in the submucosa, but mucosal arteries arose from a definite second plexus of smaller vessels deep to the muscularis. These two patterns in origin of mucosal arteries are shown in Figure 9. On reaching the mucosa, each mucosal artery divided in a stellate manner into branches which ramified in the mucosa, usually without anastomosing with each other, an appearance which suggested that mucosal arteries were end-arteries





Fig. 5(a-b). (a) Part of the anterior wall of the cat stomach. (b) Same part and orientation of the stomach as in the upper picture, but after removal of the submucosa and its contained vessels. The larger components of the plexus have been removed. A second plexus of fine vessels remains in a deeper layer. This can be clearly seen in the area in the window.

(Fig. 8). However, the existence of capillary communications between adjacent mucosal arteries could not be excluded with the techniques used in this study.

#### Size of area supplied by a single mucosal artery

In the best filled specimens, the area of mucosa supplied by a single mucosal artery was measured with an eyepiece graticule, since it was readily distinguishable from areas supplied by neighbouring arteries (Fig. 8). A total of 30 mucosal arteries was measured in each species, 10 taken at random in each of three areas, lesser curvature, mid-anterior wall and fundus (except in the rabbit, in which 50 mucosal arteries

Fig. 4(a-b). (a) Part of the anterior wall of the rabbit stomach. Six vasa recta enter along the upper edge of the picture and anastomose by many branches in the central area, with vasa recta entering the lower and side edges. (b) Same part of the stomach and same orientation as in the upper picture but after removal of the submucosa with its contained vessels. The vasa recta with their first branchings have been removed with the submucosa. All anastomotic arteries (in the central area) remain, showing that they are in a deeper layer.



Fig. 6 (a-h). Sections along the course of a single large (diameter 400  $\mu$ m) anastomosing artery from the rabbit stomach. Actual distance from (b) to (c) was 0.7 cm. A, artery; V, vein; MM, muscularis mucosae. (a) The artery lying in the submucosa; (b, c) ensheathed within the split muscularis mucosae; (d) beneath the muscularis mucosae. The odd appearance of the mucosa is due to the 15  $\mu$ m thickness of the section. (e-h) Sections along the course of a pair of smaller arteries (diameter 70  $\mu$ m). (e) The parent vessel in the submucosa. The distance between (f) and (g) is 0.5 cm.



Fig. 7. Three-dimensional impression of the concept of plexus vessels running within the muscularis mucosae.



Fig. 8. Rabbit gastric plexus showing mucosal arteries arising from the plexus, ramifying in the mucosa and supplying discrete, definable areas. P, plexus vessel; MA, mucosal artery.





Fig. 9(*a-b*). (a) Transverse section of the gastric wall in the dog (left) and the cat (right)  $\times$  25. (b) Stylised impression of the two systems. SM, submucosa; MM, muscularis mucosae; smp, submucous plexus; msl, muscle.

# Table 1. Areas $(mm^2 \times 10^{-2})$ of gastric mucosal surface supplied by a single mucosal artery in various animals

(Each value is a mean from 10 areas (except for the rabbit in which each value is a mean of 50 areas).)

	Lesser curvature	Antrum	Fundus	Combined
Rat	See	4·3 ± 1·8		4·3 ± 1·8
Cat	$5.9 \pm 3.6$	$2.7 \pm 0.7$	$3.8 \pm 2.6$	$5.5 \pm 2.0$
Primate	$9.0 \pm 3.9$	$10.0 \pm 5.0$	$7.2 \pm 3.4$	$10.1 \pm 4.1$
Ferret	10.6± 4.3	$14.2 \pm 8.7$	$7.6 \pm 5.6$	$10.8 \pm 6.2$
Swine	24·7±15·9	51.0 + 60.7	11.5+ 6.3*	$29.1 \pm 27.7$
Guinea-pig	$26.6 \pm 14.1$	24.7 + 34.9	$41.6 \pm 28.7$	31.0 + 25.9
Rabbit	16·6±11·8	$47.8 \pm 33.4$	29.4 + 21.2	32.3 + 22.1
Dog	$165.0 \pm 89.3$	$35.9\pm15.3$	$138.5 \pm 97.5$	$113.1 \pm 67.4$
		* Diverticulum ve	ntriculi.	

were measured in each of the three areas). Results are shown in Table 1. In the cat, it was decided to consider the second plexus deep to the muscularis as giving origin to the mucosal arteries. This was justified because the vessels of the plexus were of greater diameter than were the branches to the mucosa. Although there was some inconstant variation between areas within one stomach, the following grouping of species emerged, each having a different order of magnitude.

Rat, cat, monkey and ferret, 0.05 to  $0.1 \text{ mm}^2$ ; swine, rabbit and guinea-pig,  $0.3 \text{ mm}^2$ ; dog,  $1.1 \text{ mm}^2$ . The comparable figure measured previously in human stomachs was  $6.8 \text{ mm}^2$  (Piasecki, 1975).

33



Fig. 10(*a-b*). (a) Guinea-pig greater curvature, with a crop of arteries showing little or no connection with the plexus (square).  $\times 5$ . (b) Enlargement of square in (a).  $\times 12$ .



Fig. 11. An apparent end-artery in the rabbit.

The large standard deviation in Table 1 indicates a great range of sizes, and these large and small areas frequently existed side by side. In Figure 8 the right upper quadrant is occupied by one such large mucosal artery.

#### Occasional giant mucosal arteries as end-arteries of extra- or intramuscular origin

Since the principal purpose of this study was to seek end-artery systems which might supply discrete areas of mucosa, with no collateral source of supply, every visible mucosal artery in each stomach was examined for this purpose. A few isolated arteries of this type were found in guinea-pig and rabbit, and one guinea-pig had a group of these vessels on the greater curvature.

The criteria for defining an end-artery were that: (1) the stem of the artery originated from another vessel either outside the muscularis externa or within the muscle; (2) no connections were made with the intramural plexus during its passage through the submucosa and muscularis mucosae; (3) none of its branches in the mucosa anastomosed with neighbouring arteries. Although a large number of vessels almost satisfied these criteria, most failed to do so because they had a few anastomoses, 20  $\mu$ m in diameter, with neighbouring arteries. Such vessels were particularly numerous in the distal lesser curvature of rhesus monkey and swine, which had the poorest plexus in these areas and to a lesser extent in guinea-pig and rabbit.

However, unequivocal end-arteries were found in five out of twelve guinea-pigs and in three out of fourteen rabbits. In one guinea-pig, there was a group of eight such vessels in the middle of the greater curvature (Fig. 10). Their diameters ranged



Fig. 12. Another end-artery A. The artery B, which anastomosed at the arrow, is very close to A, because both arose from a common vessel within the muscularis propria.



Fig. 13. Illustrating the effect of lack of connections of mucosal arteries of extra- or intramural origin. Spasm of muscle at A would not occlude blood flow in the mucosal artery (a) because of the collateral circulation. Spasm at B involving a mucosal artery arising within it or outside the muscle might interfere with the circulation at (b).

from 80 to 150  $\mu$ m, and they supplied areas from 7 to 20 mm<sup>2</sup>. In five of these vessels, no anastomoses at all could be found (but three of them had one anastomosis each, 15 to 20  $\mu$ m in diameter). Four other guinea-pigs had one end-artery each, in the antrum. These vessels were 50 to 70  $\mu$ m in diameter, supplying areas 2.5–4 mm<sup>2</sup>. Each of the three rabbits had one end-artery in the distal lesser curvature; their diameters were 220, 100 and 80  $\mu$ m, with corresponding areas 5, 2.5 and 1 mm<sup>2</sup> (Figs. 11, 12). Figure 13 shows the difference between these end-arteries and the usual vessels. Comparison with the sizes of areas of 'normal' mucosal arteries shows that these end-arteries supplied areas some ten- to twentyfold larger than the vessels arising directly from the intramural plexus.

In monkeys and swine, some vessels in the lesser curvature appeared to be mucosal end-arteries, but the degree of injection was insufficient for a firm conclusion.

In dogs and cats, injections were satisfactory and not a single end-artery was found. In ferrets, although the extent of the injection was variable, it appeared unlikely that end-arteries were present.

â

á

ą

\*

#### DISCUSSION

In this study, relative vascularity has been assessed by visual observation and measurement of injected vessels with a micrometer eyepiece. Ideally vessels are best filled *in vivo*, but the consequent filling of capillaries and veins would have made study of arteries extremely difficult, hence the use of postmortem injection. In many injected specimens, the antral area filled either badly or not at all, or only the larger vessels were filled. Such areas were rejected on the grounds that there must have been smaller vessels in the area. Results are therefore given only for those specimens in which it was considered that the great majority of pre-capillary vessels were visibly filled. The cause of poor filling in the antral area is not known; it was experienced here in all species, and has been noted by previous workers. It appears that these vessels are different from those in the rest of the stomach, either because of smaller calibre or because they are subject to selective constriction which is maintained after death.

Past reports referring to the gastric plexus in animals are mostly incidental to other interests. Leone (1948) published a simple homogeneous description applicable to cat, dog and rabbit. The vessels in swine were described briefly by Trixl (1973) and by Busch (1973). Both authors reported a submucous plexus with primary and secondary anastomoses based on vessel diameter. Saito *et al.* (1974) noted that in swine there was a second smaller plexus in the mucosa, formed by branches of mucosal arteries. Only two reports exist of the intramural vessels in the rabbit, an incidental note by de Busscher (1948) in which he described glomerulus-like arteriovenous anastomoses, and by Bulkley, Goldman & Li (1970), who described a submucosal and mucosal plexus. The above reports do not give any indication as to regional variations.

In the dog and rat the situation is clearer. In the dog, Piasecki (1975) reported a rich submucous plexus in all areas, and demonstrated that the vessels in the lesser curvature were fewer and of smaller calibre. The rat has been the subject of many injection and ligation studies (Berg, 1946; Basu Mallik, 1955; Arabehety, Dolcini & Gray, 1959; Schnitzlein, 1957; Nylander & Olerud, 1961; Oka, 1970; Guth & Rosenberg, 1972; Hase & Moss, 1973). All agree that the two main arteries are the left gastric and right gastroepiploic, the remaining sources of supply being relatively small and insignificant. However, opinions differ as to whether or not these two main

# Blood supply of the gastric mucosa

arteries anastomose with each other. Berg claimed the anastomosis is poor, whilst Schnitzlein concluded that it is good. Berg also commented that the vessels in the antrum are fewer than in the fundus. Various ligation procedures performed *in vivo* by some of these workers resulted in an incidence of ulceration varying for 25 to 75 %, of which the vast majority eventually healed. Unfortunately, documentation is insufficient to determine whether necrosis was due to an absence of anastomoses or to other factors. In the present study, the anastomosis between left gastric and right gastroepiploic arteries over the anterior and posterior walls was found to be as good in the rat as in any other species, suggesting that post-ligation necrosis is more likely to be due to other causes.

The existence of a reduced vascularity in the lesser curvature in man has been thought to represent a reduced blood supply to the mucosa. However, the mucosa in this region is visibly flatter and thinner than elsewhere (Kirk, 1982), so the reduced vascularity may merely reflect a lower circulatory demand. A similar reduction has been demonstrated in the species studied here. This feature, together with the fact that animals do not appear to suffer from chronic peptic ulceration (Spira, 1956; Ivy *et al.* 1950), seems to suggest that reduced vascularity does not contribute to ulceration. Indeed, a reduction in vascularity need not suggest insufficiency, which has not so far been demonstrated. This does not, however, exclude vascular features localised to the area of a peptic ulcer, which could be by virtue of pattern rather than density (Piasecki, 1971, 1977).

It is customary to think of the main intramural plexus of gastric arteries as lying in the submucosa. This is known to be true in man from numerous past studies and from observation during the operation of mucosal antrectomy. It has been shown here that it is also true in dog, cat, swine and ferret. However, in guinea-pig, monkey, rat and rabbit, the major components of the plexus are embedded in the muscularis mucosae and are therefore not in the submucosa. This remarkable arrangement suggests that in these species, the muscularis mucosae may play a special role in influencing blood flow within these vessels. It would seem at first sight that contraction of the muscularis mucosae could occlude blood flow in vessels embedded in it, which would defeat the traditionally accepted purpose of a vascular plexus. The role of the muscularis mucosae has not yet been clearly ascertained, but this new finding merits further investigation.

There appear to be no previous reports describing communications between mucosal arteries within the mucosa in animals, nor of the size of mucosal areas supplied by mucosal arteries. The sizes of these areas may be relevant to the sizes of lesions suspected of being of ischaemic origin. Variations are great, ranging from  $0.04 \text{ mm}^2$  in the cat to  $1.1 \text{ mm}^2$  in the dog. These should be viewed in comparison with man, where the corresponding figure is  $6 \text{ mm}^2$ . However, the standard deviations show individual variation in species, the range in dog stomach being from  $0.2 \text{ mm}^2$ .

The pattern exemplified by the cat presents a problem in defining the vessel passing horizontally deep to the muscularis mucosae. Should it be called a mucosal artery, even though it takes part in an anastomosis, or should this term be reserved for the small branches which penetrate to the mucosa? The fact that the anastomosing artery is larger than its branches to the mucosa, even at the narrowest part of the anastomosis, suggests that the vessel is not immediately nutrient but that its purpose is the same as that of the submucous plexus in general, i.e. to distribute blood to mucosal branches from either direction and equalise pressures. The term mucosal artery

#### C. PIASECKI AND C. WYATT

should thus be limited to the functional sense of including only those arteries which directly feed the mucosa. This classification was adopted in this work. There remains, however, the problem of borderline vessels, mostly in rat and monkey, where it is difficult to assign the vessels to distinct categories according to the criterion of which vessel is the larger. If future studies show that in the cat the small mucosal arteries arising from the deep plexus are all end-arteries, this will form a convenient basis for a definitive nomenclature.

The occasional finding of isolated mucosal end-arteries of extramural origin in guinea-pig and rabbit is of interest, since similar end-arteries have been reported in human stomachs in the ulcer-bearing areas (Piasecki, 1974). If these vessels in animals can be recognised *in vivo*, it may be possible to ligate them and follow the course of changes in blood flow in the underlying mucosa. Such further work would throw light on the theory that occlusion of such vessels in man is the principal initiating factor in peptic ulceration (Piasecki, 1977).

#### SUMMARY

έ,

ő

**7** 

The form of the gastric arterial supply to the mucosa has been studied in dog, swine, ferret, cat, guinea-pig, rabbit and rhesus monkey. In all these species, the bore of vessels in the submucous plexus diminished from body to pylorus, though this was most marked in the guinea-pig and rabbit. The plexus was also continuous across the pylorus with duodenal vessels. Thus the well known poverty of vascularity in distal parts of the human stomach is shared by other species and is unlikely to be a contributory factor to the initiation of peptic ulcer, a disease limited to man.

In dog, swine, ferret and cat, as in man, the primary (largest) and secondary (smaller) components of the plexus lay entirely in the submucosa. In the cat, there was a secondary plexus of much smaller vessels deep to the muscularis mucosae. In the guinea-pig, rat, rabbit and monkey, both plexuses were mostly embedded within the muscularis mucosae. As a result, mucosal arteries had two modes of origin: (a) the first, in which they did not pass through the muscularis mucosae as exemplified in the cat, and (b) the second, where they did pass through muscularis mucosae as exemplified by the dog, ferret and swine; in other species, they passed through part of the muscularis mucosae. Areas of mucosa supplied by a single mucosal artery were measured, and ranged widely from the smallest in the cat to the largest in the dog. These features do not seem to have been reported previously, and may be associated with as yet undiscovered functional mechanisms of the muscularis mucosae.

Mucosal arteries of extramural origin were found to occur occasionally in the guinea-pig and rabbit, and hence these may provide an experimental model of the pattern existing in man.

The authors wish to thank Professor R. E. M. Bowden for her encouragement, and the Royal Free Hospital School of Medicine for a junior research fellowship held by Colin Wyatt for the duration of this work.

ĩ

#### REFERENCES

- ARABEHETY, J. T., DOLCINI, H. & GRAY, S. J. (1959). Sympathetic influences on the circulation of the gastric mucosa in the rat. American Journal of Physiology 197, 915–922.
- BARLOW, T. E., BENTLEY, F. H. & WALDER, D. N. (1951). Arteries, veins and arteriovenous anastomoses in the human stomach. Surgery, Gynecology and Obstetrics 93, 657-671.
- BASU MALLIK, K. C. (1955). Experimental study of the pathogenesis of gastric ulceration produced by pilocarpine. Journal of Pathology and Bacteriology 70, 315-324.
- BERG, B. N. (1946). Vascular changes in the mucosa in experimental nutritional gastritis. *Gastroenterology* 7, 340–354.
- BERLET, K. (1923/24). Über die Arterien des menschlichen Magens und ihre Beziehungen zur Aetiologie und Pathogenese des Magengeschwurs. Zeitschrift für Pathologie 30, 472-489.
- BULKLEY, G., GOLDMAN, H. & TRENCIS LI (1970). Gastric microcirculatory changes in haemorrhagic shock. Surgical Forum 21, 17-30.
- BUSCH, C. (1973). Gefassversorgung der Magenwand von Schwein. Veterinary Medicine Inaugural Dissertation, Munich.
- DE BUSSCHER, G. (1948). Les anastomoses artério-veineuses de l'estomac. Acta neerlandica morphologica 6, 87-105.
- DISSE, H. (1903/4). Über die Blutgefasse der menschlichen Magenschleimhaut, besonders über die Arterien derselben. Archiv für mikroskopische Anatomie und Entwicklungsmechanik 63, 512-531.
- GUTH, P. H. & ROSENBERG, A. (1972). In vivo study of gastric micro-circulation. American Journal of Digestive Diseases 17, 391-398.
- HASE, T. & Moss, B. J. (1973). Microvascular changes in the gastric mucosa in the development of stress ulcers in rats. *Gastroenterology* **65**, 224–234.
- HOFFMAN, L. & NATHER, K. (1921). Zur Anatomie der Magenarterien. Archiv für klinische Chirurgie 115, 650-671.
- Ivy, A. C., GROSSMAN, M. I. & BACHRACH, W. H. (1950). In *Peptic Ulcer*, ch. 3, Incidence of ulcer in lower animals, pp. 46–52. London: Churchill.
- JATROU, S. (1920). Ueber die arterielle Versorgung des Magens und ihre Beziehung zum Ulcus ventriculi. Deutsche Zeitschrift für Chirurgie 159, 196–223.
- KIRK, R. M, (1968). Site and localisation of duodenal ulcers: a study at operation, Gut 9, 414-419.
- KIRK, R. M. (1982). Factors determining the site of chronic gastroduodenal ulcers. *Hepatogastroenterology* 29, 75–85.
- LEONE, V. (1948). Osservazione sulla vascolarizzazione sanguigna dello stomaco. Archivio italiano di anatomia e di embriologia 53, 264–286.
- MAYO, W. J. (1908). Anaemic spot on the duodenum. Surgery, Gynecology and Obstetrics 6, 600.
- NYLANDER, G. & OLERUD, S. (1961). The vascular pattern of the gastric mucosa of the rat following vagotomy. Surgery, Gynecology and Obstetrics 112, 475-480.
- OI, M., ITO, Y. & KUMAGAI, F. (1969). A possible dual control system in the origin of peptic ulcer. A study on ulcer location as affected by mucosa and musculature. *Gastroenterology* 57, 280–293.
- OKA, S. (1970). The microcirculation of the gastro-intestinal mucosa and blood vascular system. Saishin Igaku 25, 1705–1713.
- PIASECKI, C. (1971). A possible vascular factor in the aetiology of duodenal ulceration. *British Journal of* Surgery 58, 660-662.
- PIASECKI, C. (1974). The blood supply to the human gastro-intestinal mucosa, with special reference to the ulcer bearing areas. *Journal of Anatomy* **118**, 295–335.
- PIASECKI, C. (1975). Observations on the sub-mucous plexus and mucosal arteries of the dog's stomach and first part of the duodenum. *Journal of Anatomy* 119, 133-148.
- PIASECKI, C. (1977). Role of ischaemia in the initiation of peptic ulcer. Annals of the Royal College of Surgeons of England 59, 476-478.
- REEVES, T. D. (1920). A study of the arteries supplying the stomach and duodenum and their relation to ulcer. Surgery, Gynecology and Obstetrics 30, 374-385.
- SAITO, I., MURAKAMI, T., ASHIZAWA, H., NOSAKA, D., OTSUKA, H., KURODA, H., SHINJO, T. & HAMANA, K. (1974). Studies on esophagogastric ulcers in swine. II. Blood supply to the normal gastric wall of swine. Bulletin of the Faculty of Agriculture, University of Miyazaki 21, 201–209.
- SCHNITZLEIN, H. M. (1957). The regulation of blood flow through the stomach of the rat. Anatomical Record 127, 735-748.
- SPIRA, J. (1956). In Gastro-duodenal Ulcer, ch. 21, Incidence of peptic ulcer, p. 311. London: Butterworth. TRIXL, H. (1973). Angioarchitecture of stomach of Göttingen miniature pig. Veterinary Medicine Inaugural Dissertation, Munich.