NEUROPHARMACOLOGICAL INVESTIGATIONS INTO THE MECHANISMS OF EMESIS CAUSED BY CYTOTOXIC DRUGS AND RADIATION

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A thesis submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy within the Faculty of Clinical Medicine of the University of Oxford
December 1988

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This work is lovingly dedicated to the
memory of my father, who, long ago,
awakened my interest in the natural world.

SIDNEY DAVIS

28 September 1913 - 24 May 1984

"There must be a beginning of any great
matter, but the continuing unto the end
until it be thoroughly finished yields
the true glory."

Dispatch to Sir Francis Walsingham, 17 May 1587
Navy Records Society, Vol. XI (1898), p.134

Sir Francis Drake 1540?-1596

ABSTRACT

Neuropharmacological Investigations into the Mechanisms of Emesis caused by Cytotoxic Drugs and Radiation

Submitted by Christopher John Davis
Corpus Christi College, Oxford
Michaelmas 1988
for the degree of Doctor of Philosophy

The aims of the project were to determine whether the ferret could provide an animal model for the study of emetic mechanisms in man and to investigate the mechanism of vomiting induced by cytotoxic drugs and radiation. The literature was reviewed and areas for experimental investigation identified. The ferret was shown to be emetically sensitive to a wide range of agents; e.g. copper sulphate, ipecacuanha, cisplatin, emetine, mustine, diacetoxyscirpinol, cycloheximide and X-radiation. Subsequently a detailed investigation of cytotoxic and radiation-induced vomiting was carried out using section of the abdominal vagus and greater splanchnic nerves, pharmacological interventions, (using metoclopramide, domperidone, BRL 24924, BRL 43694, cisapride) and 2-deoxyglucose (2-DG) autoradiography.

Neuropharmacological and autoradiographic studies revealed the following:

(a) Dopamine does not play a major part in the emetic mechanism of the ferret except in the case of apomorphine.

(b) 5-hydroxytryptamine (5-HT) plays a role in the genesis of cytotoxic and radiation-induced emesis as a range of drugs with 5-HT_3 receptor antagonist activity are effective anti-emetics. These effects were paralleled by abdominal vagotomy, implicating the gut as a site of action.

(c) The emetic response to 200cGy of X-radiation was abolished by both vagotomy and 5-HT_3 antagonists. In contrast, at 800cGy, although emesis was again virtually abolished by 5-HT_3 antagonists, vagotomy caused only a partial reduction. This discrepancy implies the existence of an additional emetic pathway revealed by the effect of vagotomy combined with high dose radiation (800cGy).

(d) 2-DG autoradiography showed that medullary structures previously assumed to be involved in the emetic reflex increased their metabolic activity in response to cytotoxic drugs and radiation. This study provides the first evidence that abdominal vagal afferents, previously shown to project to the Area Postrema have functional effects.

The discussion reviews the results in the ferret and critically assesses its suitability for emetic studies in the light of the literature on other animal models. The mechanisms of intragastric, cytotoxic and radiation-induced vomiting are discussed with reference to the experimental results and previous studies. It is suggested that in the ferret radiation and cytotoxic drugs cause vomiting by activation of abdominal vagal afferents via the release of 5-HT from the gut.

CONTENTS

CHAPTER 2

MATERIALS AND METHODS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

Inevitably, during the course of an extensive study such as this, one garners support and on occasions technical assistance from a variety of individuals and organisations. It is now my great pleasure to be able to thank all those who have sustained me with a helping hand or an encouraging word.

Without the moral and financial support of the Royal Navy, initiated by Surgeon Vice Admiral Sir John Harrison, formerly Medical Director General (Naval), and continued by Surgeon Vice Admiral Godfrey Milton-Thompson, Surgeon General of the Armed Forces, my studies would never have been started let alone taken to completion.

I would like first of course to thank my supervisor, Professor David Grahame-Smith, Rhodes Professor of Clinical Pharmacology, for giving me the opportunity of studying in the Department of Clinical Pharmacology and for his forbearance in overseeing the project. I would also like to thank Dr. Frank Beswick of the MOD(PE) for having sufficient faith in the project to provide the degree of financial support necessary to ensure its viability.

To Miss Lynn Maskell go my sincere thanks for her expert technical assistance especially in the area of animal care where her skill in handling the ferrets proved particularly valuable. Of Dr. Brian Jones my current superior at MOD headquarters I would like to make special mention because I cannot repay him and his senior management who have, in the face of the extreme pressures on manpower in Whitehall, given me that most valuable of all earthly commodities, time.

To Dr. Paul Andrews of the Department of Physiology, St. George's Hospital Medical School, University of London goes my profound gratitude. Without his guidance, infectious enthusiasm for experimenting and insistence on rigorous scientific attitudes I should have learned less and not laughed half so much. I would also like to thank Dr. Paul Kelly lately of the Department of Physiology, University College, Cardiff for giving so generously of his time and providing me with sound advice on the setting up of the 2-deoxyglucose autoradiography technique in the department.

It is appropriate also to thank Beechams Pharmaceuticals for their timely and generous provision of the experimental drugs, BRL 24924 and 43694, and especially Dr. Gareth Sanger and Wesley Miner for their co-operation.

I should also like to thank the Director and staff of the CRC Normal Tissue Radiobiology Research Unit, Churchill Hospital the University and NHS Departments of Radiology, the Quantitation Laboratory, Welsh National School of Medicine, Kontron Electronics (UK), the Radcliffe Science, Bodleian, Janssen, Cairns, RAMC and C.D.E. Libraries, the Medical School Animal House, John Radcliffe Hospital, The Physiological Laboratory Oxford and the Department of Anatomy, University of Sheffield for the contributions to specific parts of the study. To Mrs. Julian Cole and Mr. Nick White of the Department of Medical Illustration go my special thanks for the excellent artwork and photography in this thesis.

On a purely personal note, for which I make no apology, I would like to take space to remember the encouragement and support given by colleagues, friends and family over these past 5 years, in particular, Dr. Kent Harding, Dr. Ron Leslie, Dr. Bob Young, Dr. Jan Hawthorn, Professor Claudio Cuello, Mr. Graham Ross, Miss Sharon Bingham, Dr. Nigel Vincent, Miss Corinne Hayes, Mrs. Tamsin Dunningham, Miss Jean Waddup, Norland Nurses Sally Hayden and Sally Hughes, Dr. Ron Gleadle, Mr. Leslie Stump, Professor Tony Smith, Mrs. Elizabeth Wincott, Mr. John Rose, Mr. Peter Bateman, Dr. Guy Goodwin, Mr. Brian Cole, Dr. Nigel Lawes, Dr. Donald Wild, Hamish Killip and not forgetting my Mother Mrs. Ivy Davis and my Sister Mrs. Lorraine Eastcott.

My long-suffering typist, Mrs. Johnson, has persevered through ill-health, never quite giving up on me or the monumental task that 'writing-up' has seemed at times and deserves my heartfelt appreciation; thankyou 'Pat'.

Finally, what can any man say to his family who have over a long period put up with innumerable absences and much undeserved neglect all in the quest of someone elses goal. To my dear Wife Louise, and our children, Neil, Samuel, Hannah and Emily I owe a great debt of gratitude which I shall repay with interest - from the bottom of my heart I thank you. Yes, Hannah, as you said, I will be able to come out to play, now that the D.Phil is finished!

PUBLICATIONS

The following publications resulted from the work presented in this thesis and from associated studies carried out in Oxford.

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Kucharczyk, J. and Stewart, D.
CRC Press Boca Raton, Florida, U.S.A. (In press)

Andrews, P.L.R., Davis, C.J. and Hawthorn, J. (1988)
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J. Physiol. (In preparation)

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Proceedings of the Satellite Symposium of the Society for Neuroscience; Nausea and Vomiting : A Multidisciplinary Perspective. Ottawa, Nov. 1988 A.13 (In press)

GLOSSARY OF TERMS AND ABBREVIATIONS

ACh	- acetylcholine
ACTH	- adrenocorticotrophic hormone
AP	- area postrema
ARS	- acute radiation syndrome
ATP	- adenosine triphosphate
^{14}C	- ^{14}Carbon radioisotope
CCK	- cholecystokynin
cGy	- centiGray - 10^{-2} Gray
Ci	- Curie
CNS	- central nervous system
^{60}Co	- ^{60}Cobalt radioisotope
Cp	- concentration of glucose in the plasma over the experimental period
Cp*	- concentration of 2-DG in the plasma over the experimental period
^{137}Cs	- ^{137}Caesium radioisotope
CTA	- conditioned taste aversion
CTZ	- chemoreceptor trigger zone
χ^2	- Chi-square, a measure of the discrepancy between the observed frequency of events and their expected frequency
2-DG	- 2-deoxy-D-glucose
2-DG-6-P	- 2-deoxy-D-glucose-6-phosphate
DMVN(X)	- dorsal motor nucleus of the vagal nerve
DTIC	- Dacarbazine
DVC	- dorsal vagal complex
GABA	- gamma-amino-butyric acid
GSN	- greater splanchnic nerves
HCG	- human chorionic gonadotrophin
5-HT	- 5-hydroxytryptamine, serotonin
i.a.	- intra-arterial
i.c.v.	- intracerebroventricular
i.m.	- intramuscular
i.p.	- intraperitoneal
i.v.	- intravenous

K^+	- potassium ions
K_1^*	- Kinetic rate constant for movement of 2-DG into the CNS
K_2^*	- Kinetic rate constant for movement of 2-DG out of the CNS
K_3^*	- Kinetic rate constant for phosphorylation of 2-DG
kV	- kiloVolt - 10^3 volts
mg%	- concentration in mg of substance per 100ml of water
V	- mesencephalic nucleus of the Trigeminal nerve
NA	- nucleus ambiguous
NANC	- non-adrenergic non-cholinergic
nCi	- nano Curie
NTS	- nucleus tractus solitarii
O.D.	- optical density
p	- the probility of the occurrence of an event in a single trial
PCRF	- parvicellular reticular formation
pixel	- picture point
p.o.	- by mouth
PYR	- pyramidal tracts
PYY	- peptide YY
RF	- reticular formation
s.c.	- subcutaneous
SD	- standard deviation; the square root of the variance, which is the average of the squared deviations from the mean
SE	- standard error; the S.D. of the sampling distribution of a statistic
SNG	- subnucleus gelatinosus
SP	- substance P
SPX	- section of the greater splanchnic nerves, "splanchnectomy"
ST	- solitary tract
TRH	- thyroid releasing hormone
VC	- vomiting centre
Vg	- vagus nerve (X)
VIP	- vasoactive intestinal polypeptide
XII	- nucleus of the hypoglossal nerve

CORRIGENDA

Page	Paragraph	Line	Change
i	1.3.2	1	Change page number to read "78"
ii	-	2.4.1	"Ionising" to read "Ionizing"
ii	2.2.1	1	Change paragraph number to read "2.2.1"
v	-	4.5	"adminisitration" to read "administration"
vii	Table 21	2	sp "postrema"
x	Fig 16	3	"Administed" to read "Administered"
x	Fig 19	2	"administered" to read "Administered"
xi	Fig 30	2	"Administrid" to read "Administered"
xi	Fig 25	3	"Diacetoxyscirpinol induced" to read "Diacetoxyscirpinol-induced"
xii	Fig 41	3	"20" to read "200"
xiv	2	4	"Godfrey" to read "Sir Godfrey"
xviii	-	CCk	"cholecystokynin" to read "cholescystokinin"
xviii	-	NA	"N Ambiguous" to read "N. Ambiguus"
xix	-	NTS	"solutarii" to read "solitarii"
xix	-	P	"probility" to read "probability"
2	6	2	"causes" to read "courses"
2	(c)	1	Insert "phenomenon"
2	5	3	"alkadosis" to read "alkalosis"
3	1	4	After close of brackets delete "1981"
4	3	8	"Bond" to read "Bard"
5	1	5	"inreased" to read "increased"
5	1	10	Before "previously placed..." insert "with"
7	3	7	"repeatedly" to read "repeated"
8	3	7	"alergic" to read "allergic"
8	4	4	"humonal" to read "hormonal"
9	3	3	"side effect" to read "side-effect"
11	2	14	"encephalin" to read "enkephalin"
16	2	1	insert brackets to enclose "1923 & 1924"
18	1	11	"remains" to read "neurons"
21	2	3	"Ambiguous" to read "Ambiguus"
21		9	"of" to read "or"
25	1	5	Delete "receptive"
27	1	12	"pressure" to read "pressor""
27	1	14	"cadiovascular" to read "cardiovascular"
28	2	11	Add bracket before "Duvernoy"
34	1	3	"constractions" to read "contractions"
34	d	1	"hypothalamus" to read "Hypothalamus"
36	3	8	"flacid" to read "flaccid"
36	4	7	"verartrum" to read "veratrum"
Fig 0	Label on diagram		"psychegenic" to read "psychogenic"
36	4	5 & 6	"intrapleural pressures" to read "from the pleura"
38	2	12	"Ach" to read "ACh"
40	3	7	"desribed" to read "described"
40	3	8	"AP" to read "AP."
40	3	8	"thus" to read ".Thus"
42	3	9	"cotoxic" to read "cytotoxic"
42	3	6	"forthe" to read "for the"
43	3	6	"drugswhich" to read "drugs which"
47	1	20	"Haskeil" to read "Haskell"
47	2	4	"Syneck" to read "Synek"
47	3	4	"aemoebicidal" to read "amoebicidal"
48	1	11	"these" to read "the"
48	2	6	"ribrosome" to read "ribosome"
49	1	2	";" to read ","
49	2	9	"Graham-Smith" to read "Grahamè-Smith"
50	-	-	average latency for HN2 to read "120" not "12"
53	2	9	"hoever" to read "however"
54	2	10	"in" to read "is"
55	4	10	"Muscannic, Cholinergic" to read "Muscarinic Cholinergic"
63	1	12	"446 27" to read "446 ± 27"
63	1	17	"258 38.5" to read "258 ± 38.5"
Table 3	-	-	Move from following page 71 to page 55
71	4	1	"ferret as" to read "ferret"
77	1	8	"Korpas and Widdicombe (1983)" to read "(Korpas and Widdicombe 1983)"
92	1	17	"Barret" to read "Barrett"
93	2	5	"medoclopramide" to read "metoclopramide"
93	3	13	"latest" to read "latter"
93	3	16	"43695" to read "43694"
Faceplate	Chapter 2	9	"Musterious" to read "Mysterious"
95	1	2	"100mgm^{-1}" to read "100mgm^{-1})"
95	2	17	"1.65mm" to read "1.65mm)"
Fig 4	Legend	4	"central" to read "ventral"

Page	Paragraph	Line	Change
Fig 4	Diagram Label		"Lig" not labelled thus only as "L"
103	8	2	"0.154M" to read "0.154M,"
103	9	3	"mls" to read "ml"
107	5	3	"percentage" to read "percentage of the"
108	3	Title + 1	"ionising" to read "ionizing"
109	1	1	"ionising" to read "ionizing"
109	2	4	"ionising" to read "ionizing" (twice)
110	3	1	"ionising" to read "ionizing"
119	1	8	Delete bracket before "Hammer" and replace after "1984"
121	2	7	"was" to read "were"
121	2	5	"cannula" to read "cannulae"
123	2	15	"we" to read "were"
126	1	7	"20 m" to read "20μm"
127	2	6	"100 m" to read "100μm"
129	3 Schematic	(R) Para, 5	"temeplate" to read "template"
132	1	5	"control" to read "control or"
132	1	8	"canula" to read "cannula"
133	1	8	"canula" to read "cannula"
135	1	3	"wt/wt" to read "wt/vol"
136	2	3	euthatal line should be above in Para 1 below urethane
136	5	3	"student`s" to read "Students`"
136	5	4	"approprate" to read "appropriate"
Faceplate	Chapter 3, 2	3	"Schwabe" to read "Schwabe 1965"
144	1	2	"characterized" to read "characteristic"
151	2	2	"is" to read "are"
Table 7	-	-	"syrup" to read "Syrup"; "Ipecac" to read "Ipecac`"
Table 8	-	1	"5 ± 6" to read "7 ± 6"
152	Mini table	1	"10 ± 12" to read "12 ± 10"
153	Mini table	1	"10 ± 12" to read "12 ± 10"
154	6	7	Add "μ" to the number
Table 9	-	2	"5 ± 11" to read "5 ± 5"
Table 9	-	3	"6 ± 10" to read "6 ± 4"
Fig 19	-	Title	"4-10" to read "4-26"
155	2	2	Add "μ" to the number
155	3	2	Add "μ" to the number
155	Mini table	2	"6 ± 11" to read "6 ± 5"
Table 10	-	3	"1 ± 11" to read "1 ± 1"
Table 10	-	4	"1 ± 1.5" to read "1 ± 1"
Table 10	-	4	"6 ± 11" to read "6 ± 5"
Table 10	-	5	"9 ± 19" to read "9 ± 9"
Table 10	-	-	Dose should be in "μg"
Fig 21	Legend	Title	"Respose" to read "response"
Fig 22	Legend	1	"Cyclohexamide" to read "cycloheximide"
Fig 22	Legend	3	"to" to read "in"
159	4	Table	"Vgx" to read "VgX"
159	Minitable	Table	"1 ± 2" to read "1 ± 2^{**}_{***}"
159	Minitable	Table	"5 ± 6" to read "5 ± 6^{**}_{**}"
159	Mini table	2	"1 ± 2^{**}_{**}" to read "2 ± 1^{**}_{**}"
159	Mini table	2	"5 ± 6^{**}_{**}" to read "6 ± 5^{**}_{**}"
Fig 25	Legend	Title	add "μ" to number
Fig 28	Legend	-	Replace page correct way up
162	2nd Minitable	Table	Control n to read "=5/5"
162	2nd Minitable	Table	"VL=35.0" to read "35.8"
163	5	5	¼ of this line has dropped a space
164	4	5	"100 gkg^{-1}" to read "100μgkg^{-1}"
166	3	1	"vgx" to read "VgX"
166	Mini table	2	"1 ± 2" to read "2 ± 1"
166	Mini table	2	"14 ± 28" to read "14 ± 14"
167	Mini table	2	"2 ± 3" to read "3 ± 2"
167	Mini table	2	"9 ± 20" to read "9 ± 9"
167	Mini Table	-	"Groups" to read "Group"; insert "group" for add significant values
167	4	3	"diplayed" to read "displayed"
167	5	1	"10 gkg^{-1}" to read "10μgkg^{-1}"
167	6	1	Add "μ" to number
168	4	2	"n=8" to read "n=9"
168	Mini table	3	"5 ± 6" to read "6 ± 5"
168	Mini table	4	"2 ± 3" to read "3 ± 2"
172	Mini table	2	"3 ± 4" to read "4 ± 3"
Fig 39	Legend Title	2	"20" to read "200"
Fig 39	Legend Text	3	Move "(n=6 and 8 animals)" to end of Paragraph
Fig 39	Legend Text	4	Insert missing words "Mean Vomits and Retches"
Fig 39	Legend Text	1	"to" to read "of"
174	2	2	"pre-tested" to read "pre-treated"
174	1	Mini Table	"MCP" to read "MCP Group"$^{***}_{***}$
174	Mini table	2	"2 ± 5^{***}_{***}" to read "5 ± 2^{***}_{***}"
175	Mini table	2	"6 ± 8^{**}_{**}" to read "8 ± 6^{**}_{**}"

Page	Paragraph	Line	Change
175	Mini table	2	"2 ± 4" to read "4 ± 2***"
175	Mini table	2	"60.3 ± 4.2" to read "60.3 ± 4.3***"
175	1st Minitable	Mini Table	"60.3 ± 4.2" to read "60.3 ± 4.2***"
175	2nd Minitable	Mini Table	"2 ± 4" to read "2 ± 4***"
175	Last	Mini Table	"2 ± 4" to read "2 ± 4***"
Fig 42	Legend Title	2	"20" to read "200"
Fig 43	Legend Text	2	"Fig 43" to read "Fig 42"
176	Mini table	2	"7 ± 10" to read "10 ± 7***"
176	B	1	"200cGy" to read "200cGy"
176	4	2	Add "μ" to number
176	4	4	Insert "±"
Fig 44	Legend Title	3	"20" to read "200"
Fig 45	Legend Title	4	"g" to read "mg"
177	Mini table	2	"1 ± 5" to read "1 ± 1***"
179	1	5	"oesopagus" to read "oesophagus"
Fig 46	Legend, 1	1	Add "μ" to number
182	4	3	"hypoglycaemia" to read "hyperglycaemia"
Fig 47	Legend Title	6	Add "μ" to number
Fig 49	Colour Autorad	-	"sn" to read "nts"
183	2	6	Insert "±"
184	1	12	"Fig 51" to read "Fig 50"
184	2	2	Insert "±"
Table 14	-	-	Control Group "n" to read "6" [not "4"]
Fig 52	Legend	3	To read "DMVN"
Fig 52	-	2	To read "NTS"
Fig 52	Diagram	-	3 significance marks (*) on Xray vs AP bar
Fig 52	Legend Title	6	"x irradiation" to read "X-irradiation"
188	1	6	"lesioning" to read "lesioning,"
190	1	4	Author's name to read "Tuor"
Table 15	-	1	Apomorphine units 50 $^{-1}$50, 20-3 and 70 to read μgkg^{-1}
Table 15	-	1	Insert "μ" before numbers (three times)
Table 15	-	3	Insert "μ" before numbers (twice)
Table 15	-	4	Insert Footnote to "4mgkg^{-1}" column 2.1 this dose is "i.a."
Table 15	-	5	Insert "μ" before
Table 15	-	8	Insert "μ" before number
Table 15	-	9	Insert "μ" before number
191	1	2	"24mg" to read "25mg"
191	1	3	"g" to read "mg"
191	1	11	"2.5 g" to read "2.5μg"
191	1	12	"40 g" to read "40μg"
191	1	14	"30 g" to read "30μg"
191	1	16	"50 g" to read "50μg"; and "140 g" to read "140μg"
191	1	20	"10 g" to read "10μg"
192	1	4	Insert "μ" before number
192	1	5	Insert "μ" before number
192	1	7	Insert "μ" before number
192	1	12	Insert "μ" before number
192	1	15	Insert "μ" before number
192	2	3	Insert "μ" before number and "±" to read "4.8 ± 3.6"
193	1	3	Insert "μ" before number
193	2	4	Insert "μ" before number
194	1	1	Insert "μ" before number
194	2	5	"1976" to read "1976)"
195	1	11	"1987" to read "1987)"
196	3	7	"affectof" to read "affect of"
196	3	8	"." to read ","
196	3	12	Correct units to read "mg" and "kg
197	1	3	Insert "±" between numbers
197	1	4	"ipecec" to read "ipecac"
197	1	6	Insert missing "±"
197	3	3	Insert "≈" before number
201	1	4	"200 mgkg" to read "200mgkg^{-1}"
201	2	1	Insert "dose" after "therapeutic"
201	2	1	Insert "μ" before number
201	3	6	Insert "μ" before number and "500 g" to read "500μgkg^{-1}"
201	3	11	Insert "≈" before number
202	1	4	Insert "μ" before number and "i.c.v" to read "i.c.v."
202	2	12	Insert "±" before number
202	2	14	Insert "±" before number
203	1	3	Insert "±" before number
203	1	4	Insert "±" before number
203	2	2	Insert "μ" before number
203	2	6	Insert "±" before number
203	4	5	Insert "±" before number
206	2	3	Insert "≈" before number (twice)
206	2	4	Insert "≈" before number

Page	Paragraph	Line	Change
206	2	5	Insert "≈" before number
207	2	3	Insert "≈" before number
207	2	4	Insert "≈" before number (three times)
Table 18	Title	-	"X-radiation" to read "Radiation"
212	2	7	"completly" to read "complete"
213	1	7	Insert "β"
214	1	13	"v.s." to read "vs"
214	3	2	"glucoseand" to read "glucose and"
215	1	5	Delete line beginning "In ..... emesis.""
225	2	10	"mayhave" to read "may have"
227	2	2	Insert "and" after ")" before "when"
231	1	5	Insert "±"
231	1	7	"emetic" to read "radioemetic"
231	1	11	Insert "±" (twice)
231	1	13	Insert "±"
231	1	22	Delete "this" to read "these"
233	1	4	Insert "±"
233	1	5	Insert "±"
237	2	10	"ememtic" to read "emetic"
238	1	1	"verartrum" to read "veratrum"
245	2	19	Insert "μ" before number
245	2	21	Insert "μ" before number
248	1	6	"only" to read "only one"
249	2	8	Spelling to read "erythromycin"
254	3	1	Table number to read "21"
254	3	8	"prostrema" to read "postrema"
255	b	1	"dog" to read "monkey"
256	1	4	"cause" to read "causes"
257	3	4	Insert "μ" before number
257	3	7	Insert "μ" before number
257	3	9	Insert "μ" before number
270	1	15	Delete "sulphate;" to read
274	1	2	"emesis" to read "emesis in man"
284	1	4	"admininstered" to read "administered"
285	3	3	"systemsof" to read "systems of"
285	3	16	"sorotonim" to read "sertonin"
285	3	16	"aminobutyric acid" to read ".gamma-aminobutyric acid"
286	1	1	"here" to read "where"
287	1	10	"changes" to read "activity"
288	2	21	"establishes" to read "establish"
289	1	10	setion" to read "section"
290	1	12	"parocellular" to read "parvocellular"
290	1	12	"gelatinosu" to read "gelatinous"
293	1	7	"Borrison" to read "Borison"
293	1	19	"(1982)" to read "1982)"
293	1	19	"Kostreva" to read "(Kostreva"
299	1	12	"of" to read "or"
Fig 55	-	-	Reversed binding
303	2	11	"gastroparesis" to read "gastroparesis."
305	1	12	"20 m" to read "20μm"
306	2	3	"particles" to read "β particles"
306	2	6	"particles" to read "β particles"
306	2	22	"1984)" to read "1984)."
307	1	3	"particles" to read "β particles"
307	1	9	"quanching" to read "quenching"
307	2	4	Insert "μ" before number
308	2	2	"xray" to read "X-ray"
308	2	3	"particles" to read "β particles"
308	2	8	"AL" to read "All"
308	2	8	"con tact" to read "contact"
308	2	10	"particles" to read "β particles"
308	2	14	"particles" to read "β particles"
308	2	16	"scrath" to read "scratch"
Table 23	Column Titles	-	"125 Ci" to read "125μCi"
Table 23	2	-	Insert "β" (twice)
Table 23	3	-	Insert "β"
Table 23	5	-	Insert "≈"
309	1	6	"Table" to read "Table 23"
309	1	9	Insert "β"
309	1	11	Insert "μ" before "Ci"
309	2	6	Insert "β"
310	Titles	3	Delete "analysis"
310	1	3	"Tyhe" to read "The"
311	3	1	"consists" to read "consist"
311	3	a	"consist" to read "consist of"
311	(6) iii	3	"respons" to read "response"
Table 24	2	-	"= unity" to read ".lambda. = unity"

Page	Paragraph	Line	Change
319	3	4	"basis" to read "basic"
320	2	3	"photonconduction" to read "photoconduction"
321	3	3	"study" to read "stray"
322	2	6	"20" to read "200"
323	1	4	"particle" to read "particles"
323	2	9	Insert "†"
330	Bamburg	2	Sp "Microbial"
331	Beleslin	-	Sp "Strbac"
331	Boon	-	Sp "Ionizing"
335	Brogden	-	Sp "Pharmacokinetics"
336	Carpenter	1987 1, 3	Sp "Mechanisms"; Sp "Gastrointestinal"
336	Carpenter et al	-	Sp "Cyclic"; date of publication insert "(1988)"
338	Costal et al	1987 2	Sp "Pharmacol"
341	Fairweather	1986	"Nausea and Vomiting" to read "Nausea and Vomiting in Pregnancy"
341	Fozard	1987 2	"Tips" to read "TIPS"
342	Gaddum	1954 3	Sp "Chemothera"
342	Garcia	1961 2	Sp "Ionizing"
343	Gerstner	1956 2	Sp "Aortic"
343	Gilman	1985, 1 & 2	Sp "Gilman" {twice}
347	Hay	1979 1	Sp "metoclopramide"
347	Hay	1979 2	Sp "on:"
348	Holtzman	1968 3	Sp "paediatrics"
349	Hunt	1949 2	"2-chloromethyl" to read "($\beta\beta$) 2-chloromethyl"
349	Houck	1947 4	"chloroethyl" to read "($\beta\beta$) chloroethyl"
349	Hunt	1965 2	"membranes" to read "motivation"
349	Hunt	1965 1	Sp "Kimeldorf"
349	Ireland	1987 1	Sp "Tyers"
350	Johnson	1966 2	"emetation" to read "eructation"
350	Joss	1988 3	Insert Journal name: "Proc. ASCO 7:1128"
350	Kalia	1980 3	Sp "Laryngeal"
351	Kelly	1981 2	Sp "-2-deoxy-glucose"
352	King	1988 1	"ni" to read "in"
353	Laures	1988 1	"Neurocautery" to read "Neuranatomy"
354	Leslie	1984 2	Sp "Proc"
354	Liebermann	1970 1	Sp "Liebermann"
356	Manoguerra	1978 3	"of" to read "or"
356	McCarthy	1974 2	Sp "decerebrate"
356	McCarthy	1980 2	Sp "cannabinoids"
357	McCarthy	1984 2	"abstraction" to read "ablation"
364	Schaeppi	1973 3	Sp "Cis-Dichlorodiammineplatinum""
364	Savaki	1982 3	"Cinc" to read "Clin"
365	Schurig	1984 3	"complexes" to read "therapy"
365	Schwartz	1986 4	"mechaniics" to read "mechanisms""
367	Strabo	63BC, 1	Sp "Geographia"
369	Ueno	1968 2	Sp "Ehrlich"
369	Ueno	1968 3	Sp "Fusarium"

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

"'t'is profitable for a man that his stomach should nauseate and reject things that have a loathsome taste or smell."

Vulgarly Received Notion of Nature, London, Taylor, 1686

Robert Boyle 1627 - 1691

CHAPTER 1 - INTRODUCTION AND LITERATURE REVIEW

1.1 PREFACE

1.1.1 The Phenomenon of Vomiting in Man

"Vomiting is perhaps the most complex reflex response which makes extensive use of the autonomic and motor systems. Involved in the reflex activity are salivation, spasmodic respiratory movement effected by the antagonistic action of the inspiratory and expiratory musculature, gastrointestinal reactions of a specialised nature and posture characteristics of the head, body and appendages typically adapted to the process of expulsion of the gastric contents. In addition, there are psychological and cardiovascular effects which fit into the total integrated response" (Wang, 1980). It was Shih-Chun Wang who, in conjunction with Herbert Borison, published in 1950 the last major development in ideas concerning the mechanism of vomiting control i.e., that there existed an emetic chemosensory receptor area separate from the so-called medullary "vomiting centre" (V.C.) designated the chemoreceptor trigger zone (C.T.Z.) (Wang and Borison, 1950). Their major review of the physiology and pharmacology of vomiting published in 1953 began with the words "The vomiting act is one of the most primitive functions with which animals are endowed. The extreme variety of circumstances under which vomiting can occur defies description. It may follow simple overeating or signal approaching death. It often represents one of the chief signs of drug toxicity regardless of the route by which the drug is administered. In spite of its universal appearance and great clinical importance the nervous mechanism

of the vomiting act and the emetic action of many drugs are not well understood", (Borison and Wang, 1953).

Despite the passage of thirty-five years and the publication of five thousand scientific papers on this and related topics since Wang and Borison's paper, little had changed by the time that this project was begun in 1983. The problems that still face us include the fact that:-

(a) The system is very complex;

(b) The reflex is very often, in the clinical context, an inappropriate response to a toxic challenge, sometimes associated with its own morbidity;

(c) When the success rate of drugs against vomiting from all causes is reviewed (Milton-Thompson, 1979), emesis appears to remain relatively poorly amenable to pharmacological intervention.

Emesis constitutes a major complaint in a very large number of disorders (Grahame-Smith, 1986; Bouchier, 1985; Hanson and McCallum, 1985) which are created by virtue of physiological alterations in pathological states (see Table 1).

The spectrum of problems giving rise to vomiting is then very wide, ranging from normal pregnancy (Fairweather, 1986) at one end to the bizarre psychopathological aberration of erotic vomiting at the other (Stoller, 1982).

The complications and consequences of vomiting may include dehydration, sodium and potassium depletion, metabolic alkadosis, aspiration pneumonia, Mallory-Weiss syndrome, Boerhaave's syndrome, pneumolysis, cachexia, post-surgical bleeding and post-surgical wound disruption.

Importantly it has been noted on numerous occasions by clinical investigators that after one or more causes of

TABLE 1

The Causes of Vomiting in Man

1.	Acute Infections	Measles, pneumonia, urinary tract infections, viral, gastroenteritis, hepatitis, whooping cough.
2.	Gastrointestinal	Acute pancreatitis, alcoholic disease gastritis, appendicitis, cholecystitis, gastric cancer, gastric outflow obstruction (e.g. pyloric stenosis), peptic ulcer, post-gastrectomy syndrome, small and large bowel obstruction.
3.	Metabolic disorders	Addison's disease, diabetic ketoacidosis and hypoglycaemia, uraemia.
4.	Neurological disease	Autonomic neuropathies, diabetic neuropathy syndromes, labyrinthine and vestibular disease, meningitis, migraine, tumours, raised intracranial pressure.
5.	Psychogenic syndromes	Anorexia nervosa, Bulimia, fear, disgust.
6.	Drugs	Cancer chemotherapeutic cytotoxic drugs, aminophylline, aspirin, digoxin, iron salts, narcotic analgesics, erythromycin, oestrogens, sulphasalazine, sulphonamides, tetracycline, anaesthetic agents, poisons of many kinds.
7.	Miscellaneous	Ionizing radiation, pregnancy, motion, pain, congestive cardiac failure, food allergy, myocardial infarction, malignancy, circulatory syncope.

(Adapted from Grahame-Smith, 1986)

treatment for malignant tumours patients may actually refuse to continue with potentially life saving treatment because the side effects have become intolerable (e.g. Seigel and Longo, 1981, Laszlo, 1982)1981 With increasing efficacy of therapeutic regimes for the control of malignancy such side effects can become the 'rate-limiting' step in treatment.

Thus it was with such critical problems for treatment in mind that the subject of this thesis was chosen to be the cytotoxic and radiation-induced vomiting. Broadly speaking the aims of the project were to establish a new animal model for such studies, and to investigate in that model, the basic mechanisms of control of emesis caused by cancer chemotherapeutic cytotoxic drugs and X-radiation.

A short summary of the main features of three of the other major causes of vomiting not dealt with in this thesis will be summarised below, i.e. motion illness, vomiting of pregnancy, and post-anaesthetic vomiting.

1.1.2 Synopsis of research into vomiting secondary to motion, pregnancy and anaesthesia

1.1.2.1 Motion illness

Interest in motion sickness, extends back into the ancient world; Hippocrates noted the importance of motion on the induction of emesis and indeed the very word 'nausea' is derived from the ancient Greek for a ship 'naus'. Anarchasis writing in AD500 wrote that "The world can be divided into three classes; the living, the dead and the seasick!" Irwin first formally used the term motion sickness, recognising that many forms of motion besides the sea could cause vomiting (Irwin, 1881).

Besides man many other species exhibit susceptibility to motion sickness. Dogs have roughly the same susceptibility as man and a lesser but demonstrable reaction has been found in a variety of species e.g. horses, cows, monkeys, chimpanzees, seals, various birds, sheep and cats and even some fish. Rabbits, guinea pigs and rats are immune.

The signs and symptoms that characterise motion illness in man are pallor, cold sweats, nausea and less consistently excessive salivation, drowsiness and depression, which may be severe in some individuals ("vexed with a morbid devil in his blood that veils the world with jaundice" (Hill, (1936)).

The central nervous system was implicated in motion sickness as early as 1881 by Irwin and the suggestion that a functioning vestibular system is necessary for the development of motion sickness was strengthened by observations on deaf mutes by James in 1882. Sjobert in 1929 (cited in Tyler and Bard, 1949) was the first to demonstrate the importance of the labyrinth as the primary receptor site in motion sickness and work by Bond et al., (1947) and Wang and Chinn (1956) showed that dogs were rendered immune to motion sickness by removal of the cerebellar nodulus and uvula, the region of the cerebellum that receives most of the projections from the vestibular nuclei.

Nevertheless, the role of the cerebellar nodulus and uvula is still a subject of debate with respect to requirement for their presence for motion sickness to take place at all. Sharp (1976) has recently used the 2-Deoxyglucose (2-DG) autoradiography technique to show increased glucose uptake in

rat vestibular nuclei and vestibulo-cerebellum in response to rotation. The areas most responsive to the stimulus correlated best with the major sites of primary vestibular afferents. In another 2-DG study Brizzee and Dunlap (1983) detected in the monkey inreased glucose uptake in the medial and inferior vestibular nuclei, area postrema and nucleus tractus solitarii (NTS) in response to a motion stimulus. Miller and Wilson (1983) however used electrical stimulation of the labyrinths of decerebrate cats to produce vomiting and related emetic prodromata in animals previously placed lesions of the posterior cerebellar vermis that included the nodulus and uvula. They thus demonstrated in contradistinction to previous evidence that a transcerebellar pathway involving the nodulus and uvula is not essential in this animal for vomiting produced by stimulation of the vestibular labyrinths.

Ablation studies (Wang and Borison, 1952 and Wang and Chinn, 1954) in the dog showed that the excitatory pathway to the medulary 'vomiting centre' apparently passes via the CTZ in the area postrema (AP) and recent work in the squirrel monkey by Brizzee et al., (1980) has supported these findings.

However, more recent work (Borison and Borison, 1986) in which motion-induced vomiting was studied in cats with and without AP ablations showed that removal of the AP did not abolish motion-induced vomiting and indeed there was even some suggestion that cats without the AP were significantly more susceptible to motion sickness. The exact nature of the mediatory role of the AP in motion sickness therefore remains unresolved. Chinn and Smith (1955) have suggested that its role may be related to a chemical humoral substance liberated

in motion which triggers the CTZ receptor. Crampton and Daunton (1983) suggested that a humoral factor released into the intracerebral CSF may indeed exist but they are dubious as to the reliability of their results (Crampton and Daunton personal communication) because of the intrusive nature of their experimental technique employed. The only study showing fibre connections between the vestibular or cerebellar nuclei and the area postrema is in the rat (Shapiro and Miselis, 1985) where efferent projections from the AP to the region of the cerebellar vermis were described.

Wang et al., (1957) showed that visceral afferents from the gastrointestinal tract (in this case vagal nerves) play no essential role in the vomiting of motion sickness, but cortical structures have been implicated, as human subjects were shown to be able to delay onset of motion sickness by performing mental tasks (Money, 1970). However, although higher centres may exert powerful modifying influences over the expression of motion sickness, decorticate animals (Bard et al., 1947) and 'decorticate' man (Doig et al., 1953) still display motion sickness.

The two inputs of most importance in the generation of motion sickness are from (a) the vestibular apparatus and (b) the visual apparatus (although blind people probably have similar motion sickness susceptibility to the sighted) (Graybiel, 1970)).

These sensory modalities converge at two regions of particular significance, i.e. the vestibular nuclei and the cerebellar flocculus, nodulus and uvula. Cells of the

vestibular nuclei can be modulated by optokinetic inputs as well as vestibular stimuli. The flocculus is another region of interaction between visual and vestibular inputs where, if these inputs conflict, the result will be motion sickness.

The body uses several sensory modalities to perceive orientation in space and change in orientation (i.e. motion). Irwin (1881) suggested that motion sickness may result from conflict between these various sensory modalities. The concept was further elaborated by Claremont (1931), and more recently by Reason (1970 and 1978). The main thesis of the "neuronal mismatch" hypothesis is that all situations which provoke motion sickness are characterised by sensory rearrangement in which the motion signals transmitted by the eyes, the vestibular system and non-vestibular proprioceptors are at variance not only with one another but also, crucially with what is expected on the basis of past experience.

Treisman (1977) recognised that since the occurrence of vomiting as a response to motion is both widespread and apparently disadvantageous it presents a problem for evolutionary theory and proposed the hypothesis that motion sickness originates from conflict which arises when the normal relationship between sensory inputs from visual, vestibular and proprioceptive sources are repeated and predictably perturbed. Such perturbations might in the normal course of an animal's life be caused by ingestion of poisons of neurotoxins. Since these poisons would upset the information flow that allowed an animal to function accurately within its 3-dimensional

framework vomiting would be initiated to limit the damage to the system. The evolution of such an emetic response to sensory disorganisation would thus have survival value and the fact that certain types of motion also trigger the same protective response is seen as an unfortunate and coincidental by-product of the system. Indeed, Money and Cheung (1983) have shown that the emetic response of dogs to toluidine, L-dopa and nicotine was reduced following labyrinthectomy, a procedure which Wang and Chinn (1956) showed abolished motion-induced vomiting, citing this as proof that Triesman's theory is in fact correct.

1.1.2.2 Vomiting of Pregnancy

Strangely the high frequency of this form of vomiting in society, has not given rise to a proportionate quantity of research and information on the mechanism of these types of vomiting is sparse.

References to 'morning sickness' go back to 2,000 B.C. and even then it was distinguished from hyperemesis gravidarum (vomiting appearing before the 20th week, which is intractable and requires hospitalisation). Since that time studies have tended to concentrate on hyperemesis gravidarum and work since 1940 has led to the classification of theories under four main headings i.e. endocrine, psychosomatic, allergic and metabolic (Fairweather, 1986).

Since sex hormones can be emetic and levels of oestrogens and progesterone change radically during the first months of pregnancy it was not unreasonable that interest should have concentrated on possible humonal mediators. Numerous

investigations of the levels of various hormones have been carried out in patients suffering from excessive vomiting. Alas, cause and effect were not proved for oestrogens and progesterone substances (Fairweather, 1965). Similarly interest in human chronic gonadotrophin (HCG) and ACTH yielded equivocal results (Fairweather and Loraine, 1962 and Fairweather, 1965).

Hence we still have no satisfactory explanation of the cause of emesis either in morning sickness or hyperemesis gravidarum.

1.1.2.3 Post-operative Vomiting

Nausea and vomiting may occur in some cases in up to 50% of patients and is regarded by many as the most distressing side effect of the post-operative period (Riding, 1975; Cronin et al., 1973). Palazzo and Strunin (1984) suggested that, although the incidence of post-operative sickness may not have changed in the last 50 years, the severity probably has. Although post-operative vomiting is mostly innocuous in its outcome, aspiration of vomitus is a particular hazard to be avoided at all costs during this period of depressed consciousness and impaired reflexes. However, the study of this area is fraught with difficulty because a variety of interdependent factors affect the anaesthetic contribution to post-operative nausea and vomiting.

Unfortunately, as with vomiting of pregnancy, though some clinical research into the aetiology of anaesthetic-induced vomiting has been carried out over the past 50 years, the actual mechanisms of such vomiting have been little studied.

Nevertheless it is apparent that both general anaesthetics and the opiate drugs used in pre-medication and perioperatively may interact with the CTZ and even directly, the vomiting centre itself (Palazzo and Strunin, 1984; Cookson, 1986). Indeed it is widely accepted that a suitably deep level of general anaesthesia involving depression of the reticular activating system is the single most effective anti-emetic against such powerful stimuli as X-radiation and platinum based cytotoxic drugs (e.g. Whitwam et al., 1978).

It is generally agreed (Waters, 1936, Burtles and and Peckett, 1957; Belville, 1961) that cyclopropane and diethyl ether are the most potent emetics among the inhalational agents. Jenkins and Lahay (1971) speculated that emesis caused by these agents was related to raised CSF concentrations of endogenous catecholamines and experiments were carried out using cats. They noted vomiting followed injection of agents with alpha agonist properties whether or not the agent also had beta agonist properties, and vomiting was not observed in response to injection of beta agonists or alpha or beta antagonists. Elevated catecholamine concentrations produced by pain or hypotension may be the cause of the emesis seen under these circumstances.

Halothane tends to act as an adrenergic antagonist and is thought to have both emetic and anti-emetic effects, the latter at subanaesthetic doses. Nitrous oxide is widely used and is known to cause vomiting but investigations into its mechanism of action in this respect are few. Palazzo and Strunin (1984) concluded that it probably causes emesis by

"central and peripheral" effects whilst at the same time nitrous oxide does not directly stimulate the "vomiting centre". It may be that its more significant action is by way of gastro-intestinal distension caused either by maximal ventilation or direct transfer of gas in the gastrointestinal tract (GIT) (Palazzo and Strunin, 1984). Bellville (1961) has suggested that volatile anaesthetics may stimulate gastric vagal afferents either directly or by gastric distension.

Premedication with morphine leading to an increased incidence of post-operative emesis is well reported (Riding 1960). In the same paper studies were reported showing that atropine administered concomitantly showed significant anti-emetic properties and it has been suggested that emetic properties of opiates are mediated via sensitization of the vestibular system. However, the situation is complicated by the finding that opiates have anti-emetic as well as emetic effects (Costello and Borison, 1977). They found that the anti-emetic effects of opiates were blocked by naloxone given systemically but that the emetic effects were only blocked by naloxone given intracerebroventricularly (ICV) and suggested the existence of a complementary "anti-emetic centre" exciting endogenous "anti-emetic tone" mediated via encephalin pathways.

Bellville's findings (1961) would suggest that the "vomiting centre" must therefore have more opiate receptors so that emetic symptoms prevail at large doses. However, Harris (1982) has recently postulated that the emetic and anti-emetic effects of opiates may be explained by the differential presence of the three opiate receptor sub-types

(mu, kappa and delta) at these two centres; the μ receptor being best antagonised at the anti-emetic centre and the δ receptor being less well antagonised at the chemoreceptor trigger zone.

In conclusion, there is a paucity of animal studies from which to draw firm conclusions about mechanisms of action of anaesthetics and the many clinical studies are complicated by multiple variables and approaches.

It is probable however that anaesthetics all act, in part at least, via the CTZ to cause vomiting. However, since many are assumed to have access to deep medullary structures and the reticular activating formation itself, it is difficult to say at present to what extent these drugs are acting only at the CTZ or the "vomiting centre" or a combination of both; moreover it has to be remembered that their action, on vomiting might be two-fold, at one concentration emetic, and at another anti-emetic. All this and the possibility that some agents may also be stimulating gastric vagal afferents, remains to be investigated experimentally.

1.1.3 Background and Motivation for The Study

In clinical practice it has long been widely accepted that the most distressing complication of cancer therapy using chemotherapeutic drugs or ionizing radiation is nausea and vomiting (Laszlo, 1982). For some patients this leads to dehydration, electrolyte imbalance and even occasionally the Mallory-Weiss syndrome (Bouchier, 1985). For a few the experience is so intolerable that they refuse to complete courses of potentially curative treatment (Laszlo and

Lucas, 1981 and Laszlo, 1983). The problem has been exacerbated over the last decade with the advent of more powerful and effective chemotherapeutic drugs, especially the platinum compounds like cis-diammine dichloro-platinum II (cisplatin) and the use of highly emetic combination therapy. Remarkable improvements in results of treatment have been achieved with modern chemotherapeutic regimes, but the price for these improvements has been increased toxicity; the most important symptoms are nausea and vomiting. The advent of cisplatin in fact provided the impetus to recent research into cancer therapy-induced nausea and vomiting in the hope that the discovery of its emetic mechanism would pave the way for the development of novel anti-emetics, the use of which would raise the therapeutic index and eventually improve the quality of life of patients undergoing chemotherapy.

Despite its frequency even before the advent of agents like cisplatin and techniques like whole body irradiation, up until the 1960's treatment of vomiting was largely carried out on an empirical basis using drugs such as phenothiazines and antihistamines which were shown to have effect against gastrointestinal and vestibular based vomiting. The first controlled trial was carried out in 1963 by Moertel et al., comparing phenothiazines with placebo during the use of 5-fluorouracil, a mildly emetic drug. Before 1977 chemotherapy induced nausea and vomiting received only scant attention with a total of only 6 controlled therapeutic trials between 1963 and 1976 (Fiore and Gralla, 1984). With the appearance of novel cytotoxic agents and anti-emetics like the

cannabinoids and domperidone, there was renewed interest in the area (Siegel and Longo, 1980). There has been a similar paucity of experimental work on emetic mechanisms, and apart from Borison's paper on the site of action of mustine in the cat (Borison et al., 1958) it was not until the 1980's that attention was directed to this area of experimental physiology and pharmacology. The history of clinical studies into radioemesis is similar but much more interest has been shown in the mechanism of X-radiation-induced emesis starting with Borison's work in the cat (Borison, 1957) and continuing into the 1980's (e.g. Harding et al., 1985).

1.2 HISTORICAL REVIEW

1.2.1 History of Research into Nausea and Vomiting

1.2.1.1. The Vomiting Centre

In 1679 Wepfer observed the closure of the pylorus and contraction of the pyloric part of the stomach during vomiting in cats, dogs and wolves (cited by Cannon in 1898). Magendie (1813) recounts in the 'Memoire Sur Le Vomissement' his experiment in which a dog's stomach was replaced with a pig's bladder after which vomiting could still be induced. Much of this older literature was reviewed by Magnus in 1903 and all of it is cited by Hatcher in his review of 1924. It was accepted that there were at least two centres for vomiting one of which is automatic where apomorphine acts directly and the other a reflex centre. Giannuzzi (1865) is credited with being the first worker to suggest the existence of a vomiting 'centre' per se having made serious efforts to study the involvement of the CNS in antimony sulphate-induced vomiting in

dogs (tartar emetic) (Hatcher, 1924). Openchowski (1889) and his pupil Hlasko (1887) (quoted in Hatcher, 1924) subsequently carried out a great deal of work. They contended that there were independent centres for various acts concerned with vomiting with separate paths from each centre. Interestingly, Openchowski was unable to induce vomiting by apomorphine administration after destruction of the corpora quadrigemina although copper sulphate induced vomiting remained present. His failure to induce emesis in dogs with intragastric copper sulphate after vagotomy also led him to the general conclusion that the vagi alone carry afferent emetic impulses from the stomach. Thumas (1891) placed great emphasis on the caudal tip of the calamus scriptorius as the site of the vomiting centre and showed that the function of vomiting was intact after transection of the brain stem at the acoustic striae. Furthermore, he indicated that the emetic centre is located in deep structures and pointed out the futility of unilateral lesioning in the medulla, stating that such one-sided destruction of the ala cinerea does not abolish drug-induced vomiting. He also discussed physiological relationships between the activities of the vomiting and respiratory centres but concluded that these were not one and the same.

Thumas described a very small area (2mm wide around the midline, extending from 2mm anterior to calamus scriptorius posteriorly for 5mm, through the obex) in the posterior part of the rhomboid fossa of the dog which was more sensitive than any other to the emetic, apomorphine. When this area was destroyed apomorphine failed to cause vomiting. Openchowski

suggested that the area described by Thumas served only as a pathway for emetic impulses to the corpora quadrigemina. In 1898 Cannon described the contribution of the various muscle groups to the act of vomiting.

Hatcher and Weiss, 1923, and Hatcher, 1924 confirmed Thumas's results with apomorphine but they were also able to elicit emesis with mercuric chloride in dogs which were refractory to apomorphine. On the other hand, Hatcher and Weiss prevented vomiting induced with either apomorphine or mercuric chloride by destroying the ala cinerea (dorsal sensory nucleus of the vagus) which they considered to be the site of the vomiting centre. They felt that Thumas had erroneously implicated midline structures as a result of adventitious damage to the closely situated ala cinerea. Only acute preparations were used for these experiments and localization was determined primarily on the basis of drug application. Koppanyi (1930) subsequently demonstrated in dogs with chronic lesions in the ala cinerea that the emetic sensitivity to parenteral apomorphine was reduced whilst "irritant" emetics (e.g. zinc or copper sulphate) remained effective by the oral route. The hypothesis that the vomiting centre was in the ala cinerea was thus placed in doubt. Koppanyi intimated the existence of two separate vomiting centres, one for apomorphine and the other for'reflex' vomiting to gastric irritants.

As late as 1948 various textbooks of neuroanatomy described several different neural structures as the vomiting centre, e.g. the dorsal motor nucleus of the vagus (Tilney and Riley 1938) or the nucleus of Roller (Mettler 1948). All

these centres, however, were postulated on the basis of a unitary concept, i.e., that both the central and reflex emetic agents elicit vomiting by activating the same neural structure, despite the evidence of Koppanyi.

Prior to 1948 investigations had not been successful in obtaining emesis by electrical stimulation of the medulla oblongata. Miller and Sherrington (1915) could evoke vomiting with 'noxious fluid' but not by faradic stimulation of the inferior fovea in the floor of the fourth ventricle which only caused swallowing movements. Laughton (1929) stimulated the area identified by Hatcher and Weiss as the vomiting centre, the dorsal vagal nucleus, but elicited no vomiting response. These investigators and others limited themselves to stimulation of superficial structures in the floor of the fourth ventricle. Wang carried out a variety of stimulation experiments in the lower brain stem of more than 200 cats, dogs and monkeys but failed to elicit frank vomiting (Wang and Ranson 1939). However, an unusual augmented respiratory movement was noted during stereotactically controlled stimulation of the dorso-lateral medulla of the barbiturate anaesthetised cat (Borison 1948). Having decided that barbiturate anaesthesia caused a general CNS depression, which rendered the emetic mechanism refractory to electrical stimulation this work was repeated in the decerebrate cat. Vomiting was apparently readily observed during these medullary stimulations. In 11 out of 20 cats thus prepared the region most responsive to electrical stimulation was the nucleus

tractus solitarii (NTS), the tractus solitarius and a small area on the dorsolateral border of the reticular formation (Borison and Wang, 1949). On the basis that the NTS and its tract were already directly implicated in the emetic process, it was concluded that the area ventrolateral to the solitary tract encompassed the vomiting centre. The emetic centre was situated amid loci concerned with activities which are functional parts of the integrated vomiting act, such as salivation (Wang 1943) spasmodic inspiratory movement (Borison 1948), forced respiration (Pitts et al., 1939), vasomotor reactions (Chai and Wang 1962) and the remains of the vestibular nuclei and brain stem facilitatory and inhibitory systems (Magoun 1950) responsible for the regulation of associated functions.

The destruction of the putative 'vomiting centre' with implanted radon seeds resulted in loss of the emetic response to both central and peripheral emetic stimuli in animals previously sensitive to the stimuli (Wang and Borison, 1951). These data strengthened the concept of the existence of a VC in the reticular formation, but since that time very few studies have appeared to corroborate them. Ikeda and colleagues (Ikeda and Yamanaka, 1967 and Iwase et al., 1967) stimulated in the region delineated by Borison and Wang in decerebrate dogs and obtained vomiting-like behaviour as measured by pressure changes in a stomach balloon. Similar behaviour was obtained as a result of stimulation of the AP and an area of midline brain stem caudal to obex. Frank vomiting was not observed. In more recent studies employing techniques very similar to

those of Borison and Wang (1949) and indeed, partially in Borison's laboratory, electrical stimulation of the brain stem of 15 cats produced stimulus-bound vomiting in 4 cats, including 2 in which emesis occurred only after giving naloxone and a third that vomited when the stimulus was turned off (Miller and Wilson, 1983). This compares to a response of 11 out of 20 cats for Borison and Wang (1949) where this figure represents an underestimate of their success since some of their 9 'failures' should have been discounted for various reasons which they discuss, like early death after operation. Miller and Wilson also could not produce more than one vomiting sequence during repeated stimulation of any one animal and only obtained vomiting after long stimulus periods. Moreover they always recorded prodromal signs that they describe as salivation, swallowing and mouth opening. They concluded that there is no well localised co-ordinating centre for emesis and that a discrete vomiting centre does not exist, feeling that these results are more consistent with the concept that neurons involved in the control of vomiting are diffusely distributed in the effective region described by Borison and Wang in 1949. They suggested alternatively that electrical stimulation may be successful in eliciting emesis by direct excitation of descending pathways (Holstege and Kuypers 1982) that could produce co-ordinated activation of the 'vomiting musculature' such as the solitary-spinal tract which projects to the phrenic motor neurons in the C4-L6 ventral horn and to the thoracic ventral horn (Loewy and Burton 1978) which activate the respiratory muscles that produce the pressure changes required for expulsion of vomitus from the stomach.

To address this conflict Davis et al., (1986) have suggested an alternative organisational model of this central control system based on the idea of 'sequential activation' of the various effector nuclei comprising the vomiting act and proposed the concept of the 'VC' not as a discrete entity but a 'higher' and 'integrated' function of these separate effector nuclei. Carpenter (1987) speculates that a set of more or less diffuse neurones acting to co-ordinate the vomiting act (the "central pattern generator for emesis") may be in the N.T.S..

Brizzee and Mehler (1986) refer to this area or zone of the medulla as the parvicellular reticular formation and place it ventral to the vestibular nuclei, medial to and coextensive with the elongated nucleus of the spinal tract of the trigeminal and dorsolateral to the larger-celled medullary reticular groups which give rise to the various reticulo-spinal pathways. The parvicellular reticular formation forms a column of cells that extends from the level of the obex rostrally to the pontine parabrachial region. The motor nucleus of the trigeminal is partially embedded in the rostral pole of this cell group and it receives massive ascending axon connections from both ipsilateral and contralateral parts of it especially a subset of its cells which lie just ventrolateral to the hypoglossal nuclei in the lower medulla.

There are multiple sources of input descending to the parvicellular reticular formation from cortical and subcortical regions that represent potential somatic and visceral afferent pathways to the hypothesised vomiting integration centre

located therein. Established efferent connections made by this brain region are chiefly projections to brain-stem nuclei. Briefly its connections comprise the following -

Efferent: Facial Motor Nucleus, Hypoglossal Nucleus, Parabrachial Pontine Nuclei, Solitary Nucleus, Vagal Nucleus, Trigeminal Motor Nucleus, Ambiguous Nucleus

Afferent: Motor and Sensory Cortices, Central Amygdaloid Nuclei, Hypothalamic Nucleus, Mesencephalic Nucleus of Cranial Nerve V (Trigeminal), Vestibular Nuclei

1.2.1.2 The Chemoreceptor Trigger Zone and the Area Postrema

After their work on the 'vomiting centre' Borison and Wang and colleagues proceeded to carry out superficial lesions of the medulla in dogs which resulted in abolition of the vomiting response to apomorphine and certain cardiac glycosides given intravenously (i.v.). The lesions did not affect the response to copper sulphate given orally. Deeper lesions, also involving the lateral reticular formation, impaired the response to both intravenous apomorphine and oral copper sulphate (Wang and Borison, 1950; Wang and Borison, 1952). There was thus identified a superficial CTZ which functions as a receptor site for centrally-acting emetic agents. In low doses oral copper sulphate presumably excites peripheral receptors, in the gastrointestinal tract, which activate the 'vomiting centre' directly through afferent pathways. When the 'vomiting centre' was destroyed by implantation of radon seeds into the lateral reticular formation, leaving the CTZ undamaged, the thresholds to a variety of emetic agents including intravenous apomorphine and digitalis and oral copper

sulphate were raised (Globus et al., 1952 and Wang and Borison, 1951).

It was apparent, therefore, that the CTZ has a receptive not an integrative function and that it communicates with the 'vomiting centre' (Borison and Wang, 1953). Borison further demonstrated the functional distinction between the CTZ and the vomiting centre in the cat by showing that discrete ablation of the CTZ abolished the vomiting response to intracerebroventricular (i.c.v.) injection of adrenaline and apomorphine, while the response to oral copper sulphate remained unimpaired (Borison, 1959).

In order to determine precisely the location of the CTZ in the cat, Borison and Brizzee (1951) made a variety of medullary lesions using electrocautery and observed the effects on the ability to vomit in response to intravenous cardiac glycosides. They defined the CTZ as a bilateral structure less than $1mm^3$ in size, on either side of the fourth ventricle, contiguous medially with the AP. In the dog the CTZ was less definable. Brizzee and Neal (1954) in histological studies, found the AP in the cat to be highly vascularised and to contain neuronal elements, with loose bundles of fibres passing between it and adjacent medullary tissue; this was thought consistent with a chemoreceptor function. The first accurate description of the gross topography and cell structure of the AP was given by Retzius in 1896 when it was referred to as areae postremae or eminentiae postremae. In 'higher mammals' it presents as two mounds of highly vascular tissue protruding into the lumen of the fourth ventricle in the region

of the calamus scriptorius. The two mounds, one on each side of the midline, converge caudally ventral to the obex forming the roof of the most rostral extent of the central canal of the spinal cord. In rodents and lagomorphs the AP is a midline structure overlying the central canal. The AP has been found in all mammals examined for its presence and has also been described in various species of birds (Borison, 1984).

In 1906 Wilson suggested the name nucleus postremas for the AP in humans. It is the most caudal of the circumventricular organs (Weindl, 1965) which are situated around the periphery of the ventricular system of the brain, most of which exhibit neither the structural nor physiological atributes of the blood-brain barrier. Wislocki and Putnam (1920) showed that the blood-brain barrier in the AP is deficient relative to that of other medullalry structures. The AP has an extensive blood supply coming from the anterior inferior cerebellar arteries, posterior spinal arteries and vertebral arteries. The blood vessels within the organ form non-anastomosing loops within the extraordinarily large perivascular spaces. The vascular supply enters the AP from the pia mater on the subarachnoid side of the tela choroidea. It has been suggested that a counter-current mechanism may be in effect within the AP (Kroidl, 1968; Weindl, 1973) and the general histological appearance has been compared with that of the carotid body (DeKoch, 1959).

Ultrastructural studies have revealed fenestrated capillaries and large perivascular spaces containing fibroblasts and large amounts of collagen. Both the inner

(vascular) and outer (parenchymal) basal laminae are very conspicuous. Complicated extensions of the perivascular spaces lined by the outer basal lamina were observed to project into the parenchymal tissue (Brizzee and Klara, 1984). The cell population is composed of flattened ependymal cells exhibiting microvilli, small neurons, astrocytes, 'glialoid cells' and a very few oligodendroglia (Leslie, 1986). Mast cells are occasionally present. The glialoid cells appear to be the predominant cell type and exhibit great numbers of vascular podia. Axodendritic synapses are numerous and axosomatic synapses are occasionally seen in the parenchyma. Synaptic vesicles are mainly of the clear-cored type but large dense-cored vesicles are commonly observed in some axon terminals. Neural elements of the AP are well positioned to sample constituents of blood or cerebrospinal fluid and the tanycyte-like ependymal cells with their extensive microvillous tufts may be important in this respect (Davis et al., 1986) it is however an open question as to which cellular elements of the organ are most important in the chemoreceptive function of the AP.

Several studies have established the presence of small neurons and many nerve fibres in the mammalian AP (e.g. Brizzee and Neal, 1954; Cammermeyer, 1949). The elucidation of the neuronal connectivity of the AP began with the recognition of fibre connections between it and the subjacent NTS (e.g. Morest, 1960 and 1967; Gwyn and Leslie, 1979). An especially prominent bundle of fibres was observed in the lateral region of the AP and these were orientated in the direction of the

NTS. The AP in fact receives a significant visceral afferent input by way of the vagus nerve that includes sensory information from thoracic and abdominal viscera. The glossopharyngeal nerve also projects to the AP to provide receptive information via its carotid sinus component and the dorsal hypothalamus projects there also. The two main outputs of the AP seem to be to the subjacent NTS and the parabrachial region (nucleus of Kölliker-Fuse). A fairly well-established projection from the subnucleus gelatinosus (the dorsomedial region of the NTS) to the dorsal motor nucleus of the vagus is also of significance. In general the connectivity of the AP seems to parallel fairly closely that of the subnucleus gelatinosus of the NTS. Certainly these two brain areas are closely linked by short neuronal projections (Davis et al., 1986).

The subnucleus gelatinosus has also been referred to as the area subpostrema (Gwyn and Wolstencroft 1968) and the more caudal part of the region as the parvocellular solitary nucleus (Loewy and Burton, 1978). The neuronal connections of the subnucleus gelatinosus are of interest because they probably provide additional indirect pathways to the AP (Leslie and Gwyn, 1984). These include afferents from the vagus nerve (arising from the stomach wall, or the aortic depressor nerve), the carotid sinus branch of the glossopharyngeal nerve, the prefrontal cerebral cortex, and efferents to the parabrachial region (nucleus of Kölliker-Fuse) and to the dorsal motor nucleus of the vagus.

Physiological evidence for a neuronal connection from the AP to the 'vomiting centre' was obtained by Iwase et al., (1967) who elicited vomiting-like behaviour in the dog by electrical stimulation of the CTZ but neither earlier studies by Borison and Wang, (1949) nor Miller and Wilson, (1983) were able to detect this.

Transmitter localization and binding sites in the AP have been reviewed by Leslie (1985). Noradrenaline, adrenaline, dopamine, serotonin and GABA have been localised in the bovine AP which is consistent with the findings in other species (Leslie and Osborne, 1984).

Noradrenaline and adrenaline have also been shown to occur in the AP of the human, rabbit and rat. The presence of dopamine has been detected but this may possibly reflect its role as a precursor in the synthesis of noradrenaline or adrenaline. However, dopamine receptors have been detected in the bovine (Pedigo and Brizzee, 1985) canine (Stefanini and Clement-Cormier, 1981) and human (Schwartz et al., 1986) AP. Other studies have shown that serotonin occurs in neuronal perikarya and varicosities of the AP (see Leslie, 1985). Biochemical evidence has been found for the serotonin being a constituent of bovine and rat AP and binding sites have been found for serotonin in bovine AP (Leslie and Osborne, 1984). This correlates well with the fact that most of the serotonin of the AP appears to be localized to fibres and varicosities in the AP (Maley and Elde, 1982, Leslie and Osborne, 1984). Recent work by Lanca and van der Kooy (1985), using retrograde tracing and immunofluorescent techniques, has discovered a

serotonin-containing pathway from AP to the parabrachial region in the rat. Significant levels of GABA have also been found in the AP suggesting that this amino acid may have a role as a transmitter in the region. Certain neurons of rat AP are immunoreactive for leu-enkephalin (Armstrong et al., 1981) and substance P (Armstrong et al., 1982). In addition studies have been reported on the localisation of cholecystokinin, neurotensin, angiotensin II, prolactin, insulin or their binding sites in the AP of a variety of animals, e.g. rats, other rodents and primates (Leslie, 1985). It appears then that noradrenaline, dopamine, serotonin and possibly GABA may have mediator roles in the AP, with respectively, pressure responses, emetic responses, gastrointestinal respiratory and cadiovascular responses being implicated (Leslie, 1985).

A number of neurotransmitters and peptides have been administered to animal models with the idea of characterising the responsiveness of neurones of the AP. Thus Borison et al., (1975) recorded in cats and applied drugs systemically. Because of technical difficulties, the results were sparse but excitation in response to ouabain and ATP was found. Brooks et al., (1983) attempted recordings in an isolated brain tissue preparation and found neurones unresponsive to glutamate, serotonin, angiotensin II, dopamine, and osmotic changes. However Carpenter et al., (e.g.1983, 1984) have recently carried out extensive work in the dog. Seventeen common transmitters and peptides were employed. All neurons were silent at rest but most could be excited by

glutamate. Excitatory responses were also found to histamine, noradrenaline, serotonin, dopamine and apomorphine, angiotension II, neurotensin, leu-enkephalin, VIP, TRH, gastrin, vasopressin and substance P. Most neurons were excited by dopamine or apomorphine and approximately half were excited by the other substances. In some neurones, inhibitory responses were found to noradrenaline and histamine but no response at all was found to three substances: acetylcholine, somatostatin or cholecystokinin. Except for serotonin and noradrenaline, all the agents which excite AP neurons are also emetic. The three substances without effect on AP neurons are also not known to be emetic.

Borison and Borison (1973) showed that the ability to vomit in response to stimulation of the CTZ does not develop until the age of three to four weeks in cats. Morphological studies established that the response is dependent upon the full maturation of fibre connections in and through the AP. An as yet unresolved question is: does ablation of the CTZ simply interrupt nerve fibres connecting the AP with the reticular formation or does it destroy the chemoreceptive elements in the AP? A complicating factor involves the blood supply of the AP which is delivered and drained at the lateral pial margin Duvernoy et al., 1972 and Kroidl, 1968); hence successful ablation of the lateral zone (the putative CTZ) may render the entire AP ischaemic by gross infarction of the whole organ and its fibres of passage.

As well as the recognition of the CTZ function of the AP a number of investigators have implicated the AP in a variety of

other functions, as well as delineating a long list of causes of emesis mediated by the CTZ. (For a recent comprehensive review of the structure function relationships of the AP see Leslie, 1986). Nearly 30 causative agents and conditions in four animal species have been shown to act via the AP, although controversy still exists over the part played in syndromes such as motion sickness and radiation sickness (Borison et al. 1984) The AP was once considered as the locus of chemical feedback in CO_2 regulation based on experimental results obtained in acutely lesioned animals. However, in cats in which the AP has been chronically ablated the effect disappeared and the idea was therefore discarded. Nevertheless a number of other functions (reviewed by Borison, 1974 and Leslie, 1986) have been suggested for the AP e.g. adrenaline-induced hypoglycaemia, control of food intake, serotonin-induced synchronization of the EEG, cardiovascular effects of angiotensin, blood osmoreception and control of renal function. However, all these results have been questioned on various technical grounds and Borison maintains that the only certain activity of the AP is as the CTZ for vomiting since its removal consistently abolishes the emetic reaction to a variety of agents especially the opiates and cardiac glycosides. Moreover, this chemosensory deficiency produced by the lesion is precise and activation of the central control mechanism of vomiting through other reflex inputs is in no way disabled (Borison and Wang, 1953; Borison, 1964). He further contends that over a period of 25 years of research innumerable cats with chronic lesions of the AP appeared to suffer from no other gross disability than the above.

Nevertheless, in at least two areas which may be directly relevant to the emetic phenomenon but do not necessarily culminate in vomiting, there is good evidence that the AP plays an important role. A role for the AP has been demonstrated in radiation-induced delayed gastric emptying which was abolished by ablation of the AP in rats (Harding and Ossenkopp, 1983). Conditioned taste aversion is a sensitive behavioural indicator of influence by sickness-inducing agents in rats (Berger et al., 1973 and Garcia and Koelling, 1967). Involvement of the AP in this phenomenon has been demonstrated for a variety of ingested and injected agents as well as exposure to X-radiation (Cairne and Leach, 1982; Ossenkopp, 1983; Rabin et al., 1983). More recent work on other possible functions of the AP, especially with respect to regulation of food intake and satiety (see for instance Contreras et al., 1984 and Kenney et al., 1984); has shown its possible importance in these processes but authors are careful to point out that lesions induced in the AP do in fact involve the subjacent NTS as well. This confirms to some extent Borison's opinion that, except for the CSF connection that is tied to a more general yet uncertain role of the circumventricular organ system, the remainder, match functions attributed to the NTS (Borison, 1984).

Interestingly there is some direct evidence for the role of the AP and CTZ in man. This work was carried out in five patients suffering from untreatable vomiting due to inoperable brain tumours. At craniotomy the topography of the calamus

scriptorius was found to be very similar to that of the dog, cat and monkey and in each case the AP was lesioned with the result that vomiting was relieved and moreover the patients also became refractory to apomorphine when challenged subsequent to complete recovery from the surgery (Lindstrom and Brizzee, 1962).

1.2.1.3 Visceral Afferent Information Input to the 'Vomiting Centre'

Miller (1910) studied the afferent nerves to the 'vomiting centre' using the emetic action of mustard seeds. He concluded that the vagi alone were responsible for this function, since immediately following vagotomy, emesis was no longer capable of being elicited; this was not the case after section of the splanchnic nerves. The importance of afferents in the vagus was further stressed by Bayliss (1940) who produced vomiting by intraperitoneal injection of staphylococcal enterotoxin and found that emesis rarely occurred following division of the vagi. In an acute preparation, Goldberg (1931) induced vomiting by distending an isolated pyloric pouch and found that this reflex also disappeared after vagotomy. Somewhat paradoxically many workers (Hoffman et al., 1984 and Clark et al., 1964) have observed that vagotomy does not interfere with vomiting in man, but here of course the situation is not directly comparable to an experimental one in which every vagal fibre has been located and sectioned. In addition Walton et al., (1931) reported that in order to prevent the vomiting of experimental peritonitis, they had to section both vagi and the splanchnic nerves. These early

studies of pathways from the gastrointestinal tract were reviewed by Borison and Wang (1953) and subsequent research was summarised by Barnes (1983).

Interestingly, irritation or distension of the small intestine was more effective in inducing emesis than similar stimulation of the stomach (Keeton 1925). Generally, vagal afferents were found to be more important than those in the sympathetic nerves. Vomiting could be readily elicited through electrical stimulation of the cut central ends of the vagal branches of the stomach (Miller 1910). The vomiting due to distension of pyloric pouches could be abolished by transthoracic vagotomy but not by sympathectomy.

The vomiting due to ingestion of copper sulphate was found to be dependent upon both vagal and abdominal sympathetic afferents, but vagal transmission was the more important. Delayed vomiting produced by intragastric copper sulphate after previous combined abdominal vagotomy and sympathectomy was found to be due to its direct action on the CTZ since ablation rendered the animal refractory to even a lethal infusion of copper sulphate (Wang and Borison, 1951 and 1952).

In more recent studies concerned with afferents from the upper part of the tract Hayashi (cited in Iwase, 1971) observed swallowing in the decerebrate cat in response to pressure on or electrical stimulation of the area of the root of the tongue on the rear wall of the pharynx; stronger stimulation evoked vomiting. Thus vomiting resulted from abnormal stimulation of

the pharyngeal branch of the vagus and glossopharyngeal nerves. Abrahamsson (1973) reviewed the literature on afferent innervation of the stomach and Andrews (1986) has pointed out in his more recent review of information on the vagal innervation of the G.I.T. that although the stomach functions overall as a single organ there is some separation of function such that the gastric corpus serves as a reservoir of food where digestive acid and enzymes may act whilst the antrum maintains powerful contractions which degrade food particles and propel them into the duodenum. Specific mucosal receptors responsive to hydrochloric acid and carbohydrates have been located and receptors sensitive only to temperature have also been reported. Other polymodal reeptors have been shown to be sensitive to light touch and certain chemicals. Moreover, there appear to be a number of species differences between sheep, rats and cats (Andrews, 1986). The ferret has vagal afferents responsive to hypertonic solutions (Andrews and Wood, 1984).

In response to distension and contraction of the stomach vagal afferents with receptors in the gastric muscle increase their discharge and it is now generally assumed that these receptors behave as if they are "in series" with the smooth muscle fibres. Differences in behaviour of the two gastric regions to distension are reflected in the afferent discharge from receptors located in each of these areas (Andrews et al., 1980). Thus while receptors in both locations have the same general properties their location

determines their precise behaviour; afferents with receptors located in the antrum primarily signal the occurrence and magnitude of rhythmic constractions whilst those in the corpus and fundus indicate the overall level of distension of the stomach and could be involved in signalling pre-absorption satiety. Whilst the afferents in the antrum can respond to distension of the antrum this area is not usually subjected to gross sustained distension under physiological conditions. However, not only is the distension volume important in these considerations but also distension rate.

Lastly, a single receptor has been described on the serosal surface of the stomach or omentum in the region of the greater curve of the cat stomach. This is only activated by gastric distension, stretch and digital compression (Iggo, 1957).

The main central projections of abdominal vagal afferents are summarised here:

a. To the hindbrain: NTS (dorsomedial subnucleus and commissural part), area subpostrema or subnucleus gelatinosus (in cat rather than rat and ventral part especially) possibly Nucleus ambiguus (Leslie et al., 1982; Kalia and Mesulam, 1980; Harding and Leek, 1973; Gonzales et al., 1986)

b. To the cerebellum: Some evidence in the cat. (Hennemann and Rubia, 1978)

c. To the midbrain: Inferential connections with parabrachial nuclei via the NTS and the PCRF (Sawchenko 1983)

d. To the forebrain: hypothalamus, somatosensory cortex, amygdala (Anand and Pillai, 1967; Oomura and Yoshimatsu, 1984)

Zabara et al., (1972) introduced the concept of neuroinhibition in the regulation of emesis. Emesis preceded by retching could be induced in the dog by electrical stimulation of the abdominal vagal nerves at the supradiaphragmatic level. They suggested that failure to produce retching or emesis by similar stimulation of the cervical vagal trunk meant that, either the abdominal vagal afferents do not travel in the cervical vagus, or that inhibitory fibres are present. They concluded that inhibitory fibres were present, since the retching and vomiting resulting from stimulation of the supradiaphragmatic vagus could be prevented either by transection of the cervical vagus or by simultaneous stimulation of the cervical vagal trunk. They maintained that the excitatory system, involving the 'vomiting centre' and CTZ, acts in conjunction with an inhibitory system so that emesis is normally prevented by a dominance of inhibition over excitation. No direct support has been offered for this hypothesis but Costello and Borison (1977), found that the anti-emetic effects of opiates were blocked by naloxone given systemically but the emetic effects were only blocked by naloxone given intracerebro-ventricularly. They suggested the existence of an anti-emetic centre in the reticular formation which exerted an endogenous anti-emetic force mediated by enkephalins. Such ideas have been included in a major hypothesis concerning the mechanism of cytotoxic vomiting which will be discussed later and involves the effects of enkephalins on specific opiate receptor subtypes distributed between such

emetic and anti-emetic centres (Harris, 1982, Harris and Cantwell, 1986).

From the foregoing we can construct a basic circuit diagram linking the principal components of the vomiting reflex (Fig. 0).

1.2.1.4 The Mechanics of Vomiting

The patterns of muscular activity in vomiting have been investigated by the recording of pressure changes and by the use of electromyographic, radiographic or cineradiographic techniques. Cannon (1898) reported observations of the activity of the stomach during digestion and vomiting by means of the then newly developed fluoroscopic methods. At the onset of vomiting activity, the pre-antral portion of the stomach became completely flacid and was separated from the antrum by a constricting ring. Following this, the diaphragm flattened and the abdominal muscles underwent quick, jerking movements, which resulted in ejection of the fundus contents into the oesophagus. Subsequent observers have verified the largely passive role of the stomach and active role of the diaphragm and abdominal muscles in the emetic action.

Among more recent studies of the respiratory mechanics of the process (Smith and Brizzee, 1961, McCarthy and Borison 1974, McCarthy et al., 1974), the changes in the thoracic and abdominal venous pressures and in arterial blood pressure were recorded together with those in trachea and intrapleural pressures in decerebrate cats that were induced to vomit by intramuscularly administered verartrum alkaloids.

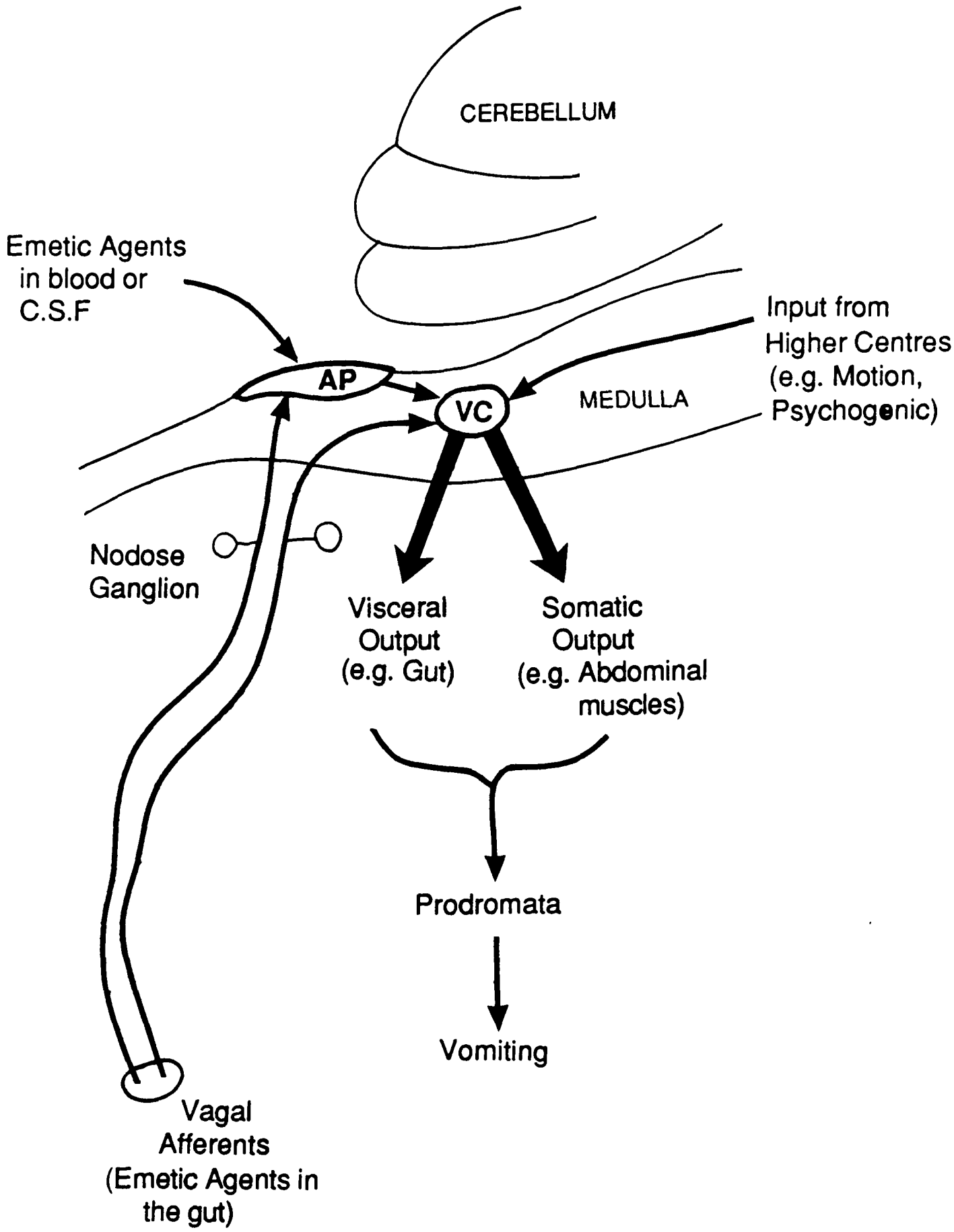


Figure O Diagrammatic Representation of The Emetic Reflex

A diagram summarizing the major pathways by which the vomiting reflex may be activated - note that the 'vomiting centre' can be activated either directly by abdominal vagal afferents or indirectly via prior involvement of the area postrema

Electromyograms were recorded from the dome of the diaphragm and from the body wall muscles of the thorax and abdomen. Radiographic, oscillographic and cineradiographic techniques were employed. In the retching phase, brief negative pressure pulses in the thorax corresponded to positive pressure pulses in the abdomen, and in expulsion there was more sustained abdominal contraction with a sudden reversal of intrathoracic pressure from negative to positive. Both retching and expulsion were effected by the same muscles. In retching the external intercostals contracted together with the diaphragm and the abdominal wall muscles. In expulsion the outstanding feature was a sudden upward shift of the diaphragm so that pressure generated by the abdominal muscles was transmitted to the thorax. It was seen to begin with the cardia already situated in the thorax as the result of preceding retching. Retching then, was seen basically as a preparatory manoeuvre in which respiratory mechanisms are used to defeat the inherent antireflux characteristics of the abdominal oesophagus and cardia prior to expulsion. However, Monges et al., (1978) showed that differences in the pattern of muscular contraction occurred between that found in retching and that in vomiting. In contrast to retching (when bursts of activity can be recorded in all areas of the diaphragm and rectus abdominis) during expulsion, the inner hiatal fibres of the diaphragm remain silent and it is this relaxation that allows the herniation of the abdominal oesophagus and the cardia into the thorax prior to expulsion of stomach contents through the mouth

as thoracic pressure reverses from negative to positive. Postural changes were noted in animals such as the cat and the dog (McCarthy and Borison, 1974, Monges et al., 1978) with abrupt flexion coincident with expulsion and indeed this has been observed anecdotally even in man. Prior to McCarthy and Monges definitive animal work in the 70's studies were carried out in man and have been reviewed by Brown, 1963, Lumsden and Holden, 1969 and Johnson and Laws, 1966.

1.2.1.5 Gastrointestinal Motility during Vomiting

Smith and Brizzee (1961) and Barnes (1983) have reviewed the data on gut activity during vomiting. More recently Willems and Lefebvre (1986) summarised the peripheral nervous pathways involved in vomiting. The extensive efferent neural control of the gastrointestinal system consists firstly of excitatory cholinergic preganglionic fibres in vagal nerves and in the pelvic nerves. They inervate both cholinergic and non-cholinergic excitatory motor neurons in the enteric plexi throughout the gastrointestial tract, but primarily in the stomach and in the large intestine. The efferent vagal activity stimulates activity in the smooth muscle cells and the excitatory transmitter is acetylcholine (Ach), but others are also involved e.g. 5-HT, substance P. The vagal nerves also contain cholinergic pre-ganglionic fibres which innervate intrinsic inhibitory neurons in the enteric plexus. Pre-ganglionic sympathetic fibres synapse in the paravertebral or prevertebral ganglia (e.g. the coeliac ganglion for the stomach and duodenum). Post-ganglionic adrenergic fibres act

to inhibit mobility by an action on both the cholinergic ganglia and smooth muscle cells.

Gastric relaxation has been observed in the period preceding evacuation of stomach contents and this is brought about by stimuli descending in the vagal fibres which innervate the intrinsic inhibitory enteric neurons. This entity has been named the non-adrenergic non-cholinergic inhibitory vagal system (NANC) and several transmitters have been proposed for these inhibitory neurons, e.g., ATP, VIP (vasoactive intestinal polypeptide) and dopamine. It has also been suggested that extrinsic 5-HT containing neurons in the vagus activate the intrinsic inhibitory neurons and thereby contribute to gastric relaxation (Andrews, 1986).

During the gastric relaxation that precedes vomiting, the electrical spike activity, which, superimposed on the basic electrical rhythm indicates contractile behaviour, disappears in the stomach and duodenum and the basic electrical rhythm is suppressed. The electrical silence is followed rapidly, before vomiting occurs, by an intense spike activity in the duodenum and jejunum which either occurs simultaneously over the whole duodenum or clearly starts initially from the duodenum or jejunum to travel back to the pylorus. Effectively this produces an antiperistaltic wave which pushes intestinal contents into the stomach during gastric relaxation prior to their evacuation during vomiting. Anti-peristalsis and duodenal spasm remain present after atropine but are abolished by hexamethonium or vagal section and it has been suggested

that this is controlled by activation of an intrinsic non-cholinergic system where 5-HT (or related substances) may play an important role.

The present project did not attempt to deal with the neural basis of human nausea because of the difficulty of making the connection between such human experience and observable correlates of changes in behaviour in animals following administration of emetic stimuli. Thus 'nausea' is only dealt with in so far as we have recorded, and attempted to define as specific, certain changes in behaviour of the ferret during the prodromal phase of vomiting, i.e., that period between administration of an emetic stimulus and the onset of evacuation of gastric contents through the mouth. These data may be useful in future attempts to study nausea using the ferret as a model for man.

It is clear that there are a number of motor changes in the GIT which accompany nausea and vomiting. Whilst these may indeed by purely secondary it has been proposed by Akwari (1983) that these motor changes are actively caused by the presence of cytotoxic agents and that it is these motor disorders when detected by the visceral afferents previously desribed evoke vomiting via the pathways outlined above, i.e. to the VC and AP thus the GIT may be the final common mediator of vomiting evoked by cytotoxics and this problem has been tackled directly in the experimental work recorded in this thesis.

1.2.2 Research into Cytotoxic- and Radiation-induced Vomiting

1.2.2.1 Cytotoxic Drugs

1.2.2.1.1 Introduction

Most reviews dealing with cytotoxic drug induced vomiting and paying more than lip-service to animal model based experimentation on basic mechanisms concede openly that relatively little work has been carried out to identify the site(s) of action of the cytotoxic drugs in current usage (e.g. Seigel and Longo 1981, Florczyk et al., 1982). The modern era of cytotoxic chemotherapy was ushered in by the introduction of the polyfunctional alkylating agents in the 1940's (Goodman et al., 1946). In spite of reports of potassium arsenite being used against leukaemias and other malignancies dating from 1865 (cited in Cline and Haskell 1980) effective chemotherapy is a relatively new discipline which has undergone rapid growth in the last 20 years.

Cytotoxic drugs, like ionizing radiation, do not kill tumour cells directly but affect cell division and thereby cell proliferation. A number of fundamental molecular processes must continue to take place for cells to proliferate. DNA must be replicated without error once every cycle. This requires an adequate supply of purine and pyrimidine nucleotides as building blocks, the enzyme DNA polymerase and lastly an intact DNA template to direct the synthesis of complementary RNA, a process catalysed by RNA polymerase. RNA is then translated into proteins through complex polymerisation that takes place on the ribosomes in the cell cytoplasm. The various chemotherapeutic

agents interfere with one or other of these essential cellular processes.

Available agents are divided into a number of classes viz., alkylating agents, antimetabolites, antibiotics, plant alkaloids, hormones, enzymes and a group of miscellaneous agents. Alkylating agents act by the transfer of alkyl groups to biologically important cell constituents. Antimetabolites interfere with the synthesis of nucleic acids. The plant alkaloids produce mitotic arrest by binding to a cytoplasmic precursor of the spindle, and many of the antibiotics bind selectively to DNA, forming complexes that block the formation of DNA-dependent RNA. The nitrosoureas exhibit alkylating agent activity. The enzyme L-asparaginase has the unique property of depleting asparagine in human cells, and the precise mode of action of the hormones is unknown.

Although all anti-cancer cytotoxic drugs can produce nausea and vomiting there are marked differences in the emetic potency between different drugs that have otherwise similar bone marrow toxicity or therapeutic effect. Drugs can therefore be ranked according to their emetic potential using average clinical doses (See Table 2). The cytotoxic drugswhich were chosen forthe present studies are Cisplatin, Cycloheximide, Nitrogen Mustard, Diacetoxyscirpinol and Emetine, substances which are highly emetic in man.

1.2.2.1.2 Cisplatin (Cis-diamminedichloroplatinum II, cisplatinum)

Platinum complexes were first synthesised in 1845 but their biological activity was discovered by accident in 1965

TABLE 2

The Relative Emetic Potential of Cytotoxic Drugs in Man

Approximate Emetic Ranking	Approximate Incidence of Nausea and Vomiting in Patients	Emetic Potential
Cisplatin	>90%	Greatest Emetic Potential
Cycloheximide		
Streptozotocin		
DTIC		
Nitrogen Mustard		
Cyclophosphamide	30-90%	
Mitomycin C		
Daunorubicin		
Adriamycin		
Ifosfamide		
5-Fluorouracil	<30%	
Cytosine Arabinoside		
Hydroxyurea		
6-Mercaptopurine		
Methotrexate		
Vincristine		
Vinblastine		Least Emetic Potential

(Adapted from Harris and Cantwell, 1986)

(Rosenberg et al., 1965). They did not however become widely available as therapeutic agents until the mid 1970's (Leh and Wolf 1976, Rozencweig et al., 1977). Cisplatin is an inorganic complex formed between platinum and chlorine and ammonium ions. It resembles the bifunctional alkylating agents derived from nitrogen mustards. Cisplatin inhibits DNA synthesis to a much greater extent than RNA or protein synthesis and it binds to DNA causing both inter and intra-strand cross linking. However, inhibition of protein synthesis occurs before DNA cross-linking takes place. It has been an extremely effective agent used alone or in combination with other cytotoxics, especially against solid tumour but studies with cisplatin revealed a wide variety of toxic reactions. Renal dysfunction was one of the principal side-effects but this has been largely overcome by mannitol diuresis. Kahn et al., (1978) showed that 21/28 subjects treated with $100mgm^{-2}$ i.v. vomited at between 2 and 4 hours and this emetic response persisted for up to 24 hours. Successful control of the major side-effects of nausea and vomiting has been difficult to accomplish (Cline and Haskeil, 1980, Laszlo, 1982 and Laszlo and Lucas, 1981).

1.2.2.1.2 Mustine (Mechlorethamine; Nitrogen Mustard)

Nitrogen mustard was one of the first really useful cytotoxic drugs to be widely used and it is the prototype for other alkylating agents like cyclophosphamide. These are highly reactive compounds with the ability to substitute alkyl groups (eg $R\text{-}CH_2\text{-}CH_2^+$) for H atoms of certain organic compounds.

Alkylation of DNA is the critical cytotoxic action, producing breaks in DNA molecules and cross-linking of twin strands thus interfering with DNA replication and transcription. Similar effects are produced by certain kinds of ionizing radiation so that alkylators are said to be 'radiomimetic'. Nitrogen mustard is also an aggressive vesicant and together with its relative, sulphur mustard, was investigated extensively during the first and second world wars for use as a chemical warfare agent (Beswick, 1983). Phillips reviewed a great deal of this toxicology and pharmacology work in 1950 (cited by Borison et al., 1958) and the important conclusions of these data were summarised by Borison and co-workers (Borison et al., 1958). They commented particularly on the parallelism of toxic effects between nitrogen mustard and ionizing radiation, i.e. the radiomimetic properties of the mustards.

1.2.2.1.4 Diacetoxyscirpinol (DAS, Anguidine)

The 12, 13-epoxytrichothecenes are a family of related sesquiterpenoid compounds produced by various species of fungi belonging to the genus Fusarium. Diacetocyscirpinol is a type A monocyclic trichothecene mycotoxin which was first discovered as a product of F. equiseti by Brian et al., in 1961, although it is produced by a number of related species. Such toxins are found naturally in diseased corn, barley, wheat, oats and other cereal grains. They are responsible for certain diseases in plants, animals and man (Bamburg and Strong 1971).

In man and animals trichothecene mycotoxins have been implicated in vomiting, diarrhoea, dermal toxicity, haematological disorders, immunological disorders and as being responsible for the clinical entity of alimentary toxic aleukia in man, caused by the consumption of overwintered wheat infected with fusarial fungi. In pigs they contribute to feed refusal and the emetic toxicoses.

In 1962 Haerri et al. showed that trichothecenes could be cytotoxic and inhibit tumour growth in animal studies. A variety of other studies confirmed this cytotoxic action in a number of systems (Liao et al., 1976). It has become established that the trichothecene mycotoxins exhibit radiomimetic injury to tissues. Toxicity has been primarily characterised by effects on the epithelial mucosa of the gastrointestinal tract and cellular injuries such as karyorrhexis in thymus, spleen, ovary, testis and lymph node (Ueno, 1983).

Trichothecenes were first observed to inhibit protein synthesis in mammalian cells in 1968 (Ueno and Fukushima). Diacetoxyscirpinol itself causes ribosomal disaggregation by inhibition of initiation. Basically all trichothecenes possess an ability to interact with the peptidyl transferase site on the 60S ribosomal subunit thereby inhibiting peptide bond synthesis (Cannon et al., 1976). As potent inhibitors of protein synthesis in eukarotic cells (e.g., verrucarin A is more potent than standard inhibitors like emetine) trichothecenes have come to be used as specific tools in biochemistry for elucidating the mechanism of protein biosynthesis in different systems.

Trichothecenes do inhibit RNA and DNA synthesis but this is thought to be a secondary effect consequent upon inhibition of protein synthesis (Liao et al., 1976).

A number of phase I clinical trials were carried out with Anguidine (diacetoxyscirpinol) (e.g., Goodwin et al., 1978, Belt et al., 1979). Nausea and vomiting were found to be major side-effects and were dose limiting. Phase II trials (Thigpen et al., 1981, Bukowski et al., 1982) failed to show the efficacy shown by the initial preclinical studies noted above and in view of the haematological, neurological and gastrointestinal side-effects it was abandoned as a therapeutic cytotoxic for cancer.

1.2.2.1.5 Emetine

Emetine is one of several alkaloids of the South American plant Uragoga or Cephaelis ipecacuanha which was first mentioned in the literature by Michael Tristram, a Portuguese monk working in Brazil in 1570, where it was used as a remedy for diarrhoea and bleeding (cited in Synek and Synek, 1969). Ipecacuanha had already been listed accurately in a medical treatise by Piso in 1658 but it was not until its import into France that it was popularised by Helvetius (1727) as a cure for dysentery. In 1817 very impure emetine was isolated from Ipecacuanha root but it was left to von Podwyssotzki (1875) to give a thorough description of the purified alkaloid in terms of its pharmacology, chemistry and physical behaviour. Circulatory effects of emetine in man were described by Wild in 1895. The use on a large scale of emetine in clinical medicine followed Vedder's discovery in 1912 of its amoebicidal properties and

Rogers' paper (1912) on the cure of amoebiasis by injections of emetine.

However, apart from its action as an emetic constituent of Ipecacuanha syrup and its inherent amoebicidal activity, emetine has been noted as having other therapeutic and pharmacological effects. These are reviewed by Syneck and Syneck (1969) and include antibiotic and antiviral properties.

Grollman (1968) showed that emetine was a powerful inhibitor of protein synthesis and nuclei acid biosynthesis, and further showed that inhibition of protein synthesis was a property of emetine that correlated closely with its aemoebicidal activity (Entner and Grollman, 1973). Attention has also been drawn by Grollman (1966) to the structural and functional similarities between the Ipecac alkaloids and the glutarimide antibiotics like cycloheximide. Both groups inhibit protein synthesis, have displayed anti-tumour activity and are emetic. In particular (±)-2, 3-Dehydroemetine (DHE) has been investigated for potential anticancer chemotherapeutic activity (e.g., Abd-Rabbo, 1969, Pannetiere and Coltman, 1971) and Grollman maintained in his 1966 paper that the then recently reported anti-tumour activity (e.g., Grollman, 1965) of emetine was consistent with inhibition of protein biosynthesis as the primary mode of action of the alkaloid. Interestingly the earliest reports of the cytotoxic action of emetine date from its recorded effectiveness in causing tumour-regression and amelioration of advanced malignancies (Lewisohn, (1918) quoted in Grollman and Jakowsky, 1974).

Lastly, a small number of reports have indicated that emetine may itself have a direct effect on neuronal transmission. Ng (1966) observed a blocking action of emetine on sympathetic nerve endings. Subsequently Achari et al., (1972), using a preparation of rabbit ileum showed that emetine will block adrenergic neuronal transmission but leave the tissues still responsive to noradrenaline. Chopra et al., (1927) had already observed increased tone and movements of the intestine with emetine and Achari et al., (1972) suggested that these gastrointestinal effects might be explained by these adrenergic neurone blocking effect. Emetine has also been suggested as an inhibitor of neuromuscular transmission by a tubocurarine-like effect (Ng, 1966).

1.2.2.1.6 Cycloheximide

Cycloheximide is one of the best known of the glutarimide antibiotics and also exhibits amoebicidal, anti-tumour and fungicidal activities (Grollman, 1966, Young and Dowling, 1975). Cycloheximide inhibits synthesis of protein in yeast, intact mammals and mammalian cell-culture systems. It acts on the 50S subunit of the ribrosome to inhibit the binding, movement and the release of tRNA. It is very similar, therefore, in its action to emetine (Grollman, 1966) although its action is reversible.

Cycloheximide has been widely used to explore the relationships between protein synthesis and such disparate physiological events as hormone action and cell injury by cytotoxic chemicals or X-irradiation (Lieberman et al., 1970).

It has been investigated as an antipyrogenic substance using fever induced in the rabbit as a model system; where it reduced fever due to injected leucocyte pyrogen and reduced protein synthesis in the hypothalamus when given by the ICV route (Cranston et al., 1981). Young and Dowling (1975) showed that it had antipyretic activity in patients with fever due to Hodgkin's Disease and a variety of other malignant neoplasms. In these patients nausea and vomiting occurred 5 - 60min following the drug given orally or i.v.. Effects were roughly dose related and at higher doses ($6mgkg^{-1}$) continuous nausea and diarrhoea became evident depending on route of administration. At the lower dose ($2mgkg^{-1}$) vomiting occurred after 5 - 60min whereas deffervescence was delayed for up to 180min.

This work of course arose out of the more fundamental discovery by Grahame-Smith (1972) that inhibition of protein synthesis, with cycloheximide, could inhibit such synthesis in the brain of the rat. Moreover, he demonstrated that CNS protein synthesis appears to be involved in neurotransmitter function and that inhibition of brain protein synthesis could have profound effect on behaviour via changes in the release of neurotransmitters, in this particular case of dopamine and 5-HT (see also Green et al., 1976, and Graham-Smith and O'Shaugnessy, 1985). It was this work that prompted Cranston's group to investigate the effect of inhibition of protein synthesis on the hyperpyrexia response and ultimately laid the foundation for the thinking upon which Harris (1982)

based his hypothesis that cytotoxic induced vomiting is mediated via the inhibiting effect of cytotoxic drugs on synthesis of a set of rapidly turning over enzymes which control the level of a critical endogenous 'emetogenic' neurotransmitter in the brain.

1.2.2.1.7 Mechanisms of Action

In 1958 Borison et al., reported that the effective emetic dose of nitrogen mustard in the dog was $0.5mgkg^{-1}$ and that ablation of the CTZ completely protected dogs against the early onset vomiting caused by this agent. In contrast to the dog, uniform effectiveness in the cat was only just attained by a dose of $5.0mgkg^{-1}$. The average latency at these doses for dog and cat was 12min (range 105 - 149) and 65min (range 15 - 150) respectively. Chronic CTZ ablation did not apparently protect cats against mustard induced vomiting. However, the cat was protected against mustard emesis by supradiaphragmatic section of the abdominal afferents in combination with dorsal rhizotomy or spinal cord transection. The effect of abdominal afferent section on mustard-induced emesis in the dog was not investigated in this group of experiments.

In 1953 Brand et al., reported on a preliminary set of experiments on a small series of cats where mid-collicular decerebration severely impaired the vomiting response to $5.0mgkg^{-1}$ of mechlorethamine administered intravenously.

Generally speaking, Borison et al., (1958) confirmed the finding that chronic frontal lobectomy reduces the incidence and increases the vomiting latency to mechlorethamine i.v. in

the cat. Borison and his co-workers suggested three possible explanations to account for these results, i.e., that the CTZ is stimulated directly by the drug, that the CTZ is stimulated indirectly by a substance released peripherally through the action of mechlorethamine (e.g. on the intestine) or that the CTZ is interposed in the afferent neural pathway from a peripheral receptor site. Obviously the effectiveness of the CTZ ablation in preventing emesis supports the first explanation. The second explanation is supported by the finding in the dog that, when mustard is denied access to the gut by tying off the blood vessels, emesis incidence was reduced (Houck et al., 1947). The third possibility remains a theoretical one substantiated only by the knowledge that there might be neural connections between the gastrointestinal tract and a medullary structure like the AP. It was also concluded that the forebrain was not acting as a central emetic site but exerted only a facilitatory effect on hind brain structures.

Subsequently Papp et al., (1966) induced emesis in the dog by intracerebroventricular administration of mechlorethamine hydrochloride ($0.5 mg kg^{-1}$) but with a mean latency of 3min (range 1 - 14). This vomiting was abolished by ablation of the AP and from these results it was concluded that the site of the emetic action of nitrogen mustard is the CTZ. More recently work with the synthetic cannabinoid Nabilone has complemented the original findings by Borison et al., (1958) which implied that the forebrain has a facilitatory role in vomiting possibly because the forebrain receives afferent

information from the gut which is then relayed back to the medulla (London et al., 1979).

Cyclophosphamide is by far the most effective member of the phosphoramide mustard series of cytotoxic compounds, analogues of nitrogen mustard. Although cyclophosphamide itself has almost no toxic activity (Cohen and Jao, 1970) phosphoramide mustard, one of its ultimate metabolites, produced in the liver upon activation, is thought to be the main cytotoxic component. Although phosphoramide mustard is a potent alkylator and has been found to cause nausea and vomiting in the cat (Fetting et al., 1982) and in clinical trials (Nathanson et al., 1967) the precise emetic factor has still not been identified.

In man emesis caused by cyclophosphamide is dose related (Borison and McCarthy, 1983) and the same turned out to be true for the incidence of emesis in ferret when the i.p. route was used (Andrews and Ostler, personal communication). Onset of vomiting via the i.p. route in the ferret was less than 30 minutes but nearly 2 hours by the i.v. route. In clinical practice using an i.v. infusion, vomiting is delayed for 6 - 12 hours. The dog has been reported to be unusually sensitive to cychlophosphamide (Friedman et al., 1979) and the emetic response in the cat inconsistent (Fetting et al., 1982). Fetting et al., suggested as a result of their work in the cat that phosphoramide mustard/cyclophosphamide either had a slow cummulative effect on the CTZ, or were acting elsewhere, e.g.,

on the gut or, indeed, were producing release of an agent from a remote site which then acts on the CTZ. However, some of the evidence did show that the CTZ was not essential for emesis after cyclophosphamide.

One study has looked at the mechanism of emetic action of the trichothecene mycotoxin Fusarinon-X, a type-B trichothecene (DAS is a type-A) which causes vomiting in dogs blockable by metoclopromide or chlorpromazine (Matsuoka et al., 1979). The authors suggest that this result implies that trichothecenes cause vomiting through stimulation of the CTZ, on the basis that at that time such compounds would have been presumed to act wholly as dopamine blockers in order to be anti-emetic at these doses. Other groups hoever,(e.g. Gylys et al., 1979) use exactly the same sort of argument about the effectiveness and site of action of metoclopramide and chlorpramazine to justify implication of a peripheral site of action for cytotoxic emetics.

Few results are available from work directed at the mechanism of cisplatin-induced vomiting. In 1979 London et al., found that Nabilone, a 9-tetrahydrocannabinol derivative of marijuana, gave good protection to cats challenged with cisplatin and suggested that this action was not on the CTZ but via the forebrain. However, Gylys et al., (1979), using cisplatin-induced emesis in the dog, failed to show any anti-emetic activity by Nabilone. Moreover, they found instead that metoclopramide was most effective against cisplatin in the dog whilst haloperidol and chlorpromazine were far less effective. The authors suggested

that a peripheral mechanism might therefore be involved in cisplatin-induced emesis. AL-1612, a potent antagonist of apomorphine-induced emesis showed no activity against cisplatin vomiting (Holmes and Gylys, 1973). Haloperidol failed to show superiority over chlorpromazine against cisplatin vomiting although it was many times more potent an antagonist of apomorphine vomiting in the dog. Metoclopramide failed to show greater potency than chlorpromazine although metoclopramide was 20 - 30 times more efficacious as an apomorphine antagonist (Pinder et al., 1976). These authors also described the lack of activity in their experimental models of Nabilone against cisplatin which is in conflict with the limited reports of its efficacy against cytotoxic induced vomiting (Herman et al., 1977) and the experimental evidence in the cat showing efficacy of Nabilone against cisplatin (McCarthy and Borison, 1981) which however did not agree with work in the dog (Stark, 1982).

Work reported in the cat (McCarthy and Borison, 1980 and 1984) and in the dog (Akwari et al., 1985) using cisplatin challenge against animals with the AP previously ablated has implied that the CTZ is a site of action for cisplatin. However, the latter study also points out the profound effect of cisplatin on gut motility (disrupting normal motility patterns and promoting oral migratory complexes, see also Akwari, 1983 and Florczyk et al., 1980) even in the absence of the AP and McCarthy and Borison in the former study warn against concluding that the CTZ in the site of action because of the relatively long latency of cisplatin. Conclusive proof

of the exact site of action of cisplatin in causing emesis was therefore lacking with both CTZ and the gastrointestinal tract being implicated as target organs.

An attempt at a summary of the evidence for various sites of action for a variety of types of cytotoxic agent is found at Table 3.

In 1982 two hypotheses were put forward to attempt a theoretical explanation of the facts that have been outlined above, including differing latencies to emesis among the cytotoxics and the variety of receptors that are blocked by the anti-emetic drugs used to ameliorate cytotoxic induced vomiting. Both studies make the initial assumption that cytotoxic-induced vomiting arises from stimulation of the CTZ (Borison and McCarthy, 1983).

In the first report (Peroutka and Snyder, 1982) conclusions are drawn about the possible efficacy of future anti-emetic drug combinations and by implication, the importance of certain neurotransmitter receptors at the AP, from the efficacy and potency of a variety of anti-emetics against different kinds of emesis. The authors point to the effectiveness of drug combinations in controlling cytotoxic induced emesis and conclude that a three pronged blockade of neurotransmitter types in the CTZ, namely Histamine (H_1) Muscarinic, Cholinergic and Dopamine (D_2), would offer the best protection against chemotherapy induced emesis. The second report (Harris, 1982) suggests that "cytotoxic drug-induced vomiting depends on the activities of rapidly turning-over

TABLE 3

The Site of Action Cytotoxic Agents

	Action at Chemoreceptor Trigger Zone	Action via Peripheral Pathways	Action via Higher Cerebral Pathways
Nitrogen Mustard	Yes (Dog) No (Cat)	N/K Yes (Cat)	N/K Yes (Cat)
Phosphoramide Mustard[1]	N/K	Yes (Cat)	N/K
Cyclophosphamide	?No (Cat)	N/K	N/K
Cisplatin	Yes (Dog) Yes (Cat)	?Yes (Dog)	N/K
Fusarenon X[2]	?Yes (Dog)	N/K	N/K

Key: "?" - source of data unreliable
"N/K" - not known, data not available

Footnotes:
1. The active principle of cyclophosphamide
2. A trichothecene mycotoxin

enzyme systems responsible for the breakdown of a critical neurotransmitter which in the absence of such enzymes increases in concentration to stimulate receptors in the CTZ."

The critical neurotransmitter was postulated to be an enkephalin. Since different agents would inhibit the synthesis of these enkephalinases at different points in their synthetic pathway this would explain why vomiting can be caused in 1 hour by cisplatin (enzyme inhibition by heavy metals) yet be delayed for much longer by the alkylating agents (when transcription of mRNA is affected). Antimetabolites which effect de novo DNA synthesis which is low in this situation are therefore found to be poor emetics.

Met-enkephalin and opiate receptors have been located in the human AP (Schwartz et al., 1986) and the emetic effects of opiates are well known. Harris discussed the link between the opioid and dopaminergic systems and thus the rationale for the use of dopamine blocking anti-emetics. He also attempted to explain the findings of Costello and Borison (1977), i.e., that opiate drugs block their own emetic effect by a separate anti-emetic action readily antagonised by naloxone. These findings are explained on the basis that there are at least 3 different types of opiate receptor, different ones being at the two sites of naloxone interaction; one receptor (μ) is best antagonised at the anti-emetic centre, and another (δ) is less well antagonised at the CTZ.

The argument is extended to explain why cannabinoids such as nabilone, whose effects are antagonised by naloxone, are effective anti-emetic agents by suggesting that cytotoxic

agents may gain access to the putative anti-emetic centre via the deficient blood brain barrier in the AP. By this route cytotoxic drugs made it possible that enkephalin production might be inhibited, thus potentiating effects already mediated by the AP.

In 1986 Harris extended the above ideas to postulate the importance of glial cells as a possible site for regulation of the emetic effects of cytotoxic drugs, pointing out that astrocytes possess dopamine receptors, dopamine-sensitive adenylate cyclase, synthesise gamma-aminobutyric acid (GABA) and take up GABA. He went on to suggest that part of the anti-emetic effect of steroids may be related to their ability to reduce leu-enkephalin release and stimulate glutamine synthetase; this could then direct glutamate from the GABA pathway thus increasing anti-emetic tone. As pointed out earlier (Borison and McCarthy, 1983) and reiterated in part by Harris (1986) these hypotheses do not take into account three important areas, viz:-

a. The possible role played by visceral afferent nerves in chemotherapy-induced vomiting despite the prediction that such chemicals act via the CTZ in the AP.
b. The complexity of synaptic transmission in the CNS; especially as approximately 30 substances (including choline esters, amines, amino acids, prostaglandins, peptides and nucleotides) have been implicated in the processing of information in the brain-stem nuclei involved in nausea and vomiting.

c. The problem of distinguishing between the effects of drug action on chemosensors and on the functional receptors in this area; complicated by the fact that the fenestrated capillaries of the AP must allow access to areas deep to it not normally open to circulating drugs or toxins.

The conflicting evidence described thus for varying mechanisms of cytotoxic drug-induced vomiting led Borison and McCarthy (1983) to state that such vomiting "might very well result from mixed input activation, that is from visceral sources and from the CTZ" leaving much open to resolution by future experiment.

1.2.2.2 Ionizing Radiation

1.2.2.2.1 Introduction

Radiation-induced vomiting has been reviewed recently by Barnes (1983) and Young (1986).

The harmful biological effects of ionizing radiation have been the subject of concern since the 1890's. Walsh first described acute constitutional symptoms in an X-ray worker in 1897. Nausea and vomiting occurs in patients undergoing radiation therapy and in the victims of exposure to nuclear weapons, detonators and nuclear reactor plant accidents. Such exposures have led to the description of a well defined pattern of signs and symptoms known as the Acute Radiation Syndrome (Court-Brown, 1953, Danjoux et al., 1979), [Fig. 1a]. The severity, scope and course of the symptoms that occur after exposure to ionizing radiation depend on the size of the dose, dose rate, radiation quality, portion of the body irradiated and sensitivity of the exposed individual.

Radiation sickness itself is characterised by three phases; an initial or prodromal phase, a latent period, and the manifest illness (Young, 1986), [Fig.1b].

Thus:-

a. Prodromal or initial phase - prodromal nausea and vomiting in man commonly begin within the first hour after irradiation recur in periodic bouts and peak 5 - 8 hours after exposure.

b. Latent period - a period of relative well-being devoid of major symptoms.

c. Manifest illness - the latent period delays the onset of this phase by days to weeks depending on the dose received. This terminal phase of radiation sickness has associated with it a secondary period of nausea and vomiting.

The work of this thesis is concerned only with radioemesis associated with the prodromal phase of the acute radiation syndrome and especially that occurring within hours of a single exposure to X-rays.

1.2.2.2.2 Dosimetry

Young (1986) points out that despite a wide range of individual sensitivities a latent period exists in man from 30min to 2¾hr between exposure and onset of vomiting. Establishing a scientifically rigorous dose response relationship to radiation-induced vomiting in man has been an almost impossible task because of individual variability compounding the paucity of reliable data. Benchmarks of

Figure 1 (a and b) ~~Acute~~ Radiation Symptoms in Man and the Components of the Acute Radiation Syndrome

a) **Acute radiation symptoms**

Nausea and
Vomiting - upper gastro-intestinal distress

Diarrhoea - lower gastro-intestinal distress

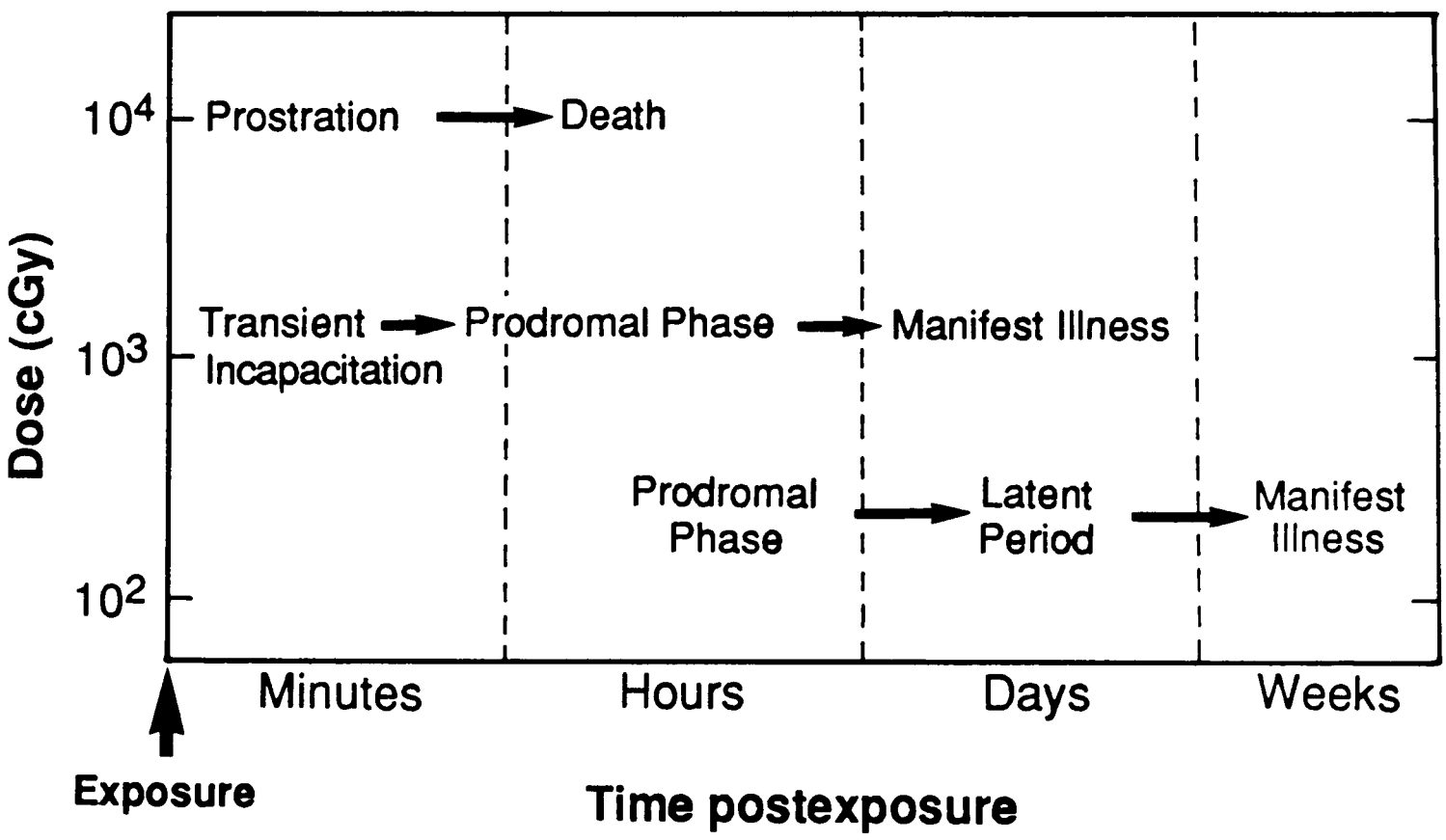
Fatiguability
Weakness

Hypotension
Dizziness
Disorientation

Infection
Bleeding
Fever
Ulceration

Fluid loss
Electrolyte imbalance
Headache
Fainting
Prostration

b) **Acute radiation syndrome**



(Reproduced from Young 1986, with permission)

800cGy and 100cGy for levels at which on the one hand most people will vomit, and on the other almost no people will vomit have been quoted (Young, 1986); Lushbaugh (1969) has estimated the median effective dose (ED_{50}) for vomiting in man to be 230cGy with 95% confidence limits between 177 and 334cGy. Recent evidence that clinical radiation exposures of 200cGy or less spare almost all vomiting (Barrett 1982) fits well with this estimate as does the evidence that above the currently accepted mid-lethal dose (LD_{50}) of 270cGy to the bone marrow (or 385cGy Free in Air) (Young, 1986) the degree of injury and individual susceptibility makes the accurate prediction of vomiting incidence almost impossible. However, yet more recently reviews of the evidence on the dose response relationship for radiation induced vomiting in man indicate that the ED_{50} may well be revised down to 180cGy (Young, personal communication).

Of the many factors that may affect the incidence of radiation-induced vomiting in man it should be noted that prodromal symptoms are produced by smaller doses when the epigastrium is irradiated than when the same volume of tissue is irradiated in other parts of the body (Gerstner, 1960).

At very high doses (of about 1000cGy or more) the pattern of radiation sickness alters slightly for in addition to decreased survival times, a period of transient functional incapacitation supervenes which precedes the prodromal emesis. Work in monkeys has shown an increasing incidence of incapacitation with increasing dose and it appears that consequently vomiting decreases. This is consistent with

observations that sedation and anaesthesia causing depression of the CNS can be an effective means of controlling radiation-induced emesis (Whitwam et al., 1978; Barrett, 1982).

The first work to investigate the mechanism of radiation-induced vomiting was carried out by Chinn and Wang in 1954, who showed that ablation of the CTZ by removal of the AP eliminated prodromal vomiting in the dog in response to 800cGy X-rays (Chinn and Wang, 1954). The response to oral copper sulphate remained largely unimpaired in these cases and the possibility that radiation might have a direct effect on the CTZ was deemed unlikely since shielding of the head did not prevent vomiting when the rest of the body was irradiated. In the monkey the response to 1200cGy X-rays was prevented by supradiaphragmatic vagotomy, but was also prevented by bilateral ablation of the AP, both of which methods were subsequently tested for their discreteness and lack of concomitant injury to neighbouring structures or fibre bundles. Again in 1958 Wang et al., observed that ablation of the CTZ consistently prevented the early vomiting response to 800cGy X-rays in the dog. Dogs with chronic AP lesions did vomit however, later in their survival period. They also found that abdominal vagotomy did not prevent early radioemesis in the dog or in the cat. No acute or delayed emesis was observed after irradiation in dogs with chronic AP lesions and abdominal visceral de-afferentation.

In the cat (Borison, 1957) it was observed that ablation of the CTZ prevented vomiting in only a minority of animals

exposed to 5500cGy. Prevention of radiation-induced vomiting by CTZ ablation did not, however, correlate with protection from apomorphine. Shielding of the abdomen prevented vomiting but shielding of the head did not.

Borison concluded from various lesions of the vagus and spinal cord in the same series, that the viscerosomatic afferent nerves' input from the upper abdomen was sufficient sensory source for radioemesis in cats and disallowed any essential role for the CTZ, crediting it only as being interposed in the afferent pathway of the radioemetic reflex. More recently Harding et al., (1985) published a study showing that discrete ablation of the AP abolished both radiation and apomorphine-induced emesis in dogs exposed to 600 and 800cGy and concluded that the CTZ is essential for radioemesis. Mention was also made in this study of the implication that radioemesis might therefore be mediated by a humoral factor.

There is a fundamental conflict in the results from different species, as one cannot ascertain from current data whether early post-irradiation vomiting is mediated by blood-borne emetic factors acting on the AP, by gastrointestinal afferent stimulation or a combination of both. Vomiting in the monkey following whole-body X-irradiation was first studied by Eldred and Trowbridge in 1954, the same year that Wang and Chinn published their work in the dog. All monkeys exposed to 600cGy vomited with none responding at 400 or 700cGy but a majority responding at 800cGy. Henscke and Morton (1957), however, found no emesis in monkeys exposed to doses of 600 - 900cGy, an anomaly thought to be due to

differences in fasting times. Middleton and Young (1975) studied the initial vomiting to supralethal doses from a neutron/gamma field of 763 - 5258cGy. The first appearance of vomiting occurred at progressively shorter intervals at doses up to 2000cGy with a minimum latency of 4min. Incidence rose up to 1000cGy after which it decreased with increasing dose (see previous discussion of transient incapacitation). In all groups most vomiting occurred in the 20 - 50min post irradiation interval. More recently, in 1980, Mattson and Yochmowitz determined the ED_{50} of ^{60}Co radiation for early radioemesis in the monkey using retching as the defined endpoint. The value obtained was 446 27cGy with an onset time of 40min. The LD_{50} for monkeys by comparison is 500 - 600cGy. Studies in the dog (Chinn and Wang, 1954; Cooper and Mattsson, 1979) using X-rays and ^{60}Co have given widely varying figures for the ED_{50} for vomiting of 540cGy and 258 38.5cGy respectively. At 800cGy all dogs vomited in the Chinn and Wang study with latencies of 9 - 107min. In the Cooper and Mattsson study latencies ranged from 37 - 244min with doses of 150 - 700cGy. Observations by Gralla et al., (1979) in the course of other studies revealed that the ED_{100} for dogs irradiated in the abdomen was 800cGy.

In the case of the cat, Borison's studies, (1957) using X-radiation have shown that it is relatively radio-resistant as a species compared with dog, monkey and man. Dose ranges of 500 - 5500cGy were used in the X-ray exposure producing an ED_{100} of 5500cGy and an ED_{33} of 500cGy with the incidence of vomiting related directly to dose and the latency inversely

related. Onset times for vomiting in these cats range from 4 - 63min at the ED_{90}.

Mention should now be made of studies that have looked at a few of the other aspects of radiation injury which may have a bearing on the genesis of vomiting, viz., the possible role of radiation-induced inflammatory effects and of peptides, the effectiveness of anti-emetics and the role of gastric motility.

1.2.2.2.3 Radiation-induced Inflammatory Effects

The involvement of inflammatory processes in post-irradiation vomiting is suggested by the success of anti-inflammatory drugs in controlling emesis. Steroids in particular have been found effective in a number of clinical studies (Barrett et al., 1979 and Barrett, 1982). Other studies have shown direct effects of radiation on the CNS causing oedema and vasculitis (Clemente and Holst 1954, Gerstner, 1956) together with evidence that non-steroidal anti-inflammatory drugs suppressed vomiting in patients (Stryker et al., 1979) and that radiation-induced delay in gastric emptying in rats was ameliorated by an anti-inflammatory drug combination. This suggests that control of inflammation in the gut and the CNS may be important in the prevention of radioemesis. It may be of course, that one mechanism may be more important than another in any given situation and this has been suggested by Young (1986) to account for the observation that Cordts (1982) made, in which a combination of cimetidine promethazine and thiethylperazine was effective in dogs irradiated with a gamma source but

ineffective in dogs irradiated with neutrons. Neutrons have already been associated with greater degrees of tissue damage and inflammation and have been estimated to be approximately three times more potent in producing the prodromal responses than gamma photons. Whether increased intracranial pressure due to radation-induced oedema plays a part in neutron radiation-induced vomiting, in particular, remains as yet unproved.

1.2.2.2.4 Radiation-induced Gastric Motility Changes

The interaction between radiation-induced changes in gastric motility and vomiting has been studied directly in the dog and monkey (Dubois et al., 1984 and Dorval, 1985) and indirectly in the rat (Hulse and Mizon, 1967, Hulse et al., 1977; Harding and Cairnie, 1980, Harding, 1981). Conditioned taste aversion induced by radiation has also been looked at as a possible model for radiation-induced vomiting (Cairne and Leach, 1982; Rabin et al., 1984). Here again we are presented with conflicting evidence from different species. In ^{60}Co irradiated dogs (800cGy) it was found that Domperidone pretreatment ($0.06mgkg^{-1}$ i.v.) suppressed vomiting but did not alter the accompanying radiation-induced delay in gastric emptying. Domperidone only reversed delay in gastric emptying induced by apomorphine or dopamine. Dubois concluded that dopaminergic receptors on the CTZ or the stomach were not involved in gastric delay under these circumstances and in addition that the two symptoms i.e. vomiting and gastric delay were independent.

In the monkey by contrast, Dorval et al. showed that

domperidone had no effect on vomiting either before or after irradiation with 800cGy of ^{60}Co. Young (1986) therefore suggested that there may be a fundamental difference in the involvement of the peripheral dopamine receptor in emesis for the two species; indeed it is already known that the dopamine receptor agonist apomorphine causes vomiting in dogs but not in monkeys (Brizzee et al., 1955).

1.2.2.2.5 Conditioned Taste Aversion as a Model for Nausea and Vomiting

Although rodents do not vomit, extensive studies have been carried out in irradiated rats using conditioned taste aversion (CTA) as a model for nausea and vomiting. Work has also been carried out in the cat on radiation-induced CTA (Rabin et al., 1986). The phenomenon of CTA to radiation was first described by Gavan et al., in 1955. Garcia et al., (1961) and Kimeldorf (1963) then suggested that CTA induced by radiation might be related to the concomitant delay in gastric emptying. This phenomenon was then studied in depth by Hulse and his associates (Hulse and Mizon, 1967 and Hulse and Patrick, 1977) and it was found that CTA to radiation could be induced in the rat, that whole body radiation was most effective and that the abdomen was most sensitive. The degree of aversion was found to be closely correlated with the severity of the radiation-induced delay in gastric emptying. This supported work published earlier by Barnes in 1962, which, after finding that CTA was much harder to achieve after de-afferentiation, concluded that the irradiation of the abdomen was the major factor in causing avoidance. Most

interestingly work by Hunt et al. in 1965 and 1966 quoted by Hulse and Mizon (1967) showed in parabiotic rats that when very large doses of whole body irradiation were used, CTA could be transferred from one animal to another thus raising the possibility that aversions might be initiated by a humoral factor. It was suggested further that the humoral factor did not act via the mechanism of delayed gastric emptying but operated independently. In their later paper Hulse and Patrick (1977) suggested a further possibility; that the gastric delay might be locally induced following cellular damage to the intestinal mucosa, which might release substances locally active on gut motility.

For the most part studies of CTA and radioemesis have focused on the role of the AP as a possible mediator of the regulation of both behaviours. Lesions of the AP have indeed been found to disrupt acquisition of radiation-induced CTA (Ossenkopp, 1983, Rabin et al., 1983) in the rat and protect against gastric stasis in the rat (Harding and Ossenkopp,1983). As has already been pointed out, such AP ablation did not prevent radiation-induced vomiting in the cat (Borison, 1957); however, recently Rabin et al., (1986) have suggested several reasons why this is so, viz., it may be that AP lesions are not effective in disrupting radiation-induced emesis in cats because the ED_{50} is so high (4500cGy) that other non-AP mediated mechanisms are at work here. Alternatively they suggest that because the CTA is a conditioned response to a conditioned stimulus while emesis is an unconditioned response to an unconditioned stimulus, totally different neural mechanisms may mediate the two responses.

Rabin and co-workers' (1986) conclusion from their experiments on cats irradiated at 100cGy and 4500cGy was that lesions of the AP do disrupt both emesis and CTA learning following radiation, consistent, they feel, with the hypothesis that both effects are mediated via the AP. Moreover, they comment that in the cat the dose and site of irradiation probably account for the differences between their data and that of Borison and co-workers. They point out that high-dose whole body irradiation was used by Borison et al. to produce emesis, conditions which might be expected to facilitate the involvement of mechanisms not requiring the AP for mediation.

1.2.2.2.6 Neuropharmacological Aspects of Radioemesis

Recently work has been carried out to investigate possible chemical mediators of radiation-induced vomiting (Carpenter et al. 1982, 1983, 1984). Systemically administered apomorphine, angiotensin II, neurotensin and leu-enkephalin produced dose-dependent emesis in dogs which was blocked by chlorpromazine (a dopamine antagonist) or AP ablation. Domperidone only blocked the response to apomorphine and saralasin only to angiotensin II and leu-enkephalin. Gastrin, substance P and VIP also produce dose dependent emesis but in addition marked signs of increased gastrointestinal motility. The responses of AP neurons in the anaesthetised dog were also tested by this group using some 17 neurotransmitters and peptides. Of the 13 that caused excitation and firing of AP neurons, 11 were inherently emetic and many have been found to be released by radiation or are

postulated mediators of radioemesis (histamine, dopamine, apomorphine, angiotensin II, neurotensin, leu-enkephalin, VIP, TRH, gastrin, vasopressin and substance P). Inhibition was seen with noradrenalin and histamine and no response with ACh, somatostatin and cholecystokinin none of which are emetic. The dog was chosen for this work because they judged it to be most similar to man in respect of their own determination of ED_{50} for vomiting, 258 ± 38.5cGy, the response to apomorphine and in histamine levels. Working through a series of compounds previously employed with varying efficacy as antiemetics clinically and experimentally, they first tried varying combination of drugs against 800cGy and then varied the radiation dose against which a single drug was tested. They clearly indicated in their results that a combination of drugs can be more effective than the same drugs given alone, but although the threshold for vomiting could be raised significantly, once this was passed the syndrome supervened as if the individual has received no treatment, indicating that not all emetic mechanisms had been suppressed. The combination that proved most effective was Cimetidine Promethezine and Thiethylperazine, producing blockade of H1, H2 and dopamine receptors and doubling the ED_{50} to gamma radiation. Curiously this combination of drugs was totally ineffective against neutron-induced emesis.

The most interesting novel candidate for a radiation-induced humoral factor has emerged during the last three years with the work of Harding and co-workers on Peptide YY (Harding et al., 1984). This intestinally derived emetic

factor originally obtained from a side fraction of porcine intestinal extract causes rapid vomiting in dogs (in 2 - 3min) which is blocked by AP ablation but not by Domperidone, spiroperidol or naloxone. This substance may well prove to be of importance as a mediator in the mucosa of the gut, at the interface with abdominal afferents or possibly as a truly humoral factor such has been implicated in the discussions above.

Some evidence for the possible neurotransmitters involved has been obtained from deductions drawn from experimental treatment studies. Perhaps the most useful in this regard are those carried out in the dog by Cooper and Mattson (1979) and Mattson et al., (1984).

In conclusion, we can say that evidence has been accumulated to support the idea that there is a 'hard-wired' pathway involved in the transmission of information about radiation-induced damage from the upper gastrointestinal tract to the brain stem vomiting centre. However, evidence from other animal models implies that the AP has a crucial role to play in conveying this information to the vomiting centre. What can not be guaranteed is that AP ablation would not remove information passing in afferents from the gut that travel through the AP to the 'vomiting centre'. If such a pathway were of crucial importance to the genesis of radiation-induced emesis, then it would be impossible to conclude from AP ablation alone that only a humoral substance was needed for this to occur. It is because so much of the work in the past has concentrated on the role of the AP in radiation-induced

vomiting, whilst freely admitting the importance of abdominal afferents that this project concentrated on the role of the abdominal vagal afferents.

Reference to the foregoing and the data summarised in (Table 3) reveals that there are many gaps in our knowledge and even that there are a variety of conflicting data from a number of animal species concerning the mechanism and site of action of cytotoxic drugs and radiation. It was therefore decided to approach the problem by performing one set of lesions in single species against a variety of stimuli in order to pick up common threads in the work and between this work and the results from other species some of which are more or less overtly similar phylogenetically.

1.3 Aims of the Project

In the light of the foregoing the principal aims of the project were seen to be the following:

a. To determine whether or not the ferret as could be an effective and reliable small animal experimental model for vomiting in man by testing, quantifying and characterising the response of the ferret to a variety of "central" and "peripheral" emetic stimuli.

b. To investigate the nervous control of cytotoxic drug and X-radiation-induced vomiting in the ferret model by -

(i) Observing and quantifying the effects of various anti-emetic drugs and peripheral autonomic nerve lesions on the response of the ferret to these emetic stimuli

(ii) Determining whether there are changes in the activity of brain stem neurones during emesis using 2-deoxyglucose autoradiography as a probe of brain function

1.3.1 Definition and Characterisation of a Novel Animal Model; Background of Use of Ferret for Biological Research

Three animal models have dominated research into nausea and vomiting for a century past, namely the dog, the cat and the primate (Borison, 1964 and McCarthy and Borison, 1981). Studies confining themselves to elucidating the structure and function of the AP have largely been carried out in the rat, a species that paradoxically does not vomit. Likewise research into conditioned taste aversion has concentrated on the rat. Studies on the physiology and pharmacology of gastro-intestinal function have been mainly carried out in carnivores and primates because of the similarities in the anatomy and histology of the GIT to that in the human.

The primate would appear to be the animal of choice for studies such as these but several factors mitigate against its universal acceptance, i.e., size may be limiting for certain types, many experiments are non-recovery in nature and large numbers may be needed, it does not respond to all known emetics (e.g. apomorphine) and the ED_{50} for radiation-induced emesis is approximately 450cGy (Mattson and Yochmowitz, 1980) *cf* 230cGy in man (Lushbaugh, 1969). As Borison observed (Borison and Borison, 1986) monkeys are, curiously enough, unlike their human relative, quite unresponsive to chemical emetic stimuli.

Brizzee also notes (Brizzee et al., 1955) "that the chemoreceptor trigger zone for emesis in the monkey is virtually non-functional from the standpoint of drug action" from his experiments in Macaca cynomologous and M. mulatta. Even with regard to motion sickness only the squirrel monkey has been found to be susceptible (Brizzee et al., 1955, Guignard et al., 1982) and had to be the monkey species of choice used by Brizzee and Dunlap (1983) in the first attempts to apply 2-DG autoradiography to the elucidation of the central control of vomiting in motion sickness. Moreover, accurate observation of vomiting as an endpoint of emetic stimulation is confused by the primate's tendency to hold vomitus in the buccal cavity for varying periods before ejection (Dubois, personal communication). Lastly, the availability and cost of using such animals can be prohibitive.

"The dog and the cat are most like man in their vomiting behaviour" states Borison et al., (1981) and it is against these that we must judge the suitability of any other model candidate. Unfortunately, the cat, although it responds to all known stimuli has an unusually high threshold to radiation-induced emesis, with the ED_{50} being of the order of 2000 - 5000cGy, an order of magnitude greater than for man. Size can also be prohibitive when studies using radiolabelled 2-DG are contemplated because of the large quantities required and consequent high cost of each individual experiment. Finally, the cost of supply is high (Bosley et al., 1983).

The dog has most often been the animal model of choice for vomiting studies in the past with references to work in this

area spanning the last 170 years (e.g., Magendie 1813 and Harding et. al., 1985). Its emetic responses are closely analogous to those in man (Borison et al., 1981), e.g. the emetic ED_{50} for radiation is 250cGy, response to cisplatin is predictable (Gylys et al., 1979) and apomorphine sensitivity is also comparable to man (Wang and Borison, 1952, Proctor et al., 1978). The dog, however, is an unsuitable animal for such studies for four reasons, i.e. for radiolabelled 2-DG studies, the high average weight of dogs means that each individual experiment is very costly, X-irradiation apparatus capable of receiving a dog are not widely available, the "unit" cost of the dog is very high, and lastly, the large number of animals required for such studies are simply not available. Leaving aside the arguments concerning the technical difficulties of irradiation and the cost of 2-DG experiments it was apparent that the other pressures were enough to affect large scale research when Florczyk et al., (1981) working on cisplatin-induced emesis in the laboratories of Bristol Myers Pharmaceuticals (USA), published the first abstract describing the use of the ferret as a substitute for the dog in vomiting research.

What was being sought was a carnivore with an upper gastrointestinal tract similar to man and vagal architecture of a similar type, in which there was good knowledge of gastrointestinal physiology and which vomited in analogous manner not only to man but to dog, the established model of choice. In the early 1980's two reports from the same group

appeared in the literature relating the same basic data on cisplatin-induced vomiting and proposing the ferret as this new animal model (Florczyk et al., 1981 and 1982). In these experiments, ferrets were challenged with subcutaneously administered apomorphine and intravenous cisplatin in an attempt to show that this species would vomit in analagous fashion to the dog and the cat. The authors concluded that while the ferret and the dog appear to have comparable emetic responses in these respects the ferret offers the advantages of lower cost and smaller size; these characteristics they concluded made the ferret more readily adaptable to a screening programme and indicated that the ferret might be a useful species in emetic testing of platinum analogues and evaluating antiemetic agents for protection against cisplatin-induced emesis. The authors also signalled their intention to challenge the ferret with other types of emetic agents in order to characterise further the ferret as an experimental animal for emesis research. Subsequently this group published results of experiments using dogs and ferrets in which they demonstrated the comparable antiemetic activity of butorphanol against cisplatinum-induced vomiting in both species (Schurig et al., 1982).

Exploitation of the efficient predatory behaviour of the ferret dates back 2000 years and its use was first formally recorded by Strabo in 63BC (cited in Andrews and Illman, 1987). Until relatively recently however it has not been widely used as an experimental animal for a variety of reasons; an unwarranted reputation for ferociousness, a requirement for a

carnivorous diet, being a seasonal breeder and there being no standard strains or breeds available to the researcher (Andrews and Illman, 1987).

Nevertheless discovery of the susceptibility of ferrets to canine distemper in the 1920's (Dunkin and Laidlaw, 1926) and the subsequent use of this species for distemper vaccine production initiated a series of events that changed the principal use of the domestic ferret from a hunting companion to a valuable model for biomedial science. Conversion of distemper vaccine production to tissue culture and the declining use of ferrets for this purpose occurred at the time when susceptibility of ferrets to influenza viruses was discovered (Smith, Andrewes and Laidlaw, 1933) and a variety of disciplines recognised the value of ferrets for research. Virological studies account for a great many of the ferrets used and attention has focused more recently on the use of organ cultures for screening anti-influenza drugs (Arroyo and Reed, 1977) and microbial agents (Mostow and Hopkins, 1979). Reproductive physiology has been a long term area of use for the ferret with the first report concerning its photoperiodicity appearing in 1932 (Bissonette). Some of the recent work on the control of gonadal function is reviewed by Donovan and Gledhill (1981). Dental research has been carried out in the ferret since 1947 when King studied the role of diet in the development of teeth and gums. This type of work continues today with recent publications on the pulpal response to temporary crown and bridge material (Tobias, 1980). Ferrets have recently been used to study the action of

teratogens and their use for this purpose has been reviewed by Beck (1978). Similarly Thornton et al., (1979) reviewed the use of the ferret as a new model in toxicological research. Studies by Truex et al., (1974) demonstrated the suitability of the ferret heart as a model for cardiac research and many pulmonary and cardiovascular studies have continued to be carried out throughout the 1980's e.g. defensive respiratory reflexes in ferrets Korpas and Widdicombe (1983).

Research on the digestive tract of the ferret has dramatically increased over the past fifteen years and the ferret is now well established as a useful animal for this type of research. The gross anatomy of the gastrointestinal tract has been described by Poddar and Murgatroyd (1977), and the gross and ultrastructural anatomy of the stomach by Stephens and Pfeiffer (1968). These authors comment on the striking similarities between the stomach of the ferret and that of man. Such similarities have made the ferret a particularly suitable model for the control of gastric motility (Andrews et al., 1980 and Andrews and Scratcherd, 1980), and such studies have also revealed useful analogies between the vagal system of man and the ferret (MacKay and Andrews, 1983 and Odekunle and Bower, 1985). Pure neurological studies have been fewer in number but overall are increasing (See Frederick and Babish, 1985) and visual physiologists in particular have been attracted by the ferret as a model (Thompson, personal communication); an increasing number of physiological and pharmacological studies have been directed towards the CNS of the ferret e.g., Bower et al., 1979, Fulker and Memrick-Luecke 1983.

1.3.2 Investigation of Cytotoxic and Radiation-induced Vomiting in a New Animal Model

1.3.2.1 Use of 2-Deoxyglucose Autoradiography as a Research Tool

Historically, studies of the central nervous system concentrated heavily on the localization of function to specific nuclei and mapping of pathways related to specific functions. These have been carried out neuroanatomically and histologically with staining and degeneration techniques, behaviourally with ablation and stimulation techniques, electrophysiologically with electrical recording and evoked electrical responses and histochemically with a variety of techniques including fluorescence and immunofluorescence methods and autoradiography of orthograde and retrograde axoplasmic flow. These methods can demonstrate only a potential for function; they do not reveal the physiological significance of a pathway.

The ability of the deoxyglucose method to map the entire brain for localized regions of altered functional activity on the basis of changes in energy metabolism offers a potent tool to identify the neural sites of actions of agents or stimuli with neuropharmacological and psychopharmacological actions. It does not, however, discriminate between the direct and indirect effects of the stimulus. An entire pathway may be activated even though the direct action of the drug for example may be exerted only at the origin of the pathway. Two simple premises provide the conceptual basis for this novel approach. First the energy requirements of cerebral tissue are derived

almost exclusively from the aerobic catabolism of glucose in a well-nourished state (Sokoloff, 1977, Siesjo, 1978). Second, functional activity within any region of the central nervous system is intimately and directly related to energy consumption within that region (Kennedy et al., 1975, Sokoloff, 1977). Thus the ability to determine the rate of glucose consumption simultaneously in all neuroanatomically defined regions of the CNS of conscious animals by the use of the 2-DG technique has been widely used to provide insight into the CNS processes.

The question of the relationship between local metabolic activities as revealed by the 2-DG technique and the electrical activity of the nervous system has been examined by Yarowsky et al., (1983) using the rat superior cervical ganglion as a model in which both the preganglionic input and postganglionic output can be isolated and electrically stimulated or monitored in vivo.

The results have indicated a clear relationship between electrical input to the ganglion and its metabolic activity, with glucose utilization in the superior cervical ganglion being enhanced by electrical stimulation of the afferent nerves. Similar effects of electrical stimulation on the oxygen and glucose consumption of the excised ganglion studied in vitro have been observed (Larrabee, 1958, Horowicz and Larrabee, 1958, Friedli, 1978). As demonstrated in the neurohypophysis (Mata et al., 1980) the effects of electrical stimulation on energy metabolism in the superior cervical ganglion are also probably due to the ionic currents associated with the spike activity and the consequent activation of the

sodium/potassium ATPase pump activity to restore the ionic gradients. It is likely that the increased extra-cellular potassium concentration and almost certainly the increased intracellular sodium concentration activate the sodium/potassium ATPase pump which in turn leads to increased glucose utilization.

1.3.2.1.1 Neurophysiological and Neuroanatomical Applications

Many of the physiological applications of the [^{14}C]-2-deoxyglucose method have been in studies designed to test the method and to examine the relationship between local cerebral function and metabolic activities. The most dramatic results have been obtained in the visual systems of the monkey and the rat. The method has for example, been used to define the nature, conformation and distribution of the ocular dominance columns in monkey striate cortex (Kennedy et al., 1976). There have also been applications of the method to other sensory systems; in studies of the olfactory system Sharp et al., (1975) have found that olfactory stimulation with specific odours activates glucose utilization in localized regions of the olfactory bulb.

Webster et al., (1978) have obtained clear evidence of selective regions of metabolic activation in the cochlear nucleus, superior olivary complex, nuclei of the lateral lemnisci, and the inferior colliculus in cats in response to different frequencies of auditory stimulation. Similar results have been obtained by Silverman et al., (1977) in the rat and guinea pig. Studies of the somato-sensory cortex have demonstrated metabolic activation of the "whisker barrels" by

stimulation of the vibrissae in the rat (Durham and Woolsey, 1977, Hand et al., 1978).

Thus far there has been relatively little application of the method to the study of motor functions. Kennedy et al., (1980) have studied monkeys that were conditioned to perform a task with one hand in response to visual cues; in the monkeys that were performing they observed metabolic activation throughout the appropriate areas of the motor as well as sensory systems from the cortex to the spinal cord.

An interesting physiological application of the [^{14}C]-2-deoxyglucose method has been to the study of circadian rhythms in the CNS. Schwartz and his co-workers (1977, 1980) found that the suprachiasmatic nucleus in the rat exhibits circadian rhythmicity in metabolic activity. These studies have been extended to natural sleep in monkeys with results that have demonstrated that during slow-wave-non-REM sleep, glucose utilization is depressed by about 25 -30% throughout the central nervous system (Kennedy et al., 1982).

1.3.2.1.2 Neuroendocrinological Applications

Studies are not extensive in this area but the hypothalamic-neurohypophyseal axis has been examined under challenge by salt-loading, showing increased glucose utilization in the posterior pituitary (Schwartz et al., 1979). Studies of the effect of hypothyroidism on the development of rat brain have also been carried out. Dow-Edwards et al., (1982) described metabolic changes consistent with the histological pattern of impaired brain

development in "cretinism". The 2-DG method has been used to demonstrate selective metabolic activation in a number of specific structures in the female rat brain elicited by vagino-cervical stimulation (Allen et al., 1981) and in an attempt to identify regions of the brain affected by oestrogen and progesterone, hormones that have potent influences on the CNS and the hypothalamus in particular.

In certain pathophysiological states with no concomitant tissue damage attempts have been made to apply quantitative and qualitative 2-DG autoradiography. For example, the 2-DG technique has been used to map the spread of seizure activity within the brain to identify structures with altered functional activity during seizure. Penicillin-induced focal motor seizures in the monkey have been studied in this way (see Sokoloff, 1984) as well as seizures produced by electroconvulsive shock in the rat (Engel et al., 1978). The phenomenon of spreading cortical depression provoked by local application of KCl on the dura or pia overlying the parietal cortex of conscious and anaesthetised rats has been studied by Shinohara et al., (1979). Several studies of the opening of the blood brain barrier have been carried out in the rat using unilateral carotid injection with a hyperosmotic mannitol solution to provide unilateral opening of the barrier. This has led to widely distributed, yet discrete regions of intensely increased glucose utilization in the ipsilateral hemisphere (Pappius et al., 1979).

1.3.2.1.3 Pharmacological Applications

The primary aim in the majority of neuropharmacological

investigations which utilized the deoxyglucose technique has been to characterise the functional consequences associated with a particular neurotransmitter system. Widespread use has been made of the technique for pharmacological applications; for example dopaminergic influences upon cerebral glucose utilization have been extensively examined. A number of general conclusions have arisen from these investigations. For instance, alterations in local glucose utilisation are highly restricted in distribution (McCulloch et al., 1982) but at the same time these alterations are not necessarily confined to those regions which are known to receive a dopaminergic innervation (Lindvall and Bjorklund, 1978) or to contain specific dopaminergic receptors (Creese, 1982). Procedures which result in decreased dopaminergic receptor activation generally result in decreased glucose utilization whereas dopaminergic receptor activation generally leads to increased glucose utilization. These observations appear to contrast sharply with the electrophysiological view of dopamine as an inhibitory neurotransmitter! It is not appropriate here to go into detail on all the areas of pharmacology, which have been pursued using the 2-deoxyglucose probe, but among the main influences on glucose utilization in the brain that have been studied are noradrenergic, cholinergic, peptidergic and GABAergic systems. In addition, general anaesthetic agents such as halothane, barbiturates, urethane and ketamine have been investigated and, indeed, it was Sokoloff and associates who, in 1977, carried out the first quantitative demonstration of a function-related alteration in glucose utilization when

they reported the widespread depression in glucose utilization which occurs in light thiopental anaesthesia (Sokoloff et al., 1977).

1.3.2.1.4 Studies of the Brain Stem and Vomiting

In marked contrast to the increasing number of studies being carried out with 2-DG autoradiography in many fields of research there is, to date, only one published study directly involving the vomiting reflex (Brizzee and Dunlap, 1983). Indeed there are relatively few that concentrate at all on the brain stem. Greatest interest here has been shown in functional mapping of the cardiovascular reflexes (Kostreva, 1983) with work being done in the rat (Savaki et al., 1982, Ciriello et al., 1983) and dog (Kostreva, 1982). In Kostreva's studies in particular it is relevant here to note that not only were the medial, dorsal and dorsolateral subnuclei of the NTS found to be important medullary targets for cervical vagal and carotid sinus afferents, but that the AP was markedly activated by the vagal pressor response elicited by electrical stimulation of the central cut end of the cervical vagus. Two papers (Sharp 1976 and Gonzalez et al., 1986) (the former using a combined [^{14}C] and [^{3}H] 2-DG technique and the latter [^{14}C]-2-DG alone) have been published on topics relevant to studies in vomiting physiology. Sharp describes rotation-induced increases of glucose uptake in rat vestibular nuclei and vestibulocerebellem. The regions of increased 2-DG uptake correlated best with major sites of termination of primary vestibular afferents; local increases occurred in the

vestibular nuclei, flocculus, nodulus, ventral uvula and accessory paraflocculus of the cerebellum. Changes in the nodulus were easiest to characterise and revealed a distinctly non-homogeneous pattern of increased glucose consumption which was confined to the granular layer. In animals that are capable of vomiting (unlike the rat) it is ablation of this area and the uvula which results in apparent immunity to motion sickness (Tyler and Bard, 1949) (but see Miller and Wilson, 1983). A modification of the original Sokoloff technique involving the use of [^{3}H]-2DG was aimed at achieving the greater resolution required to assign functionally related changes to the neuronal perikarya-rich granular layer and/or the neuropil-rich molecular layer of the cerebellum.

Gonzalez' [^{14}C]-2DG study is relevant in a different way because it attempted to look for changes in the brain stem nuclei, in particular the NTS, as a result of stomach distension in the urethane anaesthetised rat. Significant increases in 2-DG uptake were noted in the dorso-medial subnucleus and lateral commissural NTS. Possible activation of the dorsal motor nucleus of the vagus (DMVN) was noted but was not statistically significant. It is interesting to note that maximal activation of the NTS along its longitudinal axis occurred in the region of the AP. Unfortunately the fact that the distension stimulus was excessive detracts from the value of the experiment. The general result of this experiment tends to confirm that gastric distension information is conveyed to the brain at least partly through the vagus

nerve (Clarke and Davison, 1976, Iggo, 1955, Iggo, 1957, Paintal, 1964, Paintal, 1973). These findings are also compatible with present anatomical knowledge since the cervical vagal trunks are known to project to the NTS in the rat (Contreras et al., 1982, Leslie et al., 1982) and other species (Beckstead et al., 1979, Gwyn et al., 1982, Norman and Bower 1982) including the ferret (Odekunle and Bower 1985). Furthermore, neural connections from the stomach to the NTS via the vagus have also been demonstrated (Gwyn et al., 1979, Leslie et al., 1982, Scharoun et al., 1984) and the localization of the gastric distension-induced activation within the dorso-medial and commissural subnuclei of the NTS is consistent with these data.

Brizzee and Dunlap undertook to use a sequential double label ($[^{3}H]/[^{14}C]$) 2-DG technique to map the structures throughout the pons and medulla oblongata which are activated by motion patterns that elicit emesis in squirrel monkeys (Ordy and Brizzee, 1980, Brizzee et al., 1980). Significant selective increases in 2-DG uptake were revealed in the vestibular nuclei (medial and inferior) which agrees with the later work by Gonzales et al. (1986) in the rat, a species which does not vomit however. 2-DG uptake appeared to be increased in areas including the AP and the NTS but technical limitations meant that these changes were not statistically significant.

The double-label technique employed by Brizzee and Dunlap is an uncommon modification of the Sokoloff 2-DG method proposed by Agranoff et al., in 1980.

Little work at all had been attempted up to the time of Brizzee and Dunlap's work using the ferret in 2-DG experiments except in vision research (Thompson, personal communication). However it appeared that the ferret would offer itself to a 2-DG study of the central control of cytotoxic and radiation-induced vomiting, although it would be necessary concurrently to establish the viability of the technique in this species as well as assess the applicability of this form of neurophysiological investigation to the act of vomiting.

1.3.2.2. Use of Nerve Lesions and Drug Interventions

At the centre of the vomiting reflex lies the gastrointestinal tract and although vomiting continues in its absence, the gut remains the organ around which the reflex, in its present form has developed. Drawing on the ideas of the previous authors (e.g. Hatcher and Weiss, 1923), Davis et al., (1986), proposed the view that the defence of the organism against toxins is organised into a tiered system. This hierarchy of defensive measures is designed to protect the organism against increasing penetration by toxins of various types.

The mucosa of the gastrointestinal tract plays a particularly important part in this system because although it represents the second layer of defence, it is the last barrier to toxic molecules before they enter the body of the organism proper (Stewart, 1983). The gut, then, functions as both the absorptive site for food and a sensor mechanism for recognition of potentially toxic material.

Disordered gastrointestinal motility has long been suggested as being at the basis of the aetiology of several types of vomiting. A number of cytotoxic drugs, (e.g. cisplatin) disrupt gastric pacemaker potentials and initiate retroperistalsis (Akwari, 1983), both of which would lead to abnormal visceral information reaching the CNS and hence evoke vomiting (Andrews, 1986). Likewise, exposure to ionizing radiation has been noted to cause reduced gastric emptying and augmented spontaneous motor activity of the small intestine (Dubois et al., 1984, Dorval et al., 1985, Hulse and Patrick, 1977). Irradiation of the epigastrium in particular seems to give rise to the greatest susceptibility to emesis (Danjoux et al., 1979). Moreover since some cytotoxic compounds may be regarded as radiomimetic e.g. diacetoxyscirpinol, nitrogen mustard (Ueno, 1983, Borison et al., 1958) and alternatively radiation may be viewed as a cell poison or toxin (Straube and Patt, 1963), there exists the intriguing possibility that substances released from cells by both types of toxic attack are, at some point after release into circulation, capable of affecting the gastric detection mechanism from its serosal as opposed to its mucosal side.

It is with the important, yet unclear role (Young, 1986) of the abdominal vagal and splanchnic nerves in mind that we set out to explore the effect of section of these nerves on the vomiting response of the ferret. Researchers had previously concentrated their efforts on investigation of the effects of AP ablation, as emphasis had appeared to shift from the

periphery to the CNS after the delineation of the CTZ within the AP and its distinction from the 'vomiting centre'. Few papers have been published (exceptionally for instance Sharma et al., 1972, Zabara et al., 1972, Carpenter et al., 1986 and Kayashima and Hayama, 1976) which examine the effect of abdominal vagal section on vomiting since the series by Borison and his co-workers in the 1950's (e.g. Wang and Borison, 1951 and Brizzee, 1956).

Concentration on the AP and the effect of its destruction on various types of vomiting has however produced yet more controversy because the procedure is a technically difficult one and brings with it no guarantee that the animal will even survive the surgery on the brain stem because of potential damage to neuronal systems controlling respiration for instance. Arguments continue as to the ability of any one method to produce a discrete lesion (Harding et al., 1985, Carpenter et al., 1986) which does not affect the area subpostrema/medial nucleus of the tractus solitarii. Even having overcome these difficulties one must still take account of evidence that some vagal afferents may be passing through and are certainly passing to the AP (Leslie, 1986).

Vagotomy was therefore chosen as the primary approach to the surgical interruption of the information flow into the "vomiting centre".

The principal methods of pharmacological intervention employed were domperidone (a benzimidazole derivative and peripheral dopamine receptor blocker) and metoclopramide (a procainamide derivative with peripheral and central dopamine

antagonist activity) both of which are in widespread clinical use). Both drugs have been considered among the most effective anti-emetics which were minimally sedating. Varying success had been achieved with these drugs against a selection of cytotoxic drugs and X-radiation. Cisplatin-induced vomiting had proved particularly refractory to most anti-emetics but intravenous metoclopramide proved more effective against this stimulus (Strum et al.,1981) and experiments with dogs (Gylys et al.,1979) showed similarity in the effect of metoclopramide against cisplatin in man and dog. It had also been noted as an effective anti-emetic against radiation-induced vomiting (Harrington et al.,1983). However it appeared that only the higher doses of metoclopramide would be efficacious for practical clinical purposes against the most highly emetic chemotherapeutic regimes (Gralla et al., 1981). Effectiveness unfortunately brought with it some toxicity in the form of the side-effects, sedation, diarrhoea and extrapyramidal reactions. Metoclopramide was viewed then as a dopamine antagonist (Justin-Besancon and Laville, 1964, Peroutka and Snyder 1982) which exerted its anti-emetic activity through the CTZ (Seigel and Longo, 1981). Nevertheless in addition to the "central" effect, peripheral activity was thought to add to its anti-emetic efficacy both through a direct effect (e.g. prevention of copper sulphate-induced vomiting) and through enhanced gastric motility (Pinder et al., 1976). It was only the emergence of a satisfactory anti-emetic effect against highly emetic chemotherapy when high dose intravenous metoclopramide was used

that pointed the way to an anti-emetic action that was not mediated by dopamine receptors and which is now thought to be $5HT_3$ receptor mediated. The role of $5HT_3$ receptors in radiation and cytotoxic induced emesis is discussed later in the light of our own work on $5HT_3$ receptor antagonists.

Dopamine has been proposed as an inhibitory neurotransmitter in the gastrointestinal tract. Domperidone is a gastrokinetic with anti-emetic properties which selectively inhibits the relaxation induced by intra-arterial dopamine (Van Neuten, 1978) in in-vitro experiments on the isolated guinea-pig stomach, suggesting that dopamine is involved in the local feedback control of gastric motility and that the gastrokinetic effect of domperidone could be explained by its interference with dopamine receptors at the level of the stomach. The major criticism for a physiological inhibitory role for dopamine comes from the observations that after extrinsic denervation neither dopamine nor tyrosine hydroxylase activity can be found in the gastrointestinal wall. Furthermore it has been shown in dogs that the dopamine agonist apomorphine produces prodromal gastric relaxation by stimulation of a specific dopamine receptor in the CTZ (Blancquaert et al., 1982, Lefebvre et al., 1981) and that emesis in dogs induced by this mechanism can be effectively prevented by domperidone (Niemegeers et al., 1980) which is known not to cross the blood brain barrier (Laudron and Leysen, 1979).

Controlled studies with domperidone in man indicate that it is effective against vomiting caused by moderately emetic

cytotoxic drugs (Brogden et al., 1982) but less effective against substances like cisplatin and nitrogen mustard (mechlorethamine or mustine) where it proved no better than the low dose metoclopramide regimes. Side-effects have been few and in particular extrapyramidal reactions have been few and minor (Haase, 1978) as one would predict from a drug that does not cross the blood-brain barrier. However, more recent information indicates that at high i.v. doses cardiac arrythmia may occur and warnings have been given about high i.v. dosage (Joss et al., 1982). Several studies have indicated that domperidone may be useful in reducing radiation-induced vomiting in man but most of these employed the i.v. route which has now been declared unsafe at higher doses. Only one study using the oral route of administration is recorded. Good results were achieved in 80% of patients with 10 - 20mgkg^{-1} domperidone (Bernier and Juys, 1979). More recent reports do not support these findings (Barret, quoted in Harding and Davis, 1986).

The foregoing suggested therefore that there might be two distinct actions of drugs used in the treatment of upper gastro-intestinal disorders. Firstly, antagonism of dopamine-induced responses can prevent vomiting caused by stimulation of the CTZ; antagonism may also enable a drug to increase motility on occasions when normal motility is suppressed. Into this category both domperidone and metoclopramide fall. Secondly, possible stimulation of gastric motility by mechanisms which may not depend on antagonism of responses to dopamine but perhaps upon

influencing the release of acetylcholine (e.g. Hay and Man, 1979, McClelland and Sanger, 1983). Into this category comes metoclopramide. As a result of the discovery of anti-serotoninergic properties of metoclopramide at high doses it was then suggested that modulation of A.Ch release might be carried out by stimulating 5HT receptors (Kilbinger et al., 1982).

Conclusion

The anti-emetic effects of high dose metoclopramide have often been ascribed to its anti-dopaminergic activity or to an undefined action on the vomiting centre. However, more recent pharmacological studies (Fozard, 1984) have given rise to the possibility that high dose medoclopramide acts as a $5HT_3$ receptor antagonist. $5HT_3$ receptors to date have only been located on peripheral neurons (Fozard, 1984) e.g. the vagal nodose ganglion. Thus it is possible that the anti-emetic effect of metoclopramide may reside in its peripheral anti-5HT action rather than a dopaminergic one or indeed in any hitherto unknown site of action. These possibilities are explored in the present work using high dose metoclopramide in the ferret and in the latest stages more selective and potent $5HT_3$ receptor antagonists which became available in the course of the final year of the experimental work i.e. BRL24924 and BRL43695. Domperidone was also investigated in this new animal model so that data could be compared with the many previous studies in other animal models.

CHAPTER 2

MATERIALS AND METHODS

"Of all the natural phenomena to which science can turn its attention, none exceeds in its fascination the working of the human brain. Here, in a bare two-handsful of living tissue, we find an ordered complexity sufficient to embody and preserve the record of a lifetime of the richest human experience. We find a regulator and co-ordinator of the hundreds of separate muscle systems of the human body that is capable of all the delicacy and precision shown by the concert pianist and the surgeon. Most musterious of all, we find in this small sample of the material universe the organ (in some sense) of our own awareness, including our awareness of that universe, and so of the brain itself."

D. MacKay, 1967

From the Preface to the 2nd Edition of Neurological Anatomy in Relation to Clinical Medicine by Professor A. Brodal

CHAPTER 2: METHODS

2.1 ANIMALS

2.1.1 Ferrets (Fig. 2)

Experiments were performed on 350 adult ferrets obtained from the University of Oxford Farm, Park Farm, (157 males, 143 females, 176 polecats, 174 albinos). The ferret (*Mustela putorius furo L.*) may be a domesticated form of the European polecat (*Mustela putorius putorius*) which has probably been bred for hunting in England since the Roman Invasion (Porter and Brown, 1985). Both albinos ('English ferrets') and non-albinos (Polecat or Fitch ferrets) with weights ranging from 500g to 1500g were used (weights vary throughout the year by up to 40%) (Shump and Shump, 1978) corresponding to a post-weaning age of at least four months.

The animals were housed either singly or in pairs in Marmoset-type cages (50 x 50 x 70cm) or cat-breeding type cages (53 x 105 x 50cm) in conditions of controlled temperature (21 ± 1°C) and lighting (0630 - 1830 hr).

Animals were fed daily, in the afternoon, on a mixture of commercial cat food, and Laboratory Diet B (a balanced pelleted diet), supplemented with freshly-killed mice and cows milk. Tap water was allowed *ad libitum*.

Subsequent to delivery, animals were allowed to settle for at least three days before experimental procedures were started. All experiments were performed between 09.00 and 18.00hr.

Figure 2 (a and b)

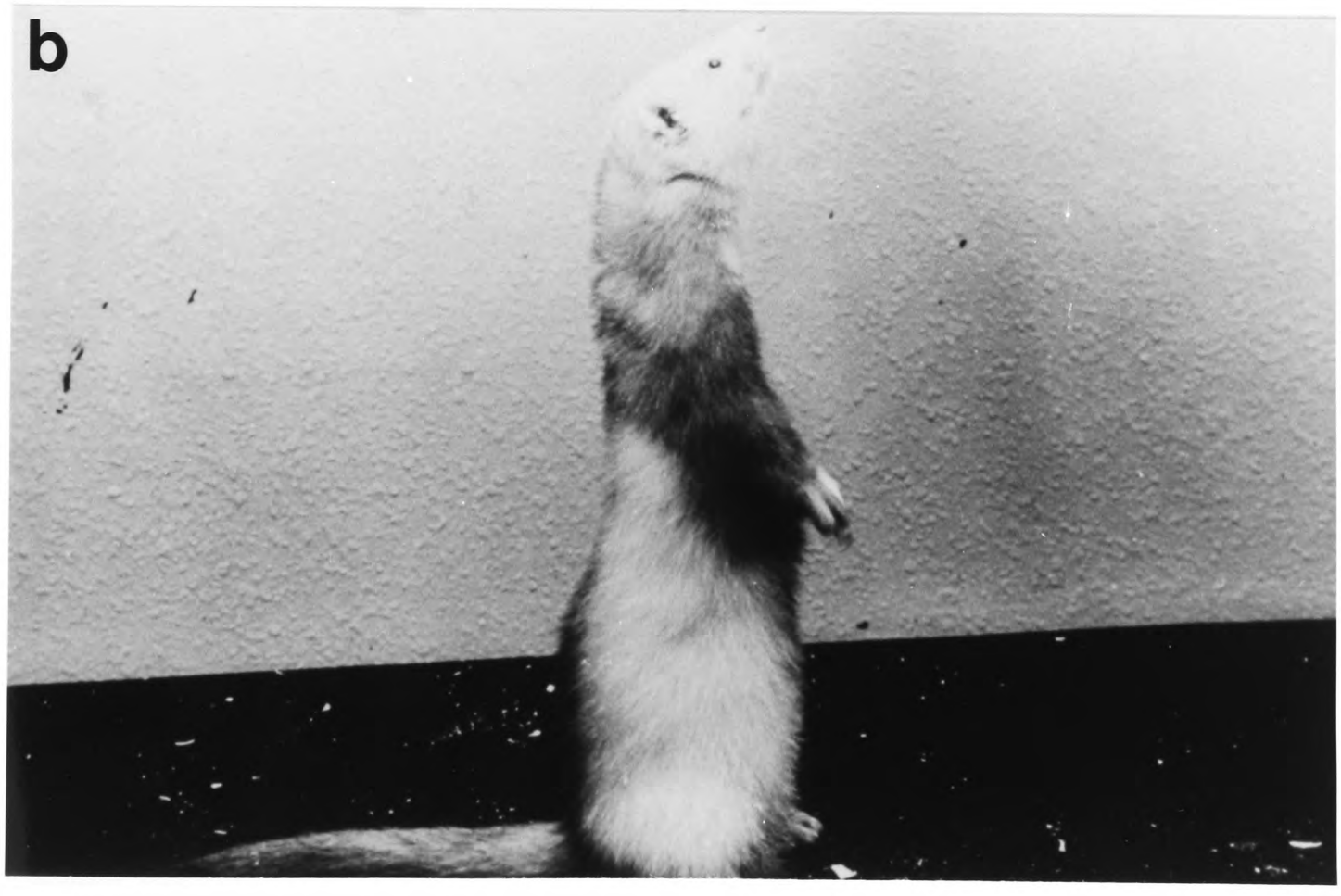
The Ferret - Mustela putorius furo L.

An experimentally naive 1kg male fitch (approx. 25cm body length) in typical postures

(a) Feeding behaviour

(b) Inquisitive behaviour


a


b

2.2. TECHNIQUES IN THE CONSCIOUS ANIMAL

2.2.1 Implantation of Venous Cannulae in the Ferret (Fig. 3)

Surgical anaesthesia was induced using ketamine hydrochloride (Ketalar, Parke Davis & Co. Gwent, $100mgml^{-1}$, $0.1ml kg^{-1}$ i.m. (Moreland and Glaser, 1985) and halothane (Fluothane, ICI Ltd) 1 - 4% with oxygen 2.0 $l min^{-1}$ and maintained using halothane 2% and oxygen 1.5 $l min^{-1}$, delivered via a face mask. The implantation procedure was a modification of that described by Florczyk and Schurig (1981).

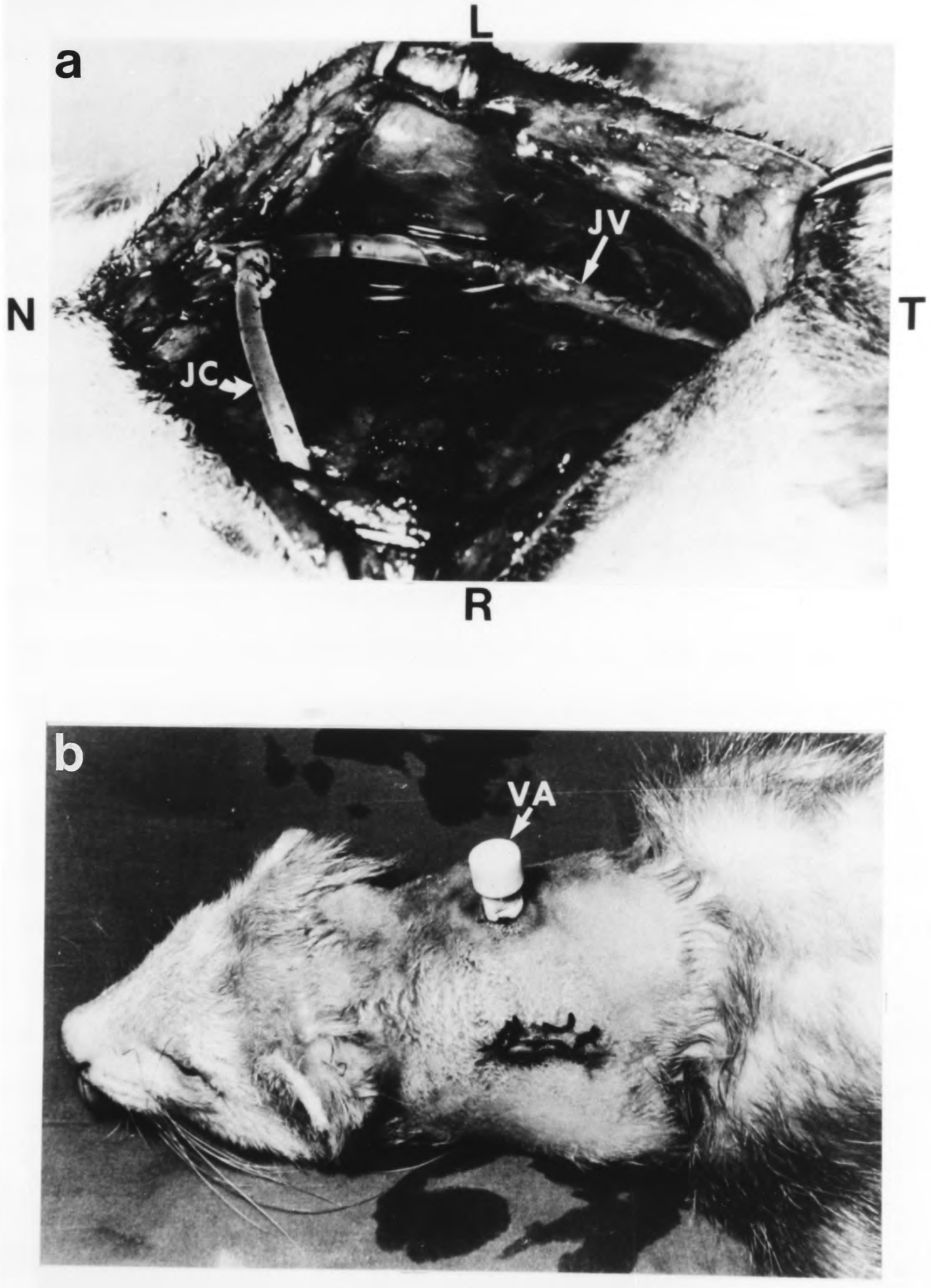
After surgical anaesthesia was attained (tested by a negative response to pinching the inter-digital webb) the ventral and dorsal surfaces of the neck were shaved and thoroughly cleansed with a bactericidal solution (Betadine Iodine/Alcohol Solution, Napp Laboratories, Cambridge). A 3cm incision was made in the ventral surface of the neck in the midline and using blunt dissection a subcutaneous tunnel was cleared around the right side of the neck to the dorsal surface taking care to avoid and pass superficial to the right external jugular vein. A 2cm incision was then made on the left side of the neck approximately 2cm lateral to the midline on the dorsal surface and a subcutaneous tunnel constructed by blunt dissection towards the midline to meet up with the subcutaneous tunnel on the right side. A medical grade silastic cannula (Dow Corning Michigan; internal diameter (i.d.) 0.76mm, external diameter (e.d.) 1.65mm filled with heparinised (20 units per ml) sterile saline (154mM NaCl) was passed through the subcutaneous tunnel with the aid of a pair of forceps. A Teflon and steel ball cat arterial valve

(Harvard Apparatus, Kent) was then attached to the cannula emerging from the dorsal incision. A 0.5cm incision was then made to the midline of the dorsum of the neck, into which the valve was positioned so that the base of the valve was flush with the skin. Then, using blunt dissection, a length of the left jugular vein was exposed and occluded at the cardiac end. After ensuring distension of the vein it was ligated with a 4/0 gauge braided silk suture (Mersilk, Ethicon, Edinburgh) at the cephalic end. A small incision was then made in the vein using irridectomy scissors and the ventral end of the cannula placed in the vein as far as the clamp. This was then removed and the cannula introduced for a further 4 - 5cm into the vein. Using the 4/0 silk suture previously attached to the cannula by silicone adhesive (Medical Adhesive Silicone Type A, Dow Corning, Michigan) it was then anchored to the underlying fascia. The cannula was further secured into position in the vein and to the underlying fascia using 4/0 braided silk sutures. The cannula was then flushed with heparinised saline and tested for patency by withdrawing blood. The skin incisions were closed with 2/0 braided silk sutures the cannula flushed and the valve then capped tight. Prior to closure of the incisions wounds were sprayed with an antibiotic aerosol preparation (Polybactrin Powder Spray (Bacitracin/Neomycin/Polymixin) Wellcome Foundation Ltd, London) and the closed incisions were treated with a prophylactic dose of antibiotic powder (Neomycin/Bacitracin) (Cicatrin, Wellcome Foundation Ltd., London).

Figure 3 (a and b) Implantation of a Venous Cannula in the Ferret

(a) Upper View: Operative field (5 x 4cm) showing exposure of left jugular vein (JV) with cannula (JC) inserted. Ventral surface

(b) Lower View: Neck region after completion of surgery with valve assembly (VA) in place
Key: N = Rostral, T = Caudal,
R = Anatomical Right, L = Anatomical Left


a
L
N
T
R
JV
JC
b
VA

2.2.2 Peripheral Nerve Lesions in the Ferret

2.2.2.1 Anatomy of the Vagus Nerve in the Abdomen of the Ferret (Mackay and Andrews, 1983)

Single ventral and dorsal vagal trunks arising from a simple supradiaphragmatic peri-oesophageal plexus are present at the level of the diaphragm and pass together with the oesophagus through the diaphragm into the abdomen. About 1cm caudal to the diaphragm the ventral trunk, found applied closely to the midline of the oesophagus, gives off its two constant divisions, namely hepatic and gastric. The hepatic division is visible against the caudate lobe of the liver as it passes in the upper part of the gastro-hepatic ligament. The gastric division is the direct caudal continuation of the ventral vagal trunk along the lesser curve of the stomach.

The dorsal vagal trunk crosses gradually from the right lateral margin of the oesophagus to lie on the right of the midline of the dorsal surface of the subdiaphragmatic portion of the oesophagus, closely applied, as in the case of the ventral trunk. The dorsal trunk gives off numerous small branches to supply the lower oesophagus and ends by dividing into two constant branches, the coeliac and gastric divisions, about 2cm caudal to the diaphragm. The coeliac division passes along the course of the left gastric vessels to reach the coeliac plexus. The dorsal gastric division is the direct caudal continuation of the dorsal vagal trunk passing over the dorsal surface of the stomach.

Macroscopically, in the abdomen of the ferret, the vagus is seen to supply lower oesophagus, liver and stomach, but

physiological studies have shown that its influence is exerted throughout the whole gastrointestinal tract including the colon (Collman et al., 1984).

2.2.2.2. Surgical Interruption of the Abdominal Vagi of the Ferret (Fig. 4)

Surgical anaesthesia was induced and maintained as described above. The ventral abdominal surface of the ferret was then shaved from the costal margin to the inguinal ligament and a midline laparotomy incision made approximately 8cm in length, subsequent to appropriate skin cleansing.

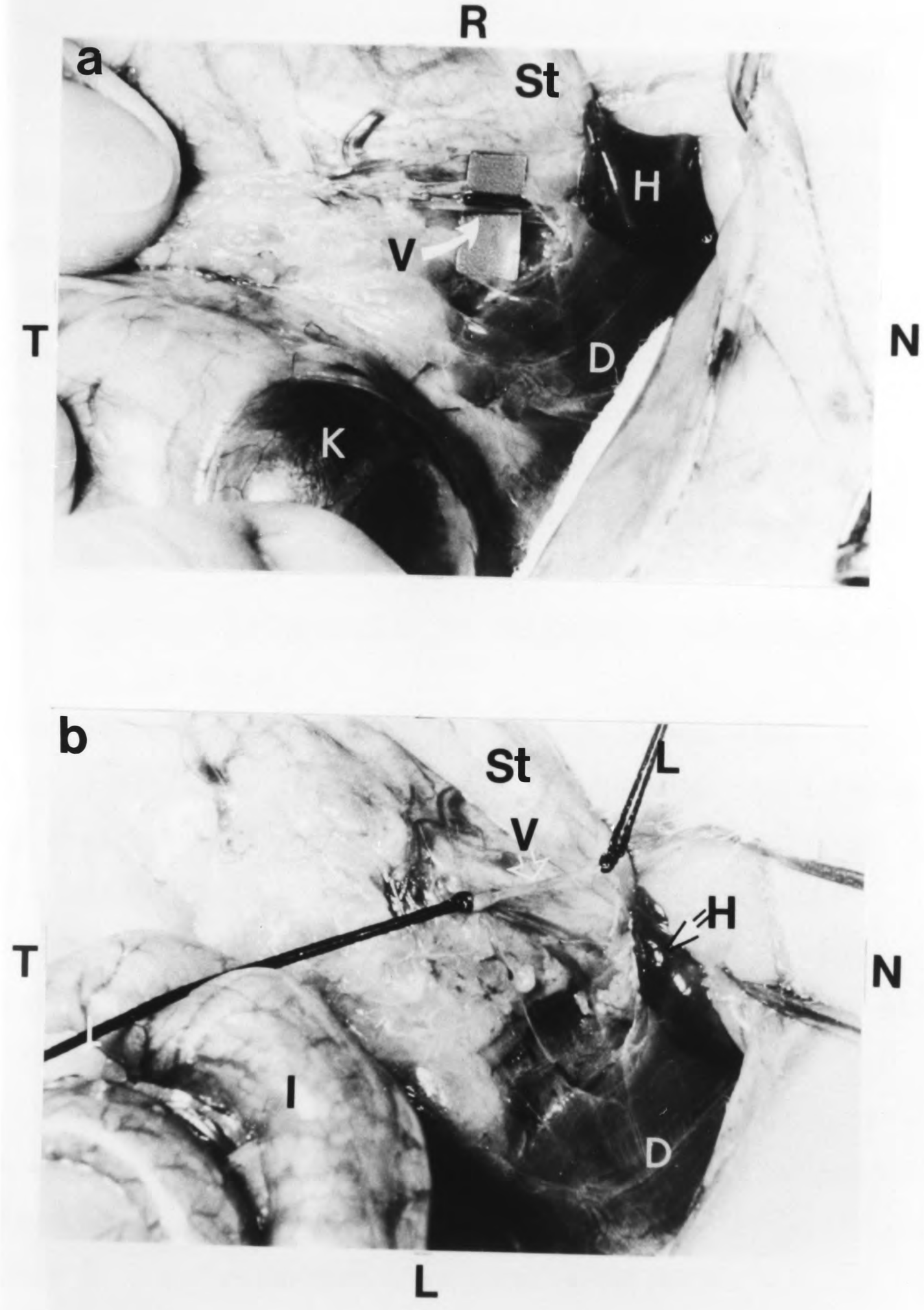
The ventral and dorsal vagal trunks were then located by blunt dissection, identified and a portion of the nerve at least 5mm in length resected as close to the diaphragm as possible. When required the hepatic branch of the ventral trunk was cut separately. The serosa of the lower oesophagus was also incised. The cut ends of the major trunks were ligated using 4/0 braided silk sutures and the abdominal contents moistened with a little sterile saline before being gently replaced within the abdominal cavity. The abdominal cavity was then sprayed with antibiotic aerosol (Polybactrin Powder Spray) before closure of the peritoneum and skin layers with 2/0 braided silk sutures. The skin wound was then dusted with antibiotic powder. Throughout the operative procedure the animal's temperature was monitored using a rectal temperature probe and maintained at a normal level for ferrets (37.8 - 40°C) with indirect heating of the operating table.

2.2.2.3 Anatomy of the Splanchnic Nerves in the Abdomen of the Ferret

The left and right greater splanchnic nerves (GSN) arise

Figure 4 (a and b) Abdominal Vagotomy in the Ferret

Operative fields in the abdomen showing the mobilization and exposure of dorsal vagus with associated blood vessels (upper view) and central vagus, ligated prior to sectioning (lower view), Key: R = Anatomical right, L = Anatomical left, T = Caudal, N = Rostral, St = Stomach, H = Liver, K = L. Kidney, Lig = Ligature, V = Abdominal Vagus, I = Small intestine, D = Diaphragm


a
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in the upper thorax at the level of the heart and course caudally to pierce the diaphragm where it adjoins the dorsal wall of the abdominal cavity.

The left GSN gradually passes medially to enter the left coeliac ganglion at the level of the coeliac artery. The right nerve, as it emerges from the diaphragm turns sharply towards the mid-line to enter the right coeliac ganglion which is often fused with the left ganglion.

Numerous nerves may be seen leaving the coeliac ganglion and coursing caudally to supply the adrenal glands, kidneys and colon. Other branches are directed towards the stomach. It should be noted of course that the GSN is pre-ganglionic in nature arising in the intermediolateral cell column of the spinal cord.

2.2.2.4 Surgical Interruption of the Greater Splanchnic Nerves in the Ferret

The left and right greater splanchnic nerves were located by blunt dissection, identified and a portion at least 2mm in length resected. The cut ends were then ligated using 4/0 braided silk sutures. The abdominal contents were then moistened, replaced and the abdominal cavity treated with antibiotic aerosol spray before closure of the peritoneum and skin as previously described.

2.2.2.5 Recovery from Anaesthesia and Post-operative Care

Animals were recovered under supervision with the aid of supplemental oxygen. On regaining mobility the animals were returned to their cages and allowed milk to drink.

In the case of the nerve lesions, following the operation, the animals' diets were restricted to 50% of their pre-operative level for one week. Subsequently diets were administered normally. This regime was imposed to minimise possible gastrointestinal motor problems known to occur in man associated with this type of lesion (Clark et al., 1964). No animal died as result of abdominal vagotomy or greater splanchnic nerve section after full recovery from anaesthesia and there was no morbidity associated with laparotomy or denervation of the gastrointestinal tract. This compares favourably with previous studies (Andrews et al., 1980). In those animals that were allowed to survive over a period of 3 - 4 weeks a small loss in weight was noticed in some of the animals but their health remained good.

In those animals with chronically implanted indwelling intravenous cannulae, the cannulae were flushed with 0.5ml of heparinised saline every other day to maintain patency.

2.2.3 Administration of Systemic Compounds to the Ferret

2.2.3.1. Intravenous (i.v.) injections of drugs were initially made directly into the cephalic vein on the dorsum of the forepaw of the ferret but this technique proved hazardous, required three animal handlers, and although successful was abandoned in favour of injection via a previously implanted cannula into the left external jugular vein. Administration of solutions was carried out by trimming down the tubing of a Butterfly-2 (Venisystems, Abbott Ireland Ltd, Ireland) to 1.5cm adjacent to the 'Luer' fitting and connecting this tightly to the adaptor of the cat arterial valve assembly. A syringe

filled with heparinised saline was then connected to the adaptor via the Luer fitting and the whole connected to the valve by screwing on the adaptor. This connects the cannula with the exterior and it is now flushed with 0.5ml of heparinised saline before administration of an experimental solution. After each administration the cannula was flushed with 0.5ml of heparinised saline. To carry out this procedure it was normal to employ two experimenters; one to hold and immobilise the ferret and the other to administer the solution.

2.2.3.2. Intraperitoneal injections in the ferret were carried out with one experimenter holding the ferret in the supine position to expose the ventral abdominal wall. The site for injection was chosen to avoid the large spleen, being half way between the costal margin and the inguinal ligament in the midline.

2.2.3.3. Subcutaneous injections in the ferret were made in the area below the dorsum of the neck between the shoulder blades whilst the animal was being held by one experimenter. Sites used in some cases were the skin of the shoulder and the dorsal surface of the forelimb.

2.2.3.4. A variety of substances were administered directly into the stomach of ferrets and a novel procedure was developed to accomplish this. A hole of approximately 0.7cm was bored in the centre of the cross bar of a polypropylene 'T' piece (Portex, Ltd., Hythe, Kent, 9mm i.d.). This was then placed in the mouth of the ferret with the cross bar across the angle of the jaw whilst a second experimenter held the ferret behind the angle of jaw so that its head was immobilised. A cannula

(Portex Size O/O, length 30cm) was then passed along the long arm of the 'T' piece through the hole in the cross bar and down the oesophagus into the stomach followed by administration of the test solution.

2.2.3.5. Intramuscular injections were made into the proximal muscles of the hind limb of the ferrets whilst they were being held immobilised by a second operator. Injection volumes were minimised in all cases and did not exceed 0.1ml.

2.2.4. Systemic Administration of Emetic Stimuli in the Ferret

All solution strengths were adjusted so that injection volumes did not exceed 2ml.

2.2.4.1 Intravenous Drugs

a. Apomorphine Hydrochloride (Apomorphine) was dissolved in 154mM sodium chloride ('normal saline') freshly for each administration and kept during use, in the dark and on ice. Doses of 10, 25, 50, 100 and 500μgkg^{-1} body weight were used.

b. cis-Platinum (II) Diammine Dichloride (Cisplatin) was dissolved in saline by agitation and gentle warming. Doses of 2, 8, 10, 12 and 20$mgkg^{-1}$ were used.

c. Mechlorethamine Hydrochloride (Mustine) was prepared as a solution by adding saline to individual sealed vials containing 10mg of the active compound. Dose of 400μgkg^{-1} and 1.2$mgkg^{-1}$ were used.

d. Peptide YY was prepared as a solution in sealed vials by adding saline. Doses of 1, 2, 5 and 10μgkg^{-1} were used.

In the case of mustine and cisplatin precautions against

contamination of the experimenter were taken which comprised, safety glasses, neck-buttoning laboratory coat, impermeable apron, surgical mask and surgical gloves, as both drugs are active through the skin, cause sensitisation and mustine is a powerful vesicant.

2.2.4.2 Intraperitoneal Drugs

a. Emetine was dissolved in saline and used at a dose of $20mgkg^{-1}$.

b. Cycloheximide was dissolved in saline and used at a dose of $20mgkg^{-1}$.

c. Cisplatin was dissolved in saline and used at a dose of $20mgkg^{-1}$.

d. DAS (Diacetoxyscirpinol or Anguidine) was dissolved in saline and used at a dose of $1.5mgkg^{-1}$.

Precautions against contamination were taken when cisplatin and DAS were being handled.

2.2.4.3. Subcutaneous Drugs

a. Apomorphine was prepared as previously described and administered in the same range of doses i.e. 10, 25, 50, 100 and $500\mu gkg^{-1}$.

2.2.4.4. Intragastric Compounds

a. Sodium chloride (NaCl) was dissolved in distilled water and used at concentrations of 0.154 0.5, 0.75 and 1M.

b. Copper sulphate ($CuSo_4.5H_20$) was dissolved in distilled water and used at concentrations of 10, 20, 40, 70, 100 and 150mg per 100mls of water (mg %). Although for consistency these concentrations should be expressed in molarity, we have used mg% to facilitate comparisons with the literature which without exception expresses dose in this manner.

c. Potassium Chloride (KCl) was dissolved in distilled water and used at a concentration of 1M. as was,

d. Choline chloride (Ch Cl)

e. Mannitol was dissolved in distilled water and used at a concentration of 2M.

f. D-Glucose was dissolved in distilled water and used at concentrations of 0.308, 1.0, 1.5 and 2.0M.

g. Syrup B.P. was obtained from the pharmacy of the Radcliffe Infirmary, Oxford. It represents a 66% solution of sucrose without preservative. It was used in this stock form (i.e. 100% Syrup B.P.) and as 25% and 50% dilution with distilled water.

h. Ipecacuanha paediatric emetic syrup B.P. (Ipecac') was obtained from the Radcliffe Infirmary pharmacy, Oxford. It was administered in several ways; as the full strength stock solution and at a dose of $0.43 ml kg^{-1}$ in 30ml of syrup B.P.

i. Emetine for intragastric administration was either dissolved in syrup B.P. at 150mg% concentration or dissolved in distilled water at 150mg% concentration.

In control experiments for intragastric challenges of emetic substances, cows milk and tap water were used. In all cases of intragastric challenge, the volume used was 30ml. This volume was chosen as it represents the quantity of fluid that can be drunk by a ferret with ease over a period of 10 min such that the stomach would be approximately one third full. This ensures that gastric distension is kept to a minimum whilst at the same time allowing complete bathing of the gastric mucosa with the infused test solution (Andrews et al., 1980).

2.2.5. Dosing Schedules for Anti-emetic Preparations in the Ferret

a. Metoclopramide was used direct from the manufacturers vial and injected subcutaneously at a dose of $5mgkg^{-1}$, 20min before emetic challenge.

b. BRL24924 was dissolved in 154mM NaCl (normal saline) and injected subcutaneously at a dose of $5mgkg^{-1}$, 30min before giving an emetic stimulus.

c. BRL43694 was dissolved in 154mM NaCl (normal saline) and injected subcutaneously at a dose of $5mgkg^{-1}$, 30min before giving an emetic stimulus.

d. Cisapride was used direct from the manufacturers vial and injected subcutaneously at a dose of $2mgkg^{-1}$, 50min before an emetic challenge.

e. Domperidone was used direct from the manufacturers vial and injected intramuscularly at a dose of $500\mu gkg^{-1}$, 20min before an emetic stimulus.

2.3 MONITORING OF RESPONSES TO EMETIC STIMULI

2.3.1 Feeding Routine for the Animals

The ferrets were fed as previously described except prior to 2-Deoxy-D-Glucose (2-DG) experiments when they were fasted for 18 hours before starting an experiment. Also when intragastric emetic challenge was used the ferrets were fasted for a similar period so that the stomach was empty before intubation.

To aid handling before the radiation challenge experiments

it was necessary to allow the ferrets access to 10ml of cow's milk.

2.3.2. Observation Methods

For observation of animals tested with emetic and anti-emetic drugs and X-radiation the animals were placed in one of three types of containment facility.

(a) Their own cages with floor area of 50 x 5cm and 53 x 105cm

(b) A specially constructed run in the laboratory with floor area of 1m x 1m

(c) A standard rat cage with floor area of 60 x 45cm

It was the practice to allocate one observer to one animal when possible. Observations of changes in behaviour and the incidence of retching and vomiting were made and noted against elapsed time from initiation of the emetic stimulus using standard electronic digital laboratory stop watches.

2.3.2.1. Pre-emetic Behaviour Patterns

Ferrets were observed throughout periods of normal waking activity and during the periods following the introduction of an emetic stimulus. As a result of this it was possible to list a number of changes in normal behaviour arising as a result of the stimulus and often culminating in vomiting. This characteristic group of features or pre-emetic behaviour patterns is referred to as the prodromata of vomiting. The results of these observations are found in section 3.1.6.. It was possible to use the classification of these prodromata when observing the results of administration of a variety of emetic stimuli to detect the presence of prodromata even if vomiting did not occur.

2.3.2.2. Definitions of Observation Terminology

Vomiting: Forceful expulsion of gastrointestinal contents through the mouth.

Retching: Forceful unproductive efforts at vomiting without resulting in expulsion of gastrointestinal contents through the mouth.

Latency: The period of time that is the delay between administration of an emetic stimulus and the onset of vomiting (or retching if so stated).

Duration: The period of time between the onset of vomiting (or retching if so stated) and its cessation.

Emetic Response: Number of animals vomiting (or retching if so stated) expressed as a fraction or a percentage number of animals tested.

Prodromata: The collection of characteristic behaviour changes in the ferret (see section 3.1.6) possibly analogous to nausea in man, initially recognised as preceding vomiting but also occurring alone when the emetic stimulus is insufficient to cause frank vomiting.

ED_{50} Vomiting: The effective dose of emetic stimulus required to induce vomiting in 50% of animals tested.

ED_{100} Vomiting: The effective dose of emetic stimulus required to induce vomiting in 100% of animals tested.

2.3.3. Post-procedure Recovery of Animals

Animals which had received intra-gastric emetics capable of resulting in morbidity and mortality under the circumstances of a peripheral nerve lesion and in which it was a requirement to test other emetics, were recovered by intra-gastric administration of 30ml of tap water between 45 and 60min post-initiation of the experiment. Using this method 100% recovery to fitness was achieved in ferrets previously vagotomised and treated with 30ml intra-gastric IM sodium chloride for instance.

2.3.4. Sacrifice of Animals

Animals were sacrificed, following any potentially lethal procedure, with an overdose of Euthatal (Sodium Pentobarbitone, B.P. (Vet) May and Maker Ltd, Dagenham, 200mgml^{-1}) given as an i.v. dose of 1ml or an i.p. dose of 3ml. Death was instantaneous in the case of i.v. injections and occurred within 2 minutes in the case of i.p. injections. For sacrifice at the end of 2-DG experiments ferrets were killed using Euthatal as above.

2.4 IRRADIATION PROCEDURES IN THE FERRET

2.4.1 Characteristics of Ionising Radiations; X-irradiation

Ionising radiation encompasses a variety of highly energetic radiations having in common the ability to eject electrons from atoms in the matter through which they pass. Radiations of this type fall into two categories, the corpuscular or particulate, and the electromagnetic. Electromagnetic radiations sufficiently energetic to produce

ionisations are called X-rays or gamma-rays. X- and γ-rays do not differ from one another in nature or properties. The X-designation indicates that the rays are produced extra-nuclearly in a device which accelerates electrons to high kinetic energy and then stops them abruptly in a target normally made of tungsten or gold; part of the kinetic energy is then converted into X-rays. Gamma rays on the other hand are produced intranuclearly by radioactive isotopes and emitted, representing the excess energy given off as the unstable nucleus breaks up and decays towards a more stable form.

X-rays may be thought of as waves of electromagnetic energy or alternatively as a stream of "photons" or packets of energy at the point of their absorption by organic systems. The critical difference between non-ionising and ionising radiations is the size of these individual photons and not the total energy involved. The energy in a beam of X-rays is divided into photons of large size, each of which is powerful enough to break a chemical bond and initiate the events which result in biological change and ultimately give rise to symptoms such as nausea and vomiting.

2.4.2. Equipment for Production of X-rays

Irradiations were carried out using two vertically positioned parallel-opposed beams produced by two X-ray sets

(Raymax 250, Newton-Victor, Motherwell) with the following characteristics:

Power Output	-	250kV
Tube Filament Current	-	15mA
Beam Filtration	-	0.5mm Copper + 1.0mm Aluminium
Half Value Layer (HVL) Equivalence	-	1.32mm Copper

2.4.3. Machine Output Dosimetry

To estimate the average dose rate achieved under the circumstances of the experiments, a saline ferret-phantom (a saline filled polythene bag of mass and dimensions similar to a 1kg ferret) was placed within the box normally used to house a ferret during irradiation (see Section 2.4.5.1.).

A 0.6ml ionisation chamber was then placed at 5 different loci within the irradiation box and connected to a dosimeter (Farmer Dosemeter Model 2570. EMI Nuclear Enterprises).

From these readings repeated on two occasions the mean dose rate achieved was calculated to be 122 ± 13cGy min^{-1} (n = 15).

2.4.4. Ferret Dosimetry

The dose-distribution throughout the body of two dead 1kg ferrets was investigated using thermo-luminescent dosimeters (T.L.D's). Prior to positioning within the ferret body the T.L.D. rods were sealed into 0.5cm lengths of appropriately size-matched catheter tubing. They were then positioned inside

the ferret at the following anatomical sites:

1. Beneath the atlanto-occipital membrane
2. In the thoracic oesophagus at the level of the heart
3. In the gastric corpus
4. In the duodenum at the mid-point
5. In the rectum 5cm from the anal margin
6. Under the skin of the right flank at the level of the last rib
7. Under the skin of the left flank at the level of the last rib

Each ferret was then irradiated, curled in the box on its right side, under standard conditions for 1 minute and the T.L.D. rods compared to callibration rods irradiated in Temex, (James Girdler & Co. Ltd., London) a tissue equivalent substance. Dosimetry was performed using 2 or 3 rods per anatomical site on each of two occasions.

Evaluation of the T.L.D.'s gave the following results for dose-rate distribution in the various anatomical sites within

the ferrets for a nominal 800cGy irradiation:

Anatomical Site	Ferret 1	Ferret 2
1 Medulla	681cGy	671cGy
2 Oesophagus	791 "	728 "
3 Stomach	830 "	792 "
4 Duodenum	759 "	759 "
5 Rectum	844 "	706 "
6 R. Flank	758 "	716 "
7 L. Flank	809 "	783 "
Mean (±SD) whole body dose	782 ± 55cGy	736 ± 44cGy

2.4.5 Administration of X-rays to the Ferret

2.4.5.1 Technique: For the administration of X-rays each ferret was placed in a sealable polypropylene box of dimensions 17 x 17 x 10cm with a 2cm ventilation hole in the side. The lid was secured with tape. This size of box restricted the size of ferret used to 1kg and below.

Within the confines of the box, ferrets could move as freely as their considerable agility will allow. The box was secured in place on the irradiation platform in the centre of the beam with tape.

Timing was begun as soon as irradiation had started and at the end of this period the ferret was removed to an observation cage. (see Section 2.3.2.). Observations were carried out for a minimum of 90min and a maximum of 360min. At the end of

this period all animals were sacrificed as previously described (see Section 2.3.4).

2.4.5.2. Dose Ranges: The following range of doses were used during construction of the dose response curve:-

Dose	Irradiation Duration
50cGy	0.41 min
75 "	0.61 "
100 "	0.82 "
125 "	1.03 "
150 "	1.23 "
200 "	1.64 "
400 "	3.28 "
660 "	5.41 "
800 "	6.56 "
1200 "	9.84 "
1600 "	13.11 "

Two doses in particular were chosen for the testing of anti-emetic agents, i.e. 200 and 800cGy. 200cGy represents a dose sufficiently in excess of the ED_{100} to guarantee a reproducible stimulus without approaching that which apparently produces a direct CNS depressant effect. 800cGy represents our high dose challenge but is still below the threshold for apparent direct CNS depressant effects; moreover it is a dose very frequently used in other animal irradiation experiments concerned with research into vomiting thus allowing us to make some inter-species comparisons of the emetic effect of X-irradiation (Chinn and Wang, 1954; Eldred and Trowbridge, 1954; Wang et al., 1958; Borison, 1957).

2.5 EXPERIMENTAL PREPARATION IN THE ANAESTHETISED FERRET

2.5.1 Anaesthesia and Preparation for Physiological Monitoring

(Andrews and Scratcherd, 1980)

Prior to experimentation food was withdrawn overnight. Anaesthesia was induced with urethane (ethyl carbamate) 1.5 gkg^{-1} i.p. (50% w/v in 154mM NaCl) and maintained when required with intermittent boli of urethane administered intravenously. A glass cannula was inserted into the trachea and the right external jugular vein was cannulated for the administration of drugs. The rectal temperature of the animal was maintained between 38.5 - 39.5°C throughout the experiment by a heating blanket (Palmer) placed beneath the body and radiant heat from a lamp above. Temperature was monitored with thermocouple thermometer (Portec Instruments Limited, Luton).

2.5.2. Electrical Stimulation of the Abdominal Vagus

The ventral abdominal vagal trunk was exposed by blunt dissection (see Section 2.2.2.1) and the cut central end laid over plastic-coated silver wire electrodes. Electrical stimulation was carried out using a Digitimer (D4030, Neurolog) and D52 Stimulator (Devices) generating a stimulation pattern of frequency 30Hz, voltage 20V and pulse width of 0.5ms. For the 2-DG experiments this pattern was repeated 1 min in every 2 min for 45 min before sacrifice with i.v. Euthatal.

2.6 2-DEOXYGLUCOSE AUTORADIOGRAPHIC TECHNIQUES

2.6.1 Background

Under normal, non-fasting conditions the adult brain uses glucose as the principal sustrate for the production of high

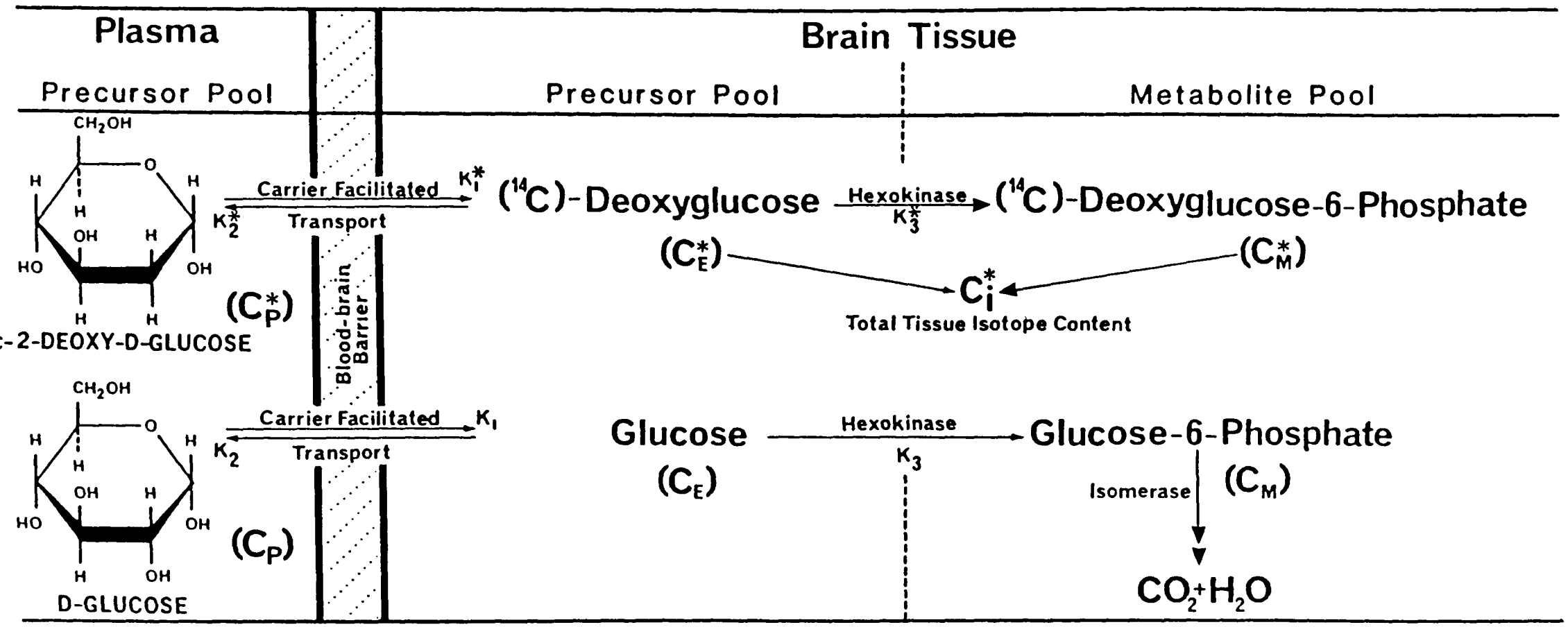
energy phosphate molecules with which to fuel the many biochemical reactions necessary for cerebral function (Ketty, 1960). Although some glucose is stored within the tissues of the brain in the form of glycogen, the relatively fixed volume afforded by the cranial bones and the requirement for 3g of water for every 1g of glycogen severely limits the availability of this energy source to meet total energy requirements (Cahill and Aoki, 1980). Thus for the maintenance of function the brain requires the continuous supply of blood-borne glucose.

2-Deoxy-D-glucose (2-DG), a structural analogue of natural glucose differing only in the hydrogen atom attached to the second carbon in place of hydroxyl group was shown many years ago to be an inhibitor of glucose uptake and phosphorylation in cerebral tissue slices (Wick et al., 1957). This inhibition was accompanied by an accumulation of 2-deoxyglucose-6-phosphate (2-DG-6-P) in the tissue (Tower, 1958). This is the basis of the method developed later by Sokoloff and his colleagues (Sokoloff et al., 1977). Sokoloff's technique relies upon the systems of brain uptake and phosphorylation being common to both glucose and deoxyglucose, and the inability of the isomerase enzyme that converts glucose-6-phosphate to act upon the anomalous structure of 2-DG-6-P which, as a result, accumulates in the tissue. If the relevant rate constants are known for the steps which take blood-borne glucose and 2-DG to phosphorylation in the tissue, and the blood concentration of both substances is known, then the rate at which 2-DG-6-P

accumulates may be directly related in a predeterminable fashion to the rate at which glucose itself passes through the preliminary stages of the glycolytic pathway, provided that the levels of deoxyglucose present are never sufficient to act as a competitive inhibitor. On the basis of the theoretical model (Fig. 5) formulated by Sokoloff and his colleagues (Sokoloff et al., 1977) an operational equation was devised which described rates of cerebral glucose utilisation in terms of the concentration of [^{14}C]-2-deoxyglucose and glucose in the arterial plasma over the experimental period (Cp* and Cp) and the concentration of tracers found within the CNS (C_i*). The distribution of tracer between the plasma and brain tissue compartments is governed by the kinetic rate constants for the movement of [^{14}C]-2-deoxyglucose into and out of the CNS its phosphorylation to 2-deoxyglucose-6-phosphate and a composite constant which, in simplistic terms, reflects the relative preference of the glucose transport and enzyme system for glucose as opposed to 2-deoxyglucose. Despite the rigour with which these constants were derived, a relatively high degree of measurement error is associated with each of them. However, within the confines of the original model and the operational equation, these errors become so small as to be negligible if a suitably long time interval is interspaced between a pulse injection of 2-deoxyglucose and ultimate sacrifice of the animal.

It was thus demonstrated that local cerebral glucose utilization measured with the 2-DG technique correlated closely with the level of functional activity in discrete areas or regions of the brain (Sokoloff et al., 1977; Plum et al., 1976

Figure 5 The 2-Deoxgyglucose Theoretical Model



Diagrammatic representation of the theoretical model with structural formulae of D-glucose and 2-deoxy-D-glucose.

(Sokoloff et al. 1977)

and Schwartz et al., 1979). It is thought that within the brain this correlation extends to the link between differential glucose uptake and electrical activity in the neuropil in general and synaptic terminals in particular. It is presumed that increased glucose utilization associated with oxygen consumption (Ritchie, 1967 and De Weer, 1975) is principally due to enhanced activity of the sodium pump in the reconstitution of electrochemical gradients.

Mata et al., (1980) then published studies indicating that the major energy-consuming function of nervous tissue is ion-pumping by the sodium pump accompanying a depolarising stimulus and that 2-DG uptake reflected this activity (see also Discussion).

The main limitations of the technique as an approach to the measurement of neuronal functional activity are the need for plasma sampling and the long time constant required for the experimental measurement. The time between the pulse of labelled deoxyglucose and sacrifice of the animal must be sufficiently long to minimise potential errors arising from uncertainty in the model (particularly the values for rate constants), but must be short enough to limit the depleting effects upon 2-DG-6-P of the small amounts of phosphatase known to be present in cerebral tissue (Nelson et al., 1986). An experimental time course of 45 minutes has been found to meet both of these requirements (Sokoloff, 1979). However, experimental design as described originally by Sokoloff and colleagues is such that events which occur within the first 10 minutes after <u>intravenous</u> isotope delivery carry greater weight in their effects upon measured glucose utilisation by virtue of

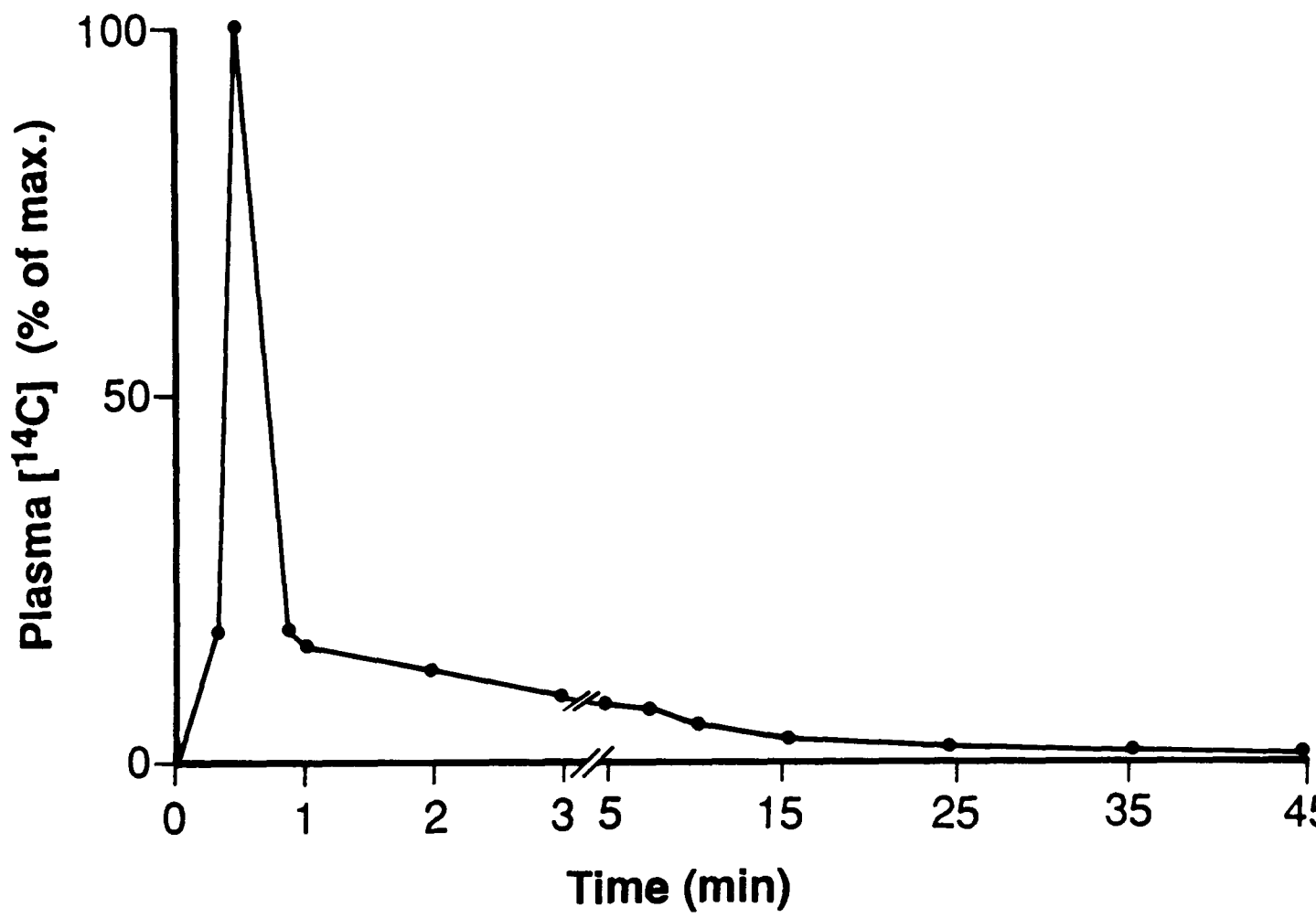
the shape of the arterial input function (Fig. 6). Thus, for example, drugs need not be infused throughout the whole 45 minutes to ensure steady-state conditions. Nevertheless, great care must be taken to ensure that the relative timings of drugs or other stimuli, and tracer administration, are meaningful in terms of the previously reported time-course of action for the specific agents.

To illustrate in a simplistic fashion how the data collected during the course of an experiment which follows the 'fully quantitative technique' (as originally described by Sokoloff and his collegues) is applied to the determination of local cerebral glucose utilisation, the operational equation may be written thus:

$$\text{Rate of Glucose Utilization} = \frac{\text{Total Tissue } [^{14}C] - \text{Tissue 2-DG}}{\text{Lumped constant} \times \text{Integrated Plasma Specific Activity}}$$

where total $[^{14}C]$ in tissue is measured densitometrically, and tissue 2-DG and integrated plasma activity are calculated from accurate measurements of plasma of 2-DG and glucose. It is worthy of note that quantification of the methodology by this approach gives an index of the rate of glucose utilisation and not merely of the accumulation of tracer which, at the moment of kill will be present both in the form of 2-deoxyglucose and 2-deoxyglucose-6-phosphate (the numerator of the equation).

Simplifying technical modifications have been advanced as feasible alternatives to the rigorous 2-DG approach. Of these, the most widely used are (a) the use of free-running animals, which often precludes the plasma sampling protocol and involves a different route for tracer administration (Meibach et al., 1980) and (b) the use of optical density



Time course of ^{14}C deoxyglucose concentrations in arterial plasma from a typical control, conscious rat injected with isotope over 30 seconds.

(Reproduced from Kelly 1982, with permission)

Figure 6 Plasma Deoxgyglucose Clearance after Intravenous Administration

ratios in the semi-quantitative analysis of autoradiographs (Brown and Wolfson, 1978, Collins, 1978, Meibach et al., 1980). The third modification employed has been the use of ^{3}H-2-DG instead of ^{14}C 2-DG as the tracer. This in itself does not preclude the use of the fully quantitative technique (Alexander et al., 1981), but until recently has most often been specifically employed to increase resolution after the method of (Hammer and Herkenham, 1984) to eliminate errors in source thickness on autoradiographic optical density and to reduce the overall cost of using the 2-DG technique.

The most widely used modification and a direct derivation the approach of Sokoloff et al., (1977) is the use of optical density ratios in the semi-quantitative analysis of autoradiographs. No attempt is made to quantify absolutely the total isotope concentration present in the CNS at the end of the experiment or the level of plasma radioactivity concentration during the measurement period. Thus when the technique is used to detect localised alterations in functional activity rather than to measure rates of glucose utilisation the demanding procedures required to estimate glucose utilisation are not necessary because:

a. The relationship between the behaviourally relevant activity of a neural system and its rate of glucose utilisation is unknown. It can implicitly be assumed to be monotonic and there is little justification for assuming more than that. For most current purposes, it does not matter what the form of this monotonic relationship may be, since alterations in metabolic activity are being used as ordinal indices of alterations in functional activity.

b. Universal or very widespread alterations in brain metabolism have no meaning for the investigator interested in the behavioural function of neural systems. At the present level of our understanding only the discrete localized alterations produced by the experimental stimulus have meaning. For these reasons the semi-quantitative use of the 2-DG technique has become more widespread than the original fully quantitative use (Gallistel et al., 1982). In such investigations, deoxyglucose uptake in a region is measured autoradiographically by the relative optical density of a region of interest in relation to some other region (such as the white matter) in which glucose utilisation is putatively unchanged. In actual fact this approach was the basis of the very first demonstration of the use of [^{14}C] deoxyglucose in mapping functional activity in the CNS.

Although normalizing transformation using optical density ratios helps to reduce errors due to variations in for example isotope dose, cryostat thickness, timing, constancy of experimental conditions, X-ray film exposure time and plasma glucose levels every effort must still be made to minimise these variations in order to maximise the discriminating power of the semi-quantitative technique which may be compromised by factors such as the reliability and comparability of optical density readings produced from a mathematical analogue of grey levels.

2.6.2 Experimental Animal Models

Ideally 2-DG experiments would be carried out in free-running unrestrained conscious animals. However, for a variety of practical reasons the majority of 'fully

quantitative' experiments have been performed in restrained, conscious rats (Kelly, 1982). For this, intravenous and intra-arterial canulae are implanted under anaesthesia and the hind-quarters of the rat immobilised in a light plaster cast which is anchored by tape to a weighted block. After return to consciousness the rats appear to remain in this state without obvious undue distress for periods of up to 6 hours.

Ferrets are stronger, more agile and potentially more aggressive animals than rats, so clearly it was necessary to appraise the various possible solutions to this problem. Of the possibilities considered, only the use of conscious unrestrained animals without indwelling cannula for blood sampling or the use of anaesthetised animals with indwelling for blood sampling was deemed suitable.

These approaches were used in the following studies:-

a. In anaesthetised ferrets with indwelling venous and arterial cannulae:-

(i) Electrical stimulation of the central abdominal vagus.

(ii) Intravenous apomorphine administration.

b. In unrestrained conscious ferrets:-

(i) Intravenous apomorphine administration (with indwelling i.v. cannula for injections only).

(ii) X-irradiation.

(iii) Intravenous mustine administration (with indwelling i.v. cannula for injections only)

(iv) Intraperitoneal cycloheximide administration

In all but the anaesthetised animals the practical constraints on the handling of ferrets precluded the use of the fully quantitative 2-DG technique as no regular plasma sampling for plasma glucose and radioactivity could be undertaken. In the anaesthetised ferret some plasma sampling was carried out with the aim of looking at the effect of this kind of experimental preparation on plasma glucose levels but insufficient samples were obtained routinely for a fully quantitative approach to be applied. Moreover the rate constants and lumped constant used in the operational equation have not been determined for the ferret. It was beyond the scope of this thesis to determine these experimentally.

The route of administration of 2-DG (i.p. or i.v.) varied between experiments so as to meet the criterion that the brain uptake profile (determined by arterial plasma DG profile) should match the type and duration of the particular phenomenon under study. For all the experiments involving i.v. administration of isotopic tracer the experimental time course was kept at 45 minutes which is the duration used in the vast majority of all 2-DG experiments. For i.v. administration a 45 minute period has been shown to be sufficiently long to minimise potential error arising from high levels of residual 2-DG in the plasma but short enough to limit the depleting effects upon 2-DG-6-P of the small amount of phosphatase known to be present in cerebral tissue (Sokoloff, 1979).

Consideration of the effect of high levels of residual unphosphorylated 2-DG when using the i.p. route of administration, led to the adoption of an extended experimental period of 60 minutes. The advantage of this was considered to

outweigh any disadvantage that might have occurred from the increasing influence of phosphatase after this length of time.

The most widely used radioisotopic tracer in 2-DG autoradiography is Carbon^{14} ([^{14}C]) labelled 2-DG and this technique was employed alongside the lesser used isotope, Tritium ([^{3}H]) labelled 2-DG. In each series of experiments a particular tracer was chosen for specific positive reasons but each has its disadvantages and special technical requirements which had to be taken into account when the decision was taken. (See also Apendix 1). Initially in the experiments on the anaesthetised ferret ^{3}H-2-DG was used because of its increased apparent resolution and relatively cheaper cost compared to the ^{14}C isotope. Its use was repeated with the matching experiments in the conscious ferret using an apomorphine stimulus so that any effect of anaesthetic on the whole procedure could if necessary be determined. Apart from the particular case of the apomorphine all conscious ferret experiments wre carried out using the [^{14}C]-2-DG isotope mainly because exposure time is much reduced whilst resolution remains acceptable. A detailed treatment of the relative merits and demerits of the two isotope systems is to be found in Section 5.6.1 and Appendix 1.

2.6.3 Preparation of Autoradiographs

At the end of the predetermined period (see below) the ferrets were killed with euthatal, given i.v. or i.p. (see section 2.3.4).

Dissecting from the supra-occipital bone, the dorsal

cranium was removed, the underlying dura reflected and the whole brain removed as quickly as possible to be frozen intact in isopentane (BDH Chemicals Ltd., Poole) pre-cooled to -45°C (Cryobath CB-60, Neslab New Hampshire). The time from sacrifice to freezing of the ferret brain varied between 10 and 20min because in the ferret the skull frequently reached a thickness of 2mm. The brain was then coated in cryomatrix (Tissue Tek OCT Compound) and stored at -70°C. Cryomatrix was used to prevent dehydration of the brain and to support it during sectioning. Sections of ferret brain were cut on a rotary microtome in a cryostat (Frigocut 2800, Reichert-Jung, Slough) maintained at -18 to -20°C (See Appendix 1). Brains were freeze-mounted by their rostral extremity on to the microtome chuck using cryomatrix. Ferret brain was cut at a section thickness of 18 and 20μm. Cut sections were picked up on No. 1 glass coverslips and rapidly dried on a hotplate at 70°C. Selected appropriate sections were then mounted on thin card together with an appropriate set of radioisotopic standards (Amersham International, Aylesbury; [^{3}H]-Microscales containing 0.07 - 6.0 and 1.3 - 3.0nCimg^{-1} tissue-equivalents of [^{3}H] and [^{14}C]-Microscales containing 40 - 1069nCimg^{-1} tissue-equivalents of [^{14}C]). [^{3}H]-Microscale blocks were sectioned using a sliding microtome at a thickness of 20μm, being a thickness easy to handle and well in excess of the 5μm which is considered infinitely thick with respect to the β-particles resulting from tritium decay. Pre-cut slices of [^{14}C]-Microscales of 120μm thickness were used as the [^{14}C] labelled polymer at this thickness is greater than the infinite thickness for [^{14}C] β-particle emissions (80μm). The cards of

coverslips were then applied to appropriately sensitive film.

In the case of [^{3}H]-2-DG the film was Ultrofilm ^{3}H (LKB, Bromma, Sweden) (See Appendix 1) a high resolution film designed for autoradiography of low energy beta-emitters such as tritium. This was then sealed in a light-tight X-ray cassette for 28 days. The exposure period exceeds that required to prevent fading of the latent image (LKB technical data) and corresponds to an optimum time for the dose of isotope chosen (confirmed by performing an exposure-response test). This exposed Ultrofilm ^{3}H was developed manually using the standard radiographic tank-based procedures.

For the [^{14}C]-2-DG isotope the X-ray film used was 20 x 25cm X-OMAT AR-5 (Eastman Kodak, Rochester, N.Y.) (See Appendix 1); a film with a high sensitivity to beta particles, a clear base and high contrast capability designed for autoradiographic imaging. As before, the film and coverslips were sealed together in light-tight X-ray cassettes but the exposure time was 5 days. At the dose of isotope used this exposure period allowed adequate definition of the autoradiographic image (confirmed by performing an exposure-response test).

The exposed X-ray film was developed automatically in a Kodak RPX-OMAT rapid processing machine with a 90 second cycle at 35.6°C.

2.6.4 Analysis of Autoradiographs

In the 2-DG experiments carried out in the ferrets optical density ratios were calculated by comparing the mean optical density (obtained from readings from multiple sections in which

the area of interest appears) of the particular brain area of interest with the mean optical density of an area of white matter within the same region. For the brain areas of interest in the ferret medulla, the white matter reference area chosen was the pyramids (pyramidal tract areas)(Meibach et al., 1980). Because the adult ferret brain is approximately 3.0cm in length and with a section thickness of 18 or 20 m it is possible to generate an unmanageable number of autoradiographic images. With the initial tritium studies it was therefore decided to set the general sampling rate at 1 in 10 but decrease it to 1 in 20 in the forebrain so that it could be increased to 1 in 3 throughout the extent of the area postrema which is approximately 0.72mm in length. This approach, it was felt, would allow inspection of the brain as whole but at the same time allow concentration on areas of particular interest in the medulla, without generating an excessive number of images.

In the later studies using [^{14}C] it was decided to increase the sampling rate to 1 in 1 throughout the extent of the area postrema with a compensating decrease in the residuum of the brain to 1 in 20 sections. The actual numbers or 'n's' achieved for each brain area analysed, also varies of course with the ability to identify the particular area within the autoradiographic section under scrutiny.

Autoradiographs were scanned with a video camera (Bosch Plumbicon TYK9B1) and illuminated with a 250watt tungsten-halogen light source under independent output control via a Variac transformer. The video signal was fed to interactive computerised image analysis system (IBAS II,

Kontron). Each autoradiographic image was averaged and stored as a digital array that was projected on a monitor in a 764 x 512 pixel (picture point) resolution format. The system gives a grey level resolution for each of the 391,168 pixels of 256. The performance of the system as a densitometer was externally calibrated against Wratten Neutral Density Standard optical density filters (0.D 0.1 -1.0) and background O.D. for each film was determined and automatically subtracted from the value derived for each brain area. The analytical routine is summarised below (Section 2.6.4.1) and a full discussion of the construction and functioning of the system as a high-resolution image analysing densitometer is to be found in Appendix 2.

The capability of the image-processing system to scan and store 768 x 512 individual readings from an area as small as 8.0mm x 5.3mm and subsequently to display these data as a 18cm x 26cm image on the monochrome and colour monitor provides potential resolution and quantification of brain structures at least as small as 100 m in width.

Where necessary for identification of cellular groupings within the brain-stem, histological staining was performed on the actual sections that were used to make the autoradiographs.

Two stains for 'Nissl substance' to show the general cell pattern of brain were chosen; 0.5% Acidified Aqueous Cresyl Fast Violet (CFV) and 1% Aqueous Thionin Y (Thionin).

It was thus necessary to remove coverslips from the card bearing them using a gentle prizing action assisted by infiltration with a few drops of Citroclear (H.D. Supplies, Aylesbury). This required extreme care as did all subsequent handling in order to avoid shattering the delicate coverslips

with consequent loss of the section. Coverslips were loaded in special racks for transport through the staining procedures. Subsequent to staining, coverslips were mounted on microscope slides, using Styrolite mounting medium (R.A. Lamb, London) and dried at room temperature in a fume cupboard in a stream of air, overnight.

Using a computer function called image positioning, histological and autoradiographic images can be superimposed, eliminating in the process linear differences such as size, orientation and position so that small structures can be accurately defined.

Alternatively, delineation can be carried out by reference to an appropriate histological atlas, in conjunction with simultaneous viewing of the histological slide giving rise to the autoradiograph, on a co-located Polyvar Photomicroscope (Reichert-Jung, Vienna, Austria). This can be done by direct viewing or via its own T.V. camera linked to the Hitachi 'slave' black and white monitor. For the ferret it was necessary to construct an histological atlas using standard techniques rather than from cryostat sections. This was carried out by Dr. J. Hawthorn who kindly provided a copy to aid the work described herein.

2.6.4.1 Computerised Analytical Routines for Densitometry

(See also Appendix 2)

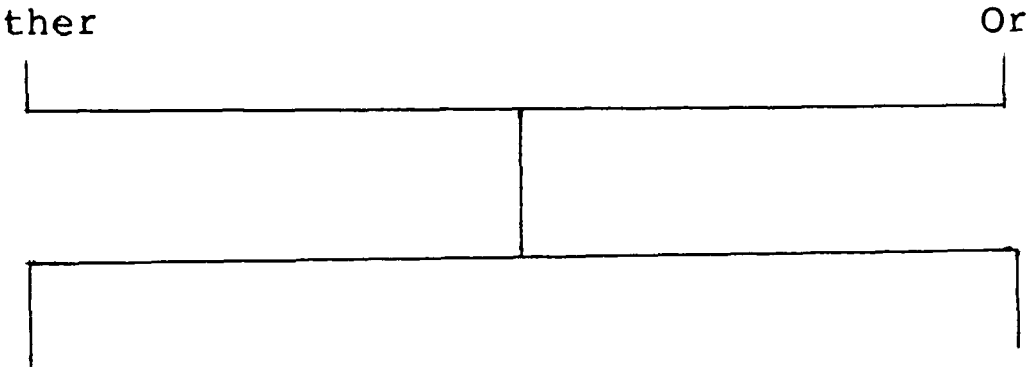
(a) Warm up and stabilize light source and camera (10min)

(b) Initiate software routine (select representation as black and white or psuedo-colour coded image)

(c) Initiate external calibration process using 0% and 100% transmission anchor points and Wratten O.D. standard Neutral Density Filters. (Implement linearity response correction if necessary)

(d) Initiate measurement process - measure background O.D. of an area of the film nearest to the image intended for analysis - capture (with signal averaging) and store image for study. Switch off light source. Carry out digital zooming of image area of interest.

Then:-



(e) Conclude measurement phase with activation of data readout on monitor and data hard copy printout. Repeat sequence d. and e. for as many images as required for sheet of film. (On completion of the analysis of one X-ray sheet of images the radioactive standard images may then be read, if required, using the same procedure as above. External calibration checks are carried out as and when required depending on the length of time for which measurements are undertaken).

(f) Activate statistical and calculation function to give mean O.D.'s of brain areas measured and appropriate normalised indices i.e. O.D. ratios, then print out results. Pseudo-colour coding can be implemented before interactive delineation of areas of interest in order to aid the discriminative process or at the end of the measurement procedure to produce photographic hard copy records of the images in colour.

Others have subsequently found the IBAS computerised image analysis to be a suitable and reliable system for carrying out densitometry (e.g. Zilles et al., 1986).

2.6.5 Experimental Protocols

2.6.5.1 [^{3}H]-2-DG Autoradiography in the Anaesthetised Ferret

Following withdrawal of food overnight for a minimum of 12h ferrets were anaesthetised and prepared as previously described; urethane anaesthesia having been chosen because of its suitability in the ferret and applicability to such investigations (Maggi and Meli, 1986) (Section 2.5.1). After

a 60min stabilization period one of the two types of stimulation was administered, i.e.

(i) Electrical stimulation of the ventral abdominal vagus (See Section 2.5.2) or

(ii) Intravenous injection of apomorphine 50μgkg^{-1} (See Section 2.2.4)

One minute after the start of vagal stimulation or injection of apomorphine, [^{3}H]-2-DG (1mCikg^{-1}) was injected as an intravenous bolus (injection volume <1.0ml) and the cannula flushed through with 0.5ml of normal saline. Control animals were prepared in a similar way but did not receive the stimulus. For the duration of the experimental period the animals remained anaesthetised under the standard laboratory conditions. They were killed by anaesthetic overdose with euthatal 45 minutes after injection of 2-DG and the brains removed and frozen as previously described.

One 500μl blood sample was taken at 45mins. The fluid loss was replaced with normal saline in each case. These samples were centrifuged in an Eppendorf 5414 Centrifuge for 15min subsequent to mixing in standard clinical blood glucose tubes containing fluoride/citrate mix to prevent oxidation and coagulation. The plasma fraction was then frozen for storage at -70° until ready for analysis in an Eris glucose autoanalyser (B.D.H. Ltd., Poole). A series of conscious animals with indwelling cannulae were also used to obtain fasting and fed blood glucose levels for comparison with the anaesthetised series.

2.6.5.2 [^{3}H]-2-DG Autoradiography in the Conscious Ferret

Following withdrawal of food overnight for the usual minimum period ferrets with an in-dwelling intravenous cannula (implanted a minimum of 7 days previously; see Section 2.2.1) were injected via the cannula with saline ($0.5 ml kg^{-1}$) as control apomorphine solution ($50 \mu g kg^{-1}/0.5 ml kg^{-1}$). The apomorphine was flushed through with 0.5ml of normal saline and immediately followed by the pulse of [^{3}H]-2-DG (1mCi, injection volume <1.0ml). Finally, the canula was flushed with 0.5ml of normal saline. For the duration of the experimental period the ferrets were allowed to move freely and unrestrained within a large observation box under our standard laboratory conditions of controlled lighting, heating and quiet. 45 minutes after the injection of [^{3}H]-2-DG the animals were killed by i.v. anaesthetic overdose as previously described, the brains removed and frozen. After administration of the anaesthetic overdose the thoracic cavity of the ferrets was incised by cutting along the costo-chondral junctions and the heart pierced using a 17 S.W.G. gauge needle. 5ml of blood was then withdrawn from the heart (right and left ventricles) and analysed for glucose concentration as described above.

2.6.5.3 [^{14}C]-2-DG Autoradiography in the Conscious Ferret

Following withdrawal of food overnight for the usual period, ferrets were treated in one of three ways according to the type of emetic stimulus to be administered. Controls were treated to the same protocol but did not receive the stimulus.

2.6.5.3.1 <u>800cGy X-irradiation</u> : Ferrets were irradiated with 800cGy as described in Section 2.4.5 then transferred to observation cages. 15 minutes after initiation of irradiation [^{14}C]2-DG was administered as an intraperitoneal injection (125μCikg^{-1}, injection volume <0.5ml) and the ferrets returned to their observation cages where they were allowed to move freely and unrestrained under the standard laboratory conditions. The time course of these experiments (time between delivery of pulse of 2-DG and sacrifice of animals) was 60 minutes, at the end of which the animals were killed with an intraperitoneal injection of euthatal as described. Subsequent to this the thoracic cavity was opened and a 5ml blood sample withdrawn from the heart to be analysed for glucose concentration as previously described.

2.6.5.3.2 <u>Mustine Administration</u> : Ferrets with an in-dwelling intravenous cannula (implanted a minimum of 7 days previously) were injected with mustine (1200μgkg^{-1}, injection volume <1.0ml) which was flushed through with 0.5ml of normal saline. 24 minutes after challenge with mustine [^{14}C]-2-DG was administered as an intraperitoneal injection (125μCikg^{-1}, injection volume <0.5ml) and the ferrets placed in the large observation box where they could move freely and unrestrained under our standard laboratory conditions of controlled lighting, heating and quiet. The time course for these experiments was 60 minutes from injection of the 2-DG, at the end of which the animals were killed with an intravenous injection of euthatal. A blood sample was then obtained via cardiac puncture and analysed for glucose concentration as described above.

2.6.5.3.3 Cycloheximide administration : Ferrets were injected intraperitoneally with cycloheximide (20mgkg^{-1}, injection volume <1.0ml) and transferred for observation to large observation boxes. 15 minutes after this challenge [^{14}C]-2-DG was administered as an intraperitoneal injection (125μCikg^{-1}, injection volume <1.0ml) and the ferrets returned to their observation boxes where they were allowed to move freely under standard laboratory conditions. The time course of the experiments from injection of 2-DG to sacrifice was 60 minutes. At death a blood sample was obtained from the ferret by cardiac puncture and analysed for glucose concentration as described above.

2.7 DRUGS, CHEMICALS AND RADIOCHEMICALS

2.7.1 Drugs

Domperidone (Motilium; 5-Chloro-1-1-[3-(2,3-dihydro-2-oxo-1-H-benzimidazol-1-yl)propyl] -4-piperidinyl -1,3 dihydro-2H-benzimidazol-2-one and Cisapride (cis-4-amino-5 chloro-N-[1-[3-(4-fluorophenoxy)propyl]3 methoxy-4-piperidinyl]-2methoxyenzamidemonohydrate) were obtained from Janssen Pharmaceutical Limited, (U.K.), Wantage, Oxfordshire. The serotonin M-receptor ($5HT_3$) antagonists BRL 24924 ([±-endo]-4-amino-5-chloro-2-methoxy-N-(1-azabicyclo [3,3,1]non-4-yl)benzamide monohydrochloride) and BRL 43694 (endo-N-(9-methyl-9azabicyclo[3.3.1]non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride) were generous gifts from Beecham Pharmaceuticals Research Division, Harlow, Essex. Metoclopramide(Parmid;4-amino-5-chloro-N-(2-diethyl-aminoethyl) -2-methoxybenzamide) was obtained from Lagup Pharmaceuticals, Guildford.

Syrup B.P. and Paediatric Ipecacuanha Emetic Mixture were

supplied ready-constituted by the Pharmacy of the Churchill Hospital, Oxford, having been prepared to the formulae:-

Syrup B.P. :- Sucrose 66% in water wt/wt

Ipecac' B.P. :-

Ipecacuanha Tincture	0.7ml
Hydrochloric Acid	0.025ml
Glycerol	1.0ml
Syrup B.P.	to 10.0ml
(Total alkaloids, as Emetine,	$1.4 mgml^{-1}$)

2.7.2 Chemicals

The following compounds were obtained from the Sigma Chemical Company, St. Louis, U.S.A.:-

Apomorphine (hydrochloride)

Cisplatin (cis-Platinum (II) Diammine Dichloride)

Cycloheximide (32(3,5-Dimethyl-2-oxocyclohexyl) -2-hydroxyethyl] glutarimide)

Diacetoxyscirpinol (DAS or Anguidine; 4β, 15-Diacetoxy-3d-hydroxy-12,13 epoxy-trichothec-9-ene)

Emetine Dihydrochloride

Mustine (Mechlorethamine hydrochloride, Nitrogen Mustard; Methyl-bis-(β-chloroethyl)-amine HCl)

Peptide YY (5, 6, 6a, 7-Tetrahydro-b-methyl-4H-diterzo [da,g] quinoline-10, 11-diol)

The following compounds were obtained from BDH Chemicals Limited, Poole, England:-

Choline Chloride (2-Hydroxy-N, N, N-trimethyl ethanamium chloride)

Copper Sulphate ($CuSO_4 . 5H_2O$ Analar grade)

D-Glucose

D-Mannitol

Isopentane (2-methyl butane, GPR grade)

Sodium Chloride (NaCl, Analar grade)

Urethane (Ethyl carbamate)

The histological stains, Thionin Y and Cresyl Fast Violet were obtained from R.A. Lamb, London

Euthatal (Sodium Pentobarbital 200mgml^{-1})

2.7.3 Radiochemical Tracers

All radio-labelled tracers were supplied by New England Nuclear (Dupont Ltd), Dreieich, F.R.G..

2-[1,2-^{3}H]-deoxy-D-glucose (specific activity 30.2Cimmol^{-1});
2-[1-^{14}C]-deoxy-D-glucose (specific activity 48.6 and 55.0mCimmol^{-1}).

Both radioisotopes were supplied in aqueous form. For injection sodium chloride was added to this aqueous solution of 2-DG to approximately 0.9% or 0.154mM.

2.8 STATISTICAL ANALYSIS

Results were expressed as the mean value ± one standard deviation. For statistical comparison of data extensive use was made of Students' unpaired sample 't' test but where appropriate a variation of this, Dunnett's test was used (Dunnett, 1955). This is a specialised multiple-comparison test which can be employed in those instances when the only comparisons desired are between the control group and various experimental groups.

Analysis of variance was also used where the data required it as was the Chi-square test.

Significance levels are indicated in the text thus:-

*	indicates	$p \leqslant 0.05$
**	indicates	$p \leqslant 0.01$
***	indicates	$p \leqslant 0.001$ or better

CHAPTER 3

EXPERIMENTAL RESULTS; CLASSICAL STUDIES OF EMETIC CHALLENGE

"What is not a poison? All things are poisons and nothing is without toxicity. Only the dose allows something not to be poisonous. For example, every food and every drink is a poison if consumed in excess."

Theophrastus Bombastus von Hohenheim; otherwise known as PARACELSUS, 1538. In: The Works of Paracelsus in Five Volumes, (Ed. Peukert, W.) vol. 2, p.510, Schwabe

CHAPTER 3 EXPERIMENTAL RESULTS; CLASSICAL STUDIES OF EMETIC CHALLENGE

3.1 INTRAGASTRIC EMETIC STIMULI

3.1.1 Controls

Controls for injection volume and speed of injection were carried out using 30ml of 0.154mM NaCl (isotonic saline) cows' milk and 50ml of tap water. 30ml of isotonic saline, tap water or milk were injected at a slow rate and found to produce no vomiting or retching. 50ml of tap water was injected at the slow rate (over a 30sec period) and found to produce no retching or vomiting. 30ml of water injected with maximum force (in <2sec) did not cause retching and vomiting. In some cases, during control testing, animals occasionally displayed very brief initial periods of subdued activity, somewhat akin to those that are part of the observed prodromata of vomiting. It was concluded that the test solutions administered in the above fashion were adequate controls for the intragastric emetic stimuli tested in respect of volume and injection speed.

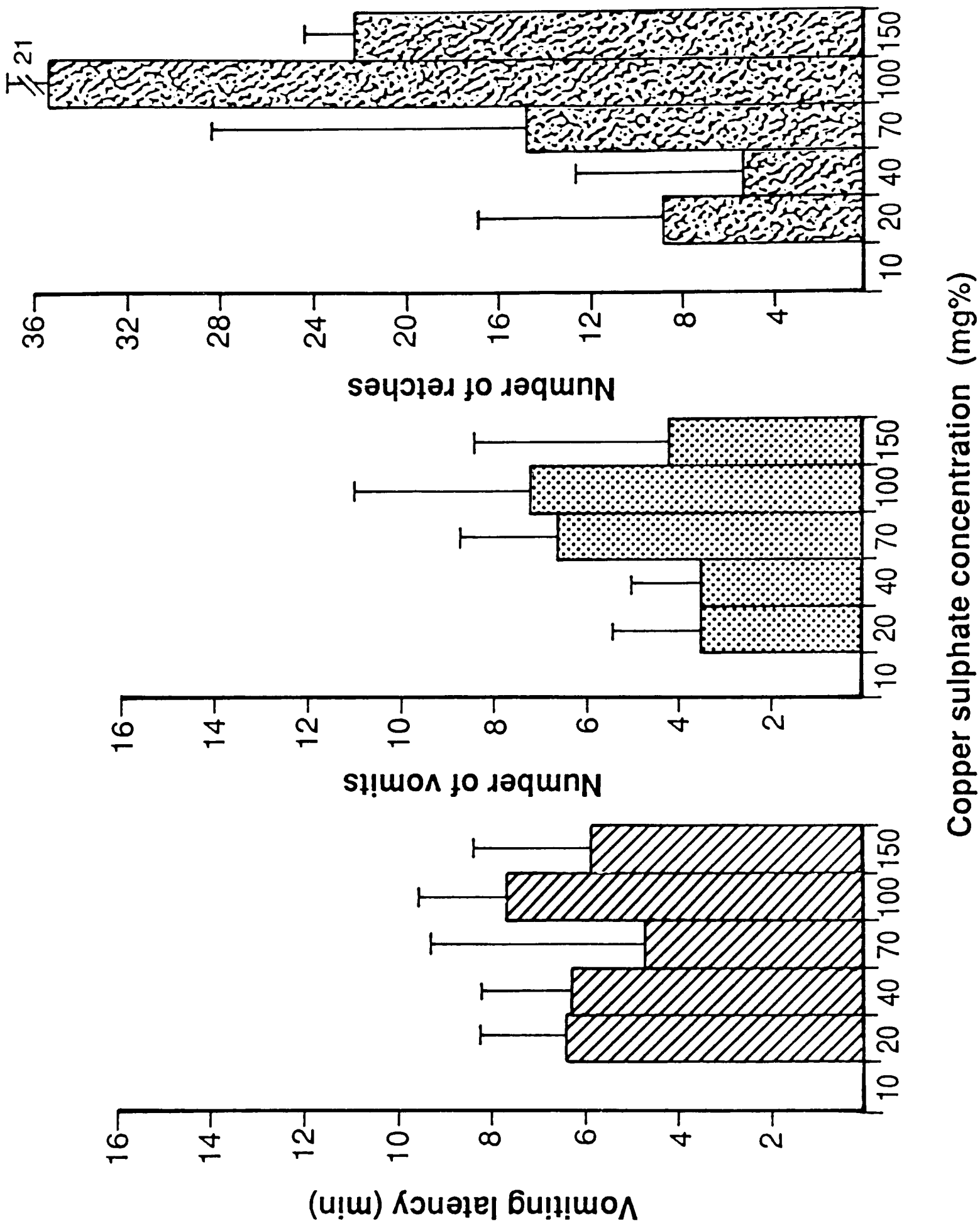
For intragastric stimuli the basic standard observation period upon which calculations of numbers of retches and vomits were based was 30min. In the case of ipecac-based experiments the observation time was lengthened to 60min. For systemic stimuli the short duration of effect substances were also observed for 30min but for the various cytotoxic drugs and X-radiation this was lengthened to 90 and 120min (cycloheximide, mustine, cisplatin and DAS-120min; emetine and X-rays-90min). Total retches and vomits relate directly to these time periods in each case.

3.1.2 Copper Sulphate

Copper sulphate has been used as an standard emetic test substance extensively in the past (and therapeutically, see Meester, 1980 and Decker, 1971) as can be seen from the foregoing and it was therefore necessary to investigate the ferret's response to the compound across a range of concentrations. 30ml of a series of concentrations from 10 - 150mg% was administered to 6 groups of ferrets (n = 5 - 20) using the method previously described. The ferrets were then observed for signs of retching, vomiting and changes in behaviour for 30min. The results of this dose-response trial are seen in Fig. 7. At all doses including and above 20mg% retching and vomiting were noted in most animals tested, ranging from 4/5 animals tested at 20mg% to 20/20 at 40mg%. 40mg% was chosen as the dose to be used in further testing; this being low enough to avoid fatal toxicity yet effective enough to produce a consistent retching and vomiting response (latency 6.3 ± 1.9min; n = 20). Diarrhoea was not a feature of challenge with $CuSO_4$. The pattern of retching and vomiting in a typical animal tested with 40mg% copper sulphate is illustrated in (Fig. 8).

Having chosen 40mg% as a concentration giving a reliable emetic response it was decided to test the time-dependent reproducibility of this response in a defined group of 5 animals. Each of the five ferrets was tested once a week for four weeks with 30ml of 40mg% copper sulphate. The results are shown in Table 4. There was no significant difference in vomiting latency or emetic response for the group during testing over the four weeks.

Figure 7 The Emetic Dose-Response Relationship for Oral Copper Sulphate in the Ferret
(n = 5 - 20 animals)



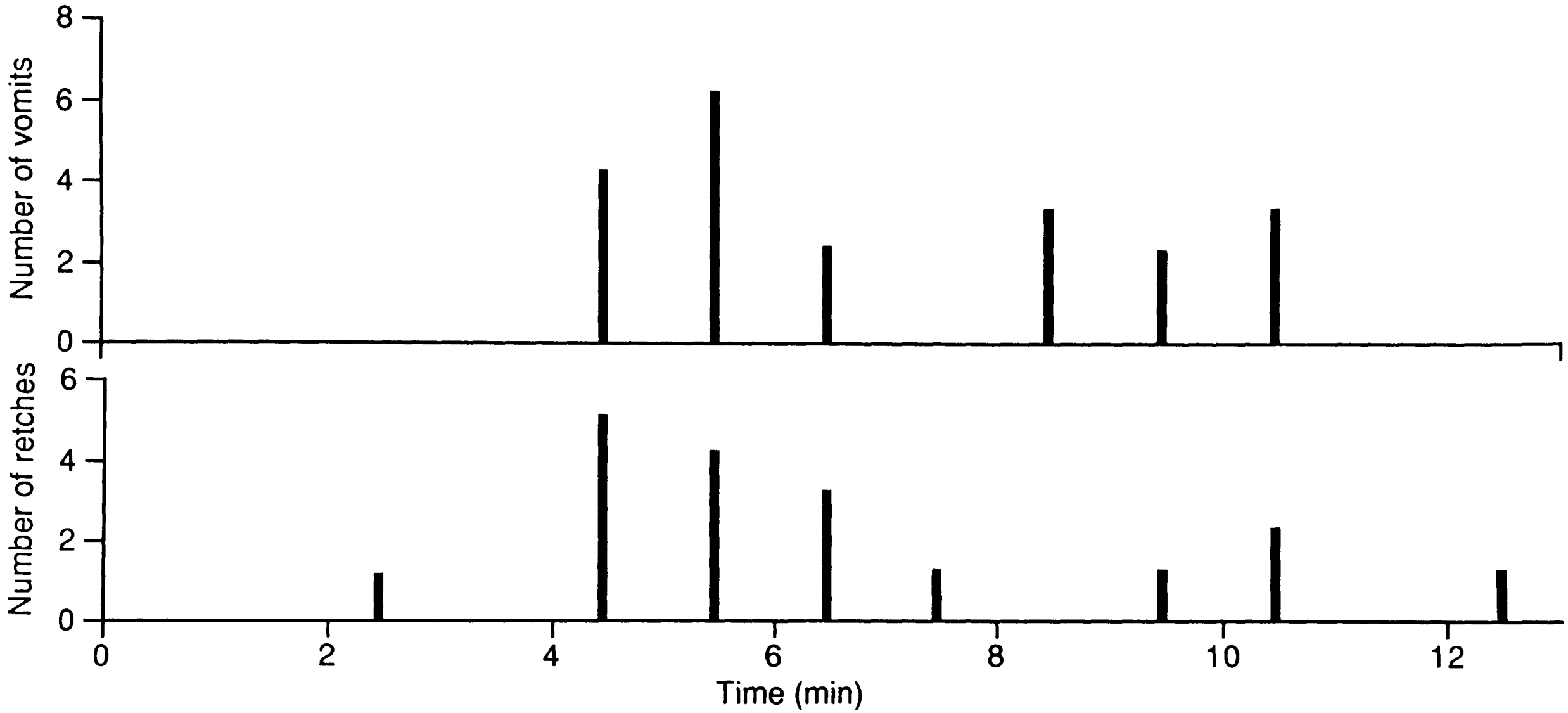


Figure 8 The Emetic Response of a Single Ferret to 30ml of 40mg% Oral Copper Sulphate

TABLE 4

The Reproducibility of the Emetic Response to 40mg% Copper Sulphate

Week No.	Emetic Response	Vomiting Latency (min)	Number of Vomits	Number of Retches
1	5/5	6.8 ± 2.5	4 ± 2	11 ± 9
2	4/5	7.8 ± 2.1	3 ± 2	5 ± 5
3	4/5	9.7 ± 4.4	3 ± 2	4 ± 3
4	4/5	10.4 ± 1.9	3 ± 2	8 ± 6

However, during each of the 2nd, 3rd and 4th weeks a different animal dropped out of the responder group. The fact that individual animals may not necessarily be consistent in their response on each occasion that they are tested means that large group sizes should be used when possible and animals having had a peripheral nerve lesion or an anti-emetic should undergo multiple testing whenever possible, to prevent a response being missed when it may appear at a second testing or vice versa.

3.1.3 Sodium Chloride

Historically sodium chloride, originally in the form of sea water, is probably the oldest known natural emetic and it has been used therapeutically (reviewed in Meester, 1980) and as test substance on many occasions.

1.0M NaCl was found to be a reliable emetic stimulus with a latency of 4.4 ± 3.0min (n = 15). Thus:-

NaCl Concentration	Vomiting Response
0.154M	0/5
0.500M	1/5
0.750M	5/5
1.000M	15/15

See also Fig. 9

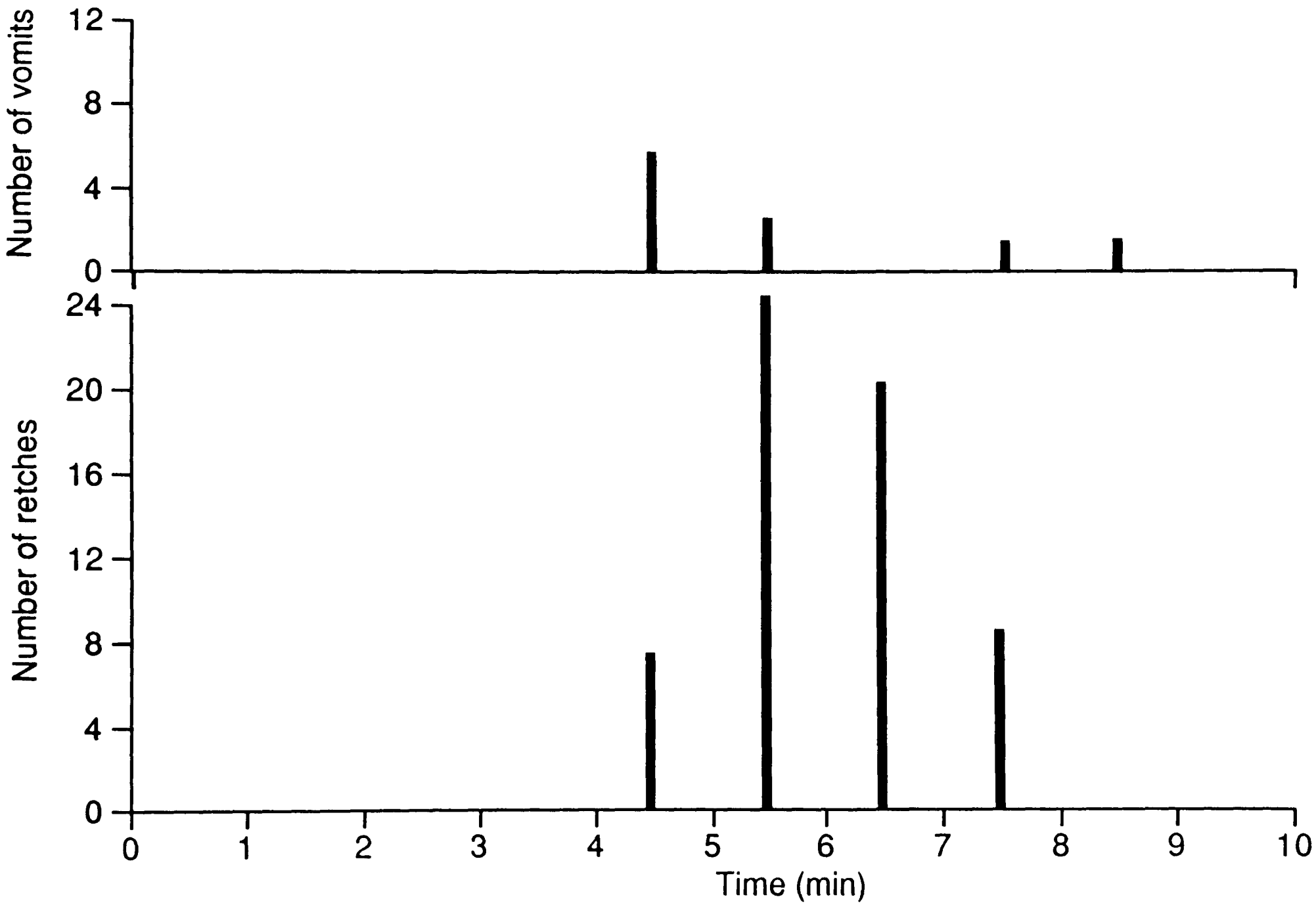


Figure 10 The Emetic Response of a Single Ferret to 30ml of 1M Oral Sodium Chloride

In normal animals 1.0M NaCl did not result in morbidity or mortality. However, subsequent studies in a number of groups of ferrets throughout the seasons of the year revealed that at particular times, (e.g. Autumn and Winter) ferrets became refractory to 1M NaCl, (e.g. in one trial of 20 animals 10 did not respond). Time did not permit us to further characterise this apparent seasonal relationship with respect to emetic response to 1M NaCl but the association has been reported by other groups of workers using ferrets obtained from entirely different sources (Andrews, personal communication). It is noteworthy that the emetic response of the same groups of ferrets to apomorphine and X-rays during these refractory periods was within the normal limits established during these studies.

The pattern of retching and vomiting in a typical animal tested with 1M NaCl is illustrated in Fig. 10.

3.1.4 Glucose

D-Glucose was prepared as 0.308M, 1.0M, 1.5M and 2.0M solutions and administered in the way previously described. Response rates are summarised thus:-

Glucose Concentration	Vomiting Response
0.308M	0/5
1.000M	1/5
1.500M	1/5
2.000M	6/6

Prodromata were displayed by all other animals in these groups. At the 2.0M concentration the mean vomiting latency was 8.7 ± 4.1min with a mean number of vomits and retches of 9 ± 5 and 28 ± 21 respectively. Glucose has been previously shown to cause activation of abdominal vagal afferents (Andrews 1986) and it is apparent from the present results that sufficient stimulation of such a kind will result in a consistent period of vomiting (duration 5.1 ± 4.6min) at a concentration of 2.0M. The pattern of retching and vomiting in a typical animal tested with 2.0M Glucose is illustrated in Fig. 11.

3.1.5 Mannitol, Choline Chloride and Potassium Chloride

Based on the effective emetic concentration of NaCl and glucose the effect of 1M potassium chloride (KCl), 1M choline chloride and 2M mannitol was investigated. At these concentrations the three compounds all caused vomiting. The vomiting latency and emetic response data are all shown in Fig. 12. The pattern of retching and vomiting in a typical animal tested with each of these three compounds is illustrated in Figs. 13, 14 and 15.

The emetic response data on all 6 intragastric emetics tested are summarized in Fig. 16 for comparison.

3.1.6 Prodromata of Emesis

We have defined the prodromata of emesis as a group of pre-emetic behaviours which may culminate in vomiting, but not invariably. Initially the following features were identified in animals following challenge with intragastric emetic stimuli. In order of chronological appearance these were:

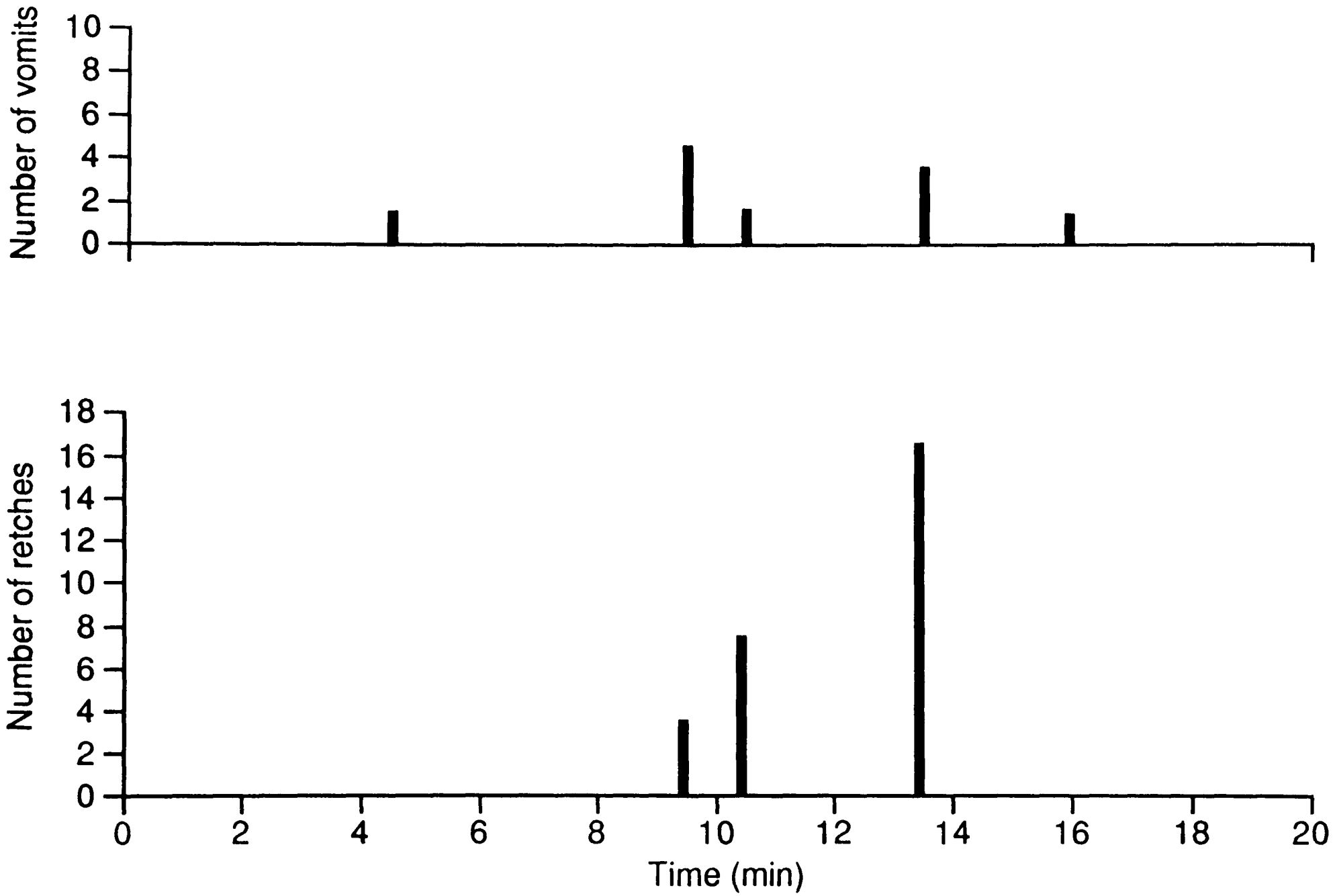


Figure 11 The Emetic Response of a Single Ferret to30ml of 2M Oral Glucose

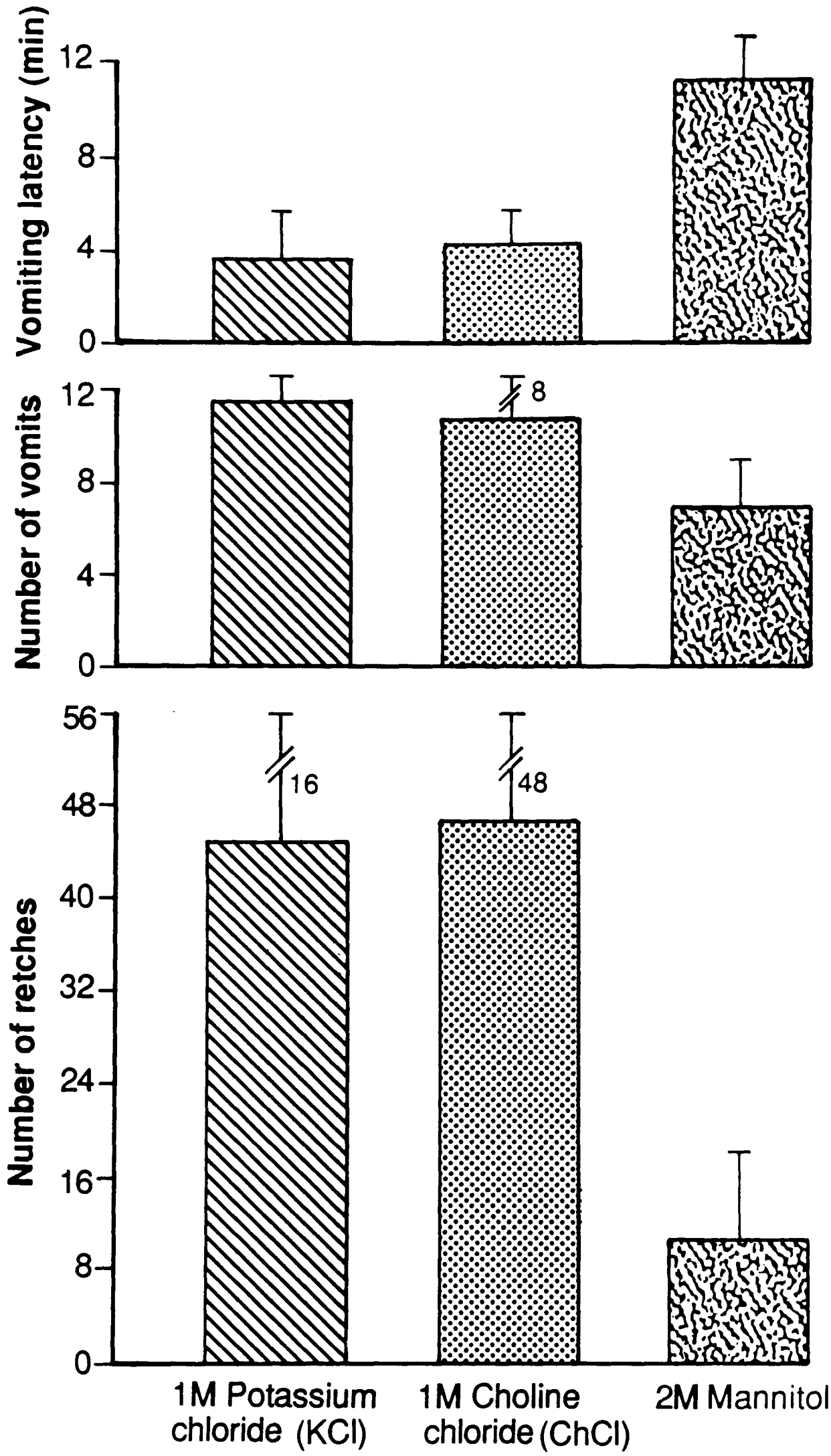


Figure 12 A Summary of the Emetic Responses of the Ferret to 30ml of Orally Administered 1M Potassium Chloride, 1M Choline Chloride and 2M Mannitol

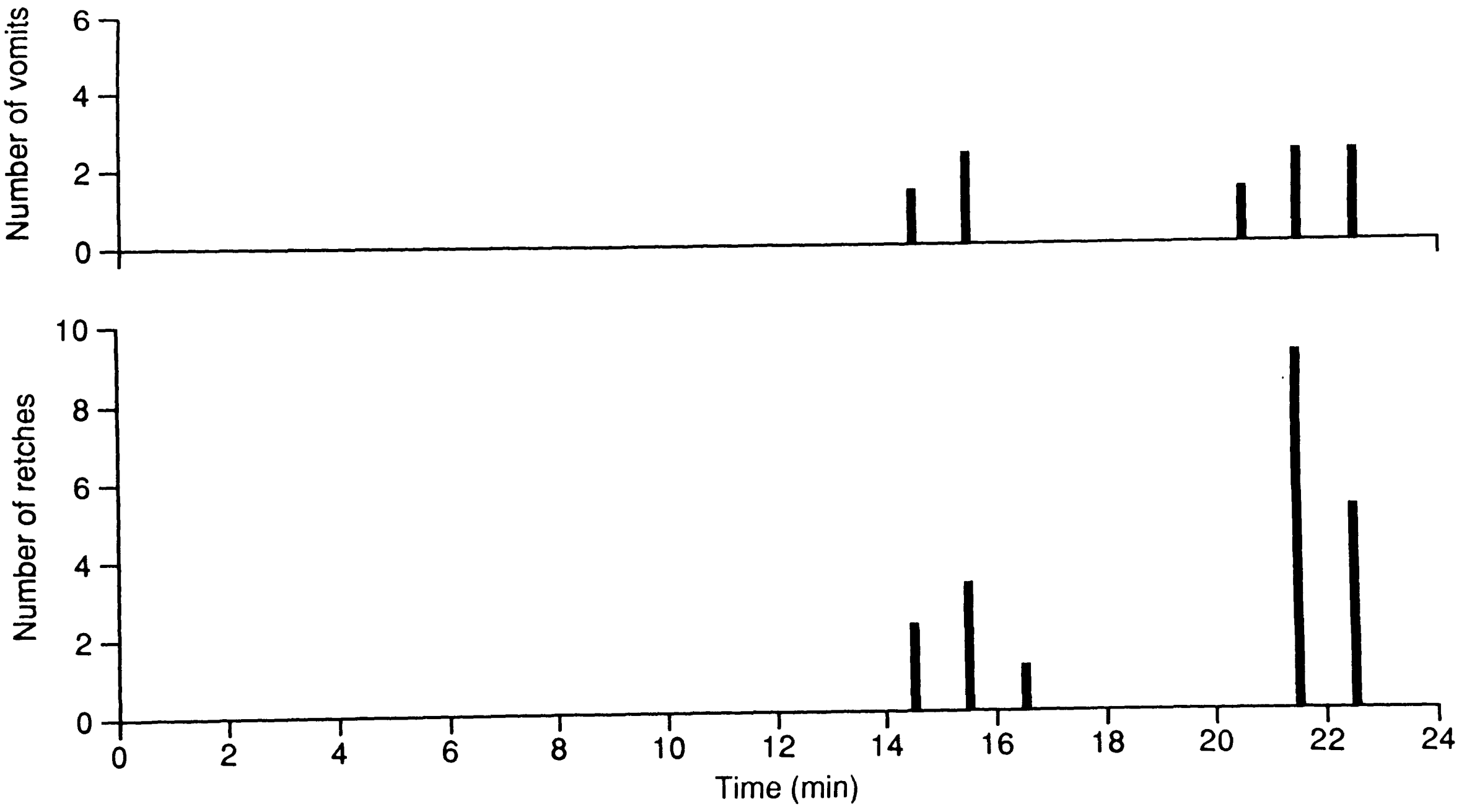


Figure 13 The Emetic Response of a Single Ferret to 30ml of Oral 2M Mannitol

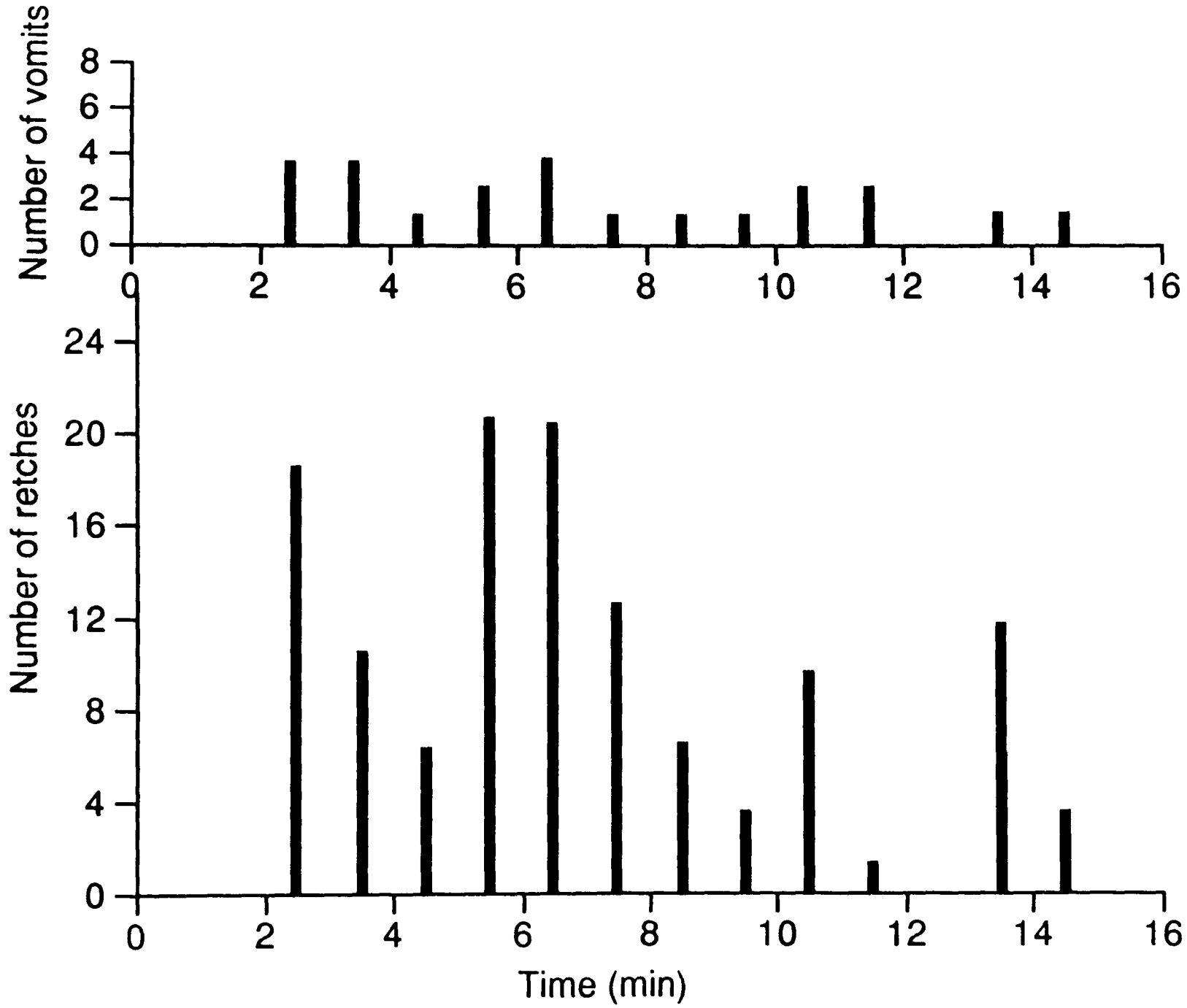


Figure 14 The Emetic Response of a Single Ferret to 30ml of Oral 1M Choline Chloride

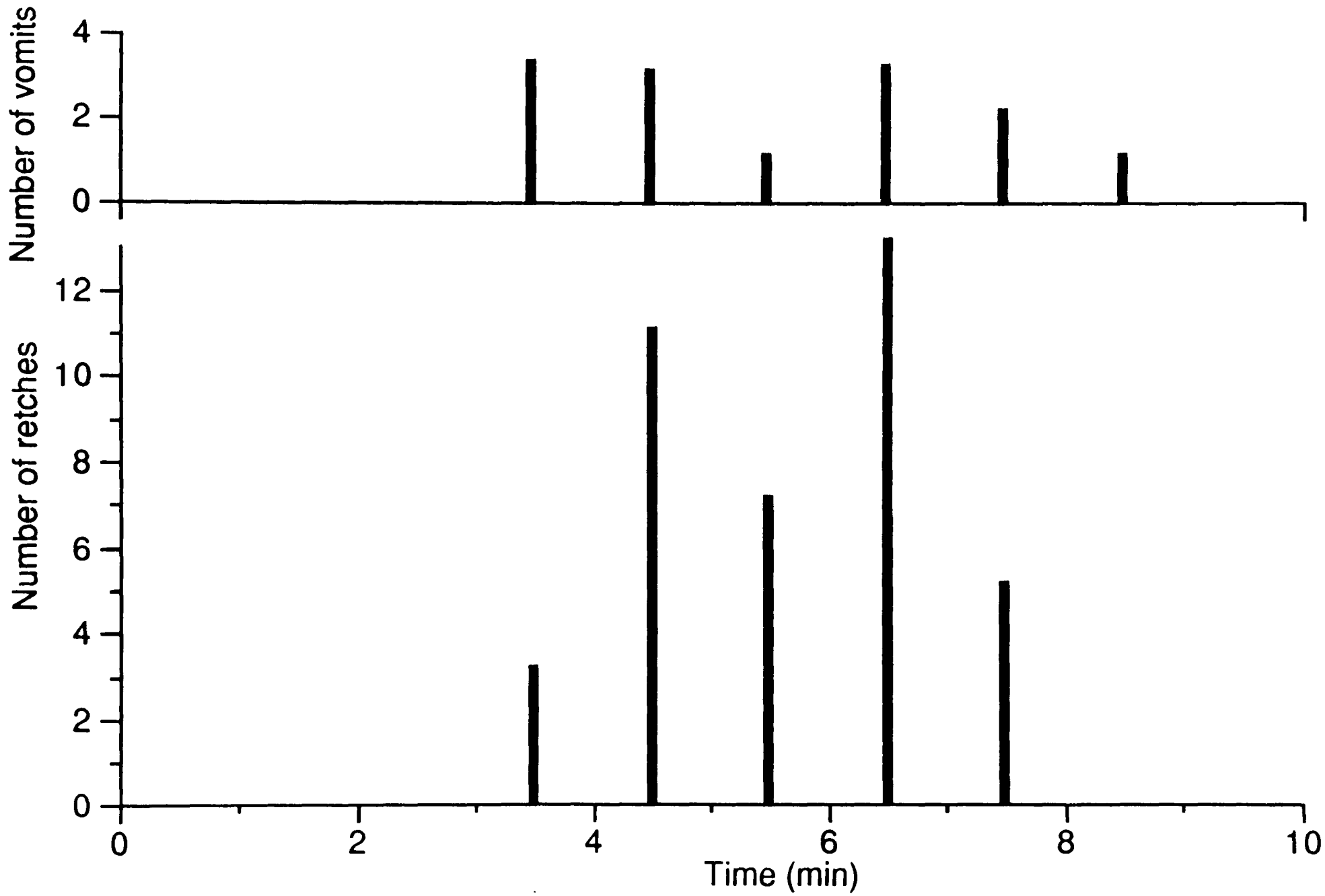
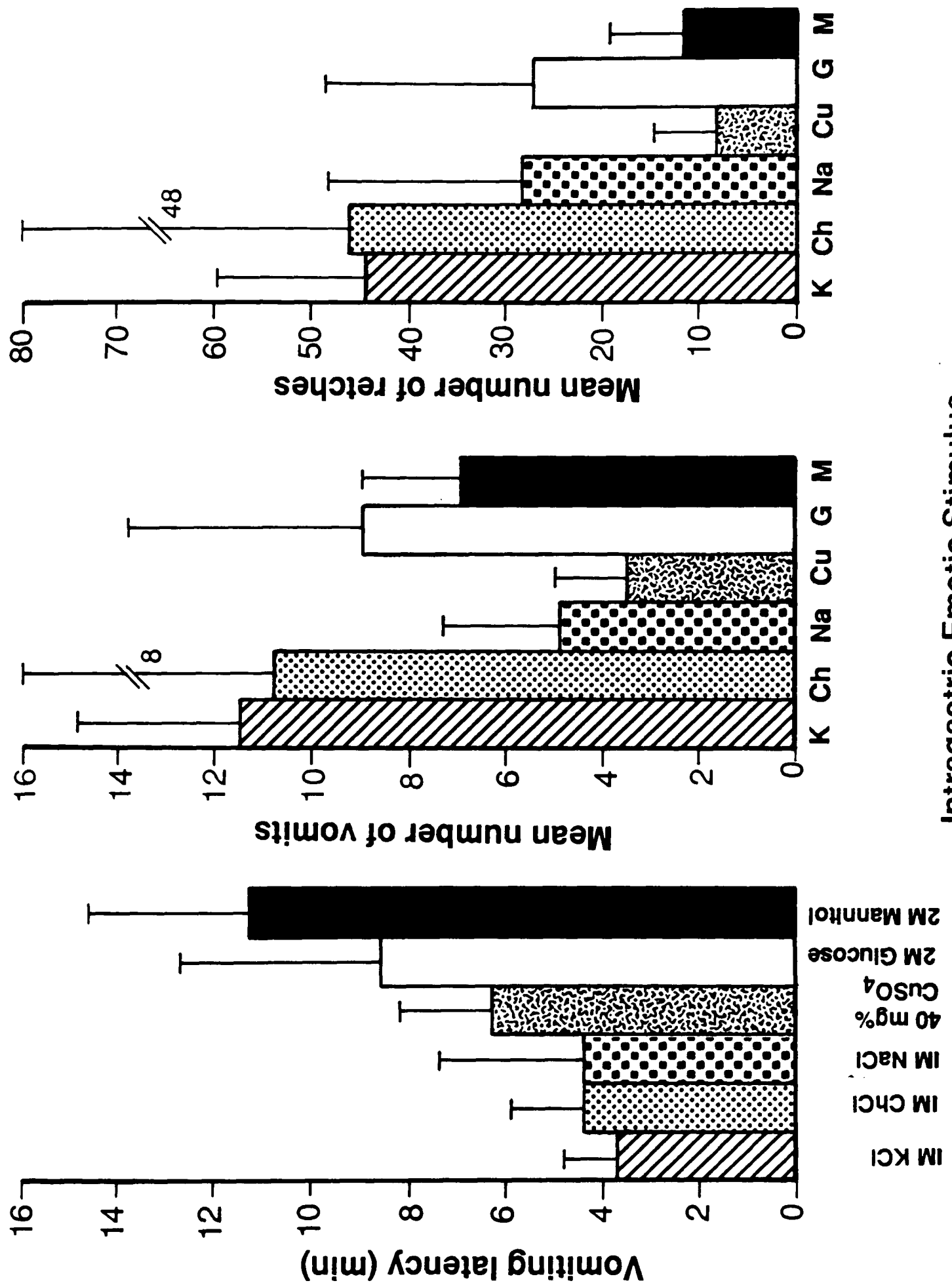


Figure 15 The Emetic Response of a Single Ferret to 30ml of Oral 1M Potassium Chloride

Figure 16 A Summary and Comparison of the Emetic Responses of the Ferret to a Variety of Orally Administered Emetics



- Hypomotility:

  A slowing down and cessation of exploratory and running behaviour

- Piloerection:

  The fur was seen to stand up on end and become 'fluffy'

- Bilateral narrowing of the palpebral fissures:

  'Narrowing' of the eyes to a "slit" in the most extreme cases

- Wet-dog shakes:

  A fast co-ordinated shaking of the body as seen typically by a wet dog

- Digging with the forepaws:

  Animals stopped running freely and dug, often very vigorously, in the bedding material of their cages

- Chin dropping:

  Animals stopped running and became quite still with their heads placed, chin down, on the floor of the cage

- Hunched back:

  Animals curled the body whilst still in the upright position so that in the extreme case front and back paws were approximated

- 'Tip-toe' gait:

  This arose out of the hunched back position and meant that the animals moved around as if on 'tip-toe' with a shuffling gait instead of their normal free flowing running style

- Burrowing:

  This was vigorous burrowing and pushing of the head through the bedding material using the snout

- Prostration:

  A sudden flattening of the ventral surface of the body on to the floor

- Retrograde motion:

  A distinctive, rapid, walking backwards, referred to as 'backing-up'

- Licking and Swallowing:

  These terms are self-explanatory; excess performance of the occasional licking and swallowing movements that take place during normal daily activity in a ferret

- Pre-emetic posturing:

  Referred to as 'posturing' and meaning the taking up of the particular stance which immediately precedes the act of vomiting or retching. It comprised immobility, bracing the body with the fixation of the hind and forepaws and the 'stretching-out' of the body

These features were then categorised into those which were characterised prodromata of emesis and those which were diagnostic prodromata of emesis. Thus:-

Characteristic Behaviours; Behaviour exhibited as part of the prodromata of emesis but which is also seen occasionally during periods of normal activity.	Diagnostic Behaviours; Behaviour exhibited only as part of the prodromata of emesis and seen at no other time.
- Forepaw Digging - 'Backing-up' - 'Burrowing' - 'Wet Dog Shakes - Excess Licking - Excess Swallowing - 'Hunched Back' - 'Tip-toe' gait - 'Chin-dropping'	- 'Eye Narrowing' - Prostration - Pre-emetic Posturing

Therefore by a process of exclusions based on the above defined categories it is possible to identify just three basic behaviours, i.e. Eye "narrowing", prostration and pre-emetic posture, that are alone always present when an animal has been treated with an emetic stimulus whether or not vomiting has actually occurred, or will occur.

It is axiomatic that it is not possible to apply the term nausea to the experiences of animals in the period leading up to

emesis or suffered as a result of receiving an emetic stimulus. However, even in man the subjectively reported sensation of nausea is known to be accompanied by certain objectively observable physiological and behavioural changes. So it is reasonable to assume that at least the 3 behavioural features listed as the 'diagnostic' prodromata of emesis in the ferret are most likely to be the correlates of nausea-associated behaviour in man. In addition the presence of the 9 'characteristic' behavioural features listed will add weight to the conclusion that any causative stimulus is emetic in nature.

This opens the way to apply a scoring system to the presence or absence of these features in response to any given stimulus and possibly to the severity of the features displayed. It may then be possible to go some way to quantifying that difficult characteristic but occasionally very significant feature of emesis, i.e. nausea, and thus gauge the effect of putative anti-emetic drugs and nerve lesions on this particular side-effect in comparison to their effect on frank retching and vomiting. An initial attempt was made by (Costall et al., 1987) during the progress of this work to evolve a system to identify and quantify 'nausea' in the monkey but the criteria used represent a much less discriminating detector of behavioural significance with respect to emetic stimuli than those suggested above. Moreover as has already been discussed the monkey is in any case very often less than ideal as a model for vomiting.

3.1.7 The Effect of Anti-emetics and Peripheral Nerve Lesions on the Response of Ferrets to Intragastric Emetics

From the list of intragastric emetic stimuli used two compounds were selected for the study of the nervous pathways involved. These were 1M NaCl and 40mg% CuSO4. Unless otherwise stated the doses of drug used were; Domperidone 500μgkg^{-1} and Metoclopramide 5$mgkg^{-1}$.

3.1.7.1 The Effect of Anti-emetics on Sodium Chloride and Copper Sulphate Induced vomiting

Domperidone did not abolish the emetic response to 1M NaCl in a group of 5 ferrets tested before and after treatment with the drug. Likewise metoclopramide also did not abolish the emetic response of 1M NaCl (n = 4). Both compounds also failed to abolish the prodromata of vomiting. Domperidone and Metoclopramide were also without apparent significant effect on the emetic response to 40% CuSO4. Domperidone did not affect the prodromata of vomiting which were consistently present but metoclopramide appeared to abolish prodromata in individual animals.

3.1.7.2 The Effect of Nerve Lesions on Sodium Chloride and Copper Sulphate Induced Vomiting

The effects of abdominal vagotomy and section of the greater splanchnic nerve ("splanchnectomy") either alone or in combination, on the emetic response to NaCl or CuSO4 were examined at various times after lesioning. Two major groups of animals were used.

One group was tested prior to lesioning and a second group was not thus tested in order to avoid criticisms of sensitization or de-sensitization of animals with respect to the stimulus. As no difference was observed in the responses of the two groups after lesioning all these data were considered as a single entity.

Because of the inherent variability of the magnitude of the emetic response to these oft-used stimuli and the relatively small group numbers the responses are considered only in terms of incidence of emesis (Tables 5 and 6) and the overall pattern of the response, including latency (Figs. 17 and 18).

Table 5 summarizes data on the incidence of vomiting in response to 1M NaCl after various nerve lesions. Three days after abdominal vagotomy none of the animals tested with NaCl actually responded. Approximately 60min after NaCl administration the animals exhibited signs of sodium poisoning and the animals were killed humanely by anaesthetic overdose. However, by seven days after lesioning the emetic response to NaCl had returned and was still shown to be present when testing took place after approximately 30 days. Section of the greater splanchnic nerve was found to be without effect on the incidence of emesis on animals tested 7 - 10 days after lesioning. The lack of effect of section of the greater splanchnic nerve alone contrasts markedly with the effect of a combined vagotomy and "splanchnectomy" lesion which abolished the response to NaCl challenge in the 7 - 10 days post-lesion time frame. However, the response had returned by 21 - 30 days post-lesion. These results show that the delay after nerve lesioning is important for the production of a response, particularly for vagotomy and the

TABLE 5

The Effect of Peripheral Nerve Lesions on the Incidence of Vomiting in Response to 30ml of Intragastric IM NaCl (ED_{100}) at Varying Times after Lesioning

	3 days	7-10 days	21-30 days
Vagotomy	0/5 (0%)	8/9 (89%)	12/12 (100%)
Section of the Greater Splanchnic Nerves	-	5/5 (100%)	-
Combined Vagotomy and "Splanchnectomy"	-	0/5 (0%)	3/4 (75%)

TABLE 6

The Effect of Peripheral Nerve Lesions on the Incidence of Vomiting in Response to 30ml of Intragastric 40mg% $CuSO_4$ (ED_{100}) at Varying Times after Lesioning

	7-10 days	21-30 days
Vagotomy	11/17 (65%)	16/16 (100%)
Section of the Greater Splanchnic Nerves	5/5 (100%)	-
Combined Vagotomy and "Splanchnectomy"	3/4 (75%)	2/4 (50%)

Figure 17 The Effect of Time Interval to Testing, after Abdominal Vagotomy, on the Vomiting Response of the Ferret to Oral Copper Sulphate

Key: Vomiting episodes are indicated by the small vertical bars. Dose of Copper Sulphate was 30ml of 40mg% solution

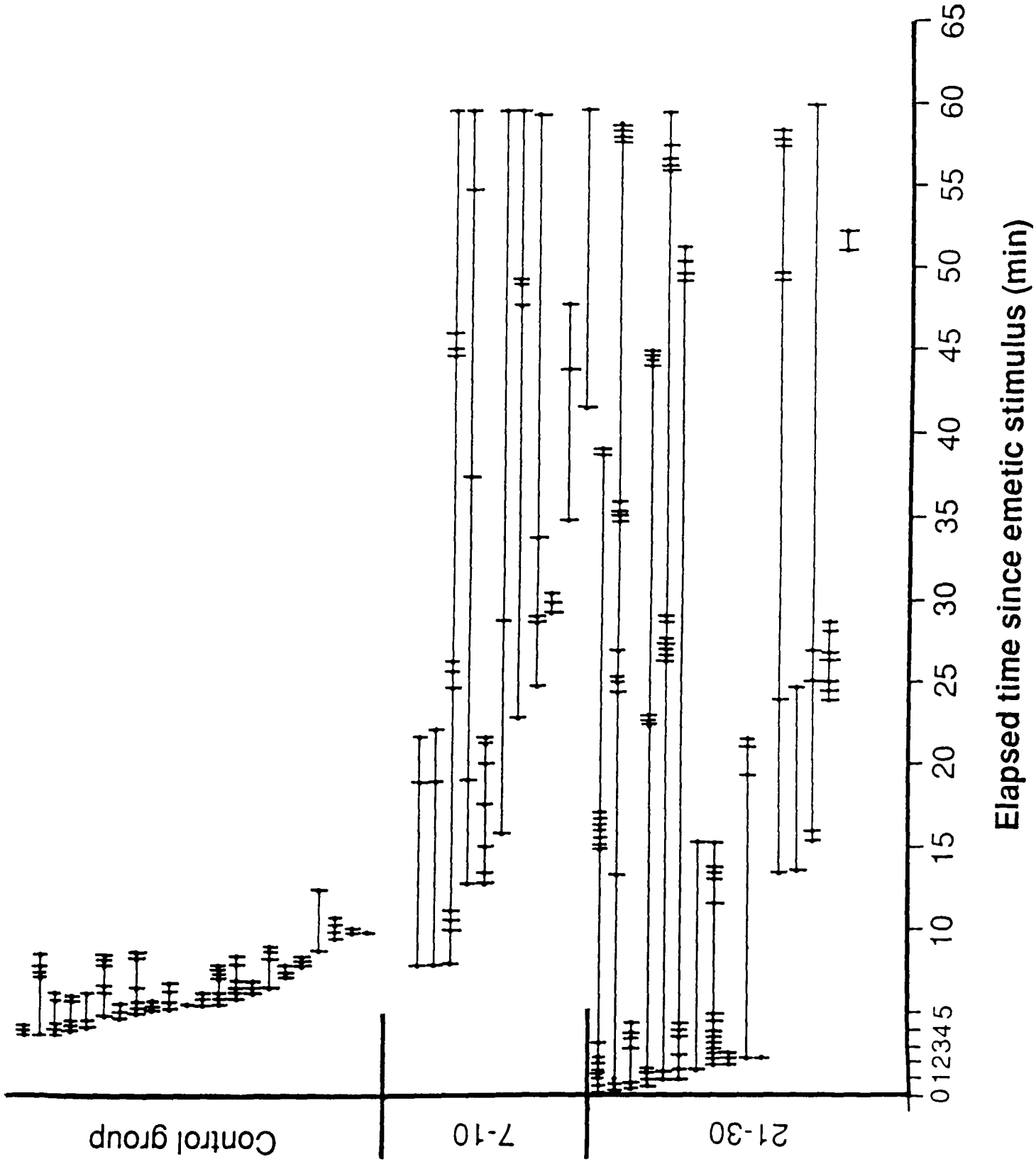
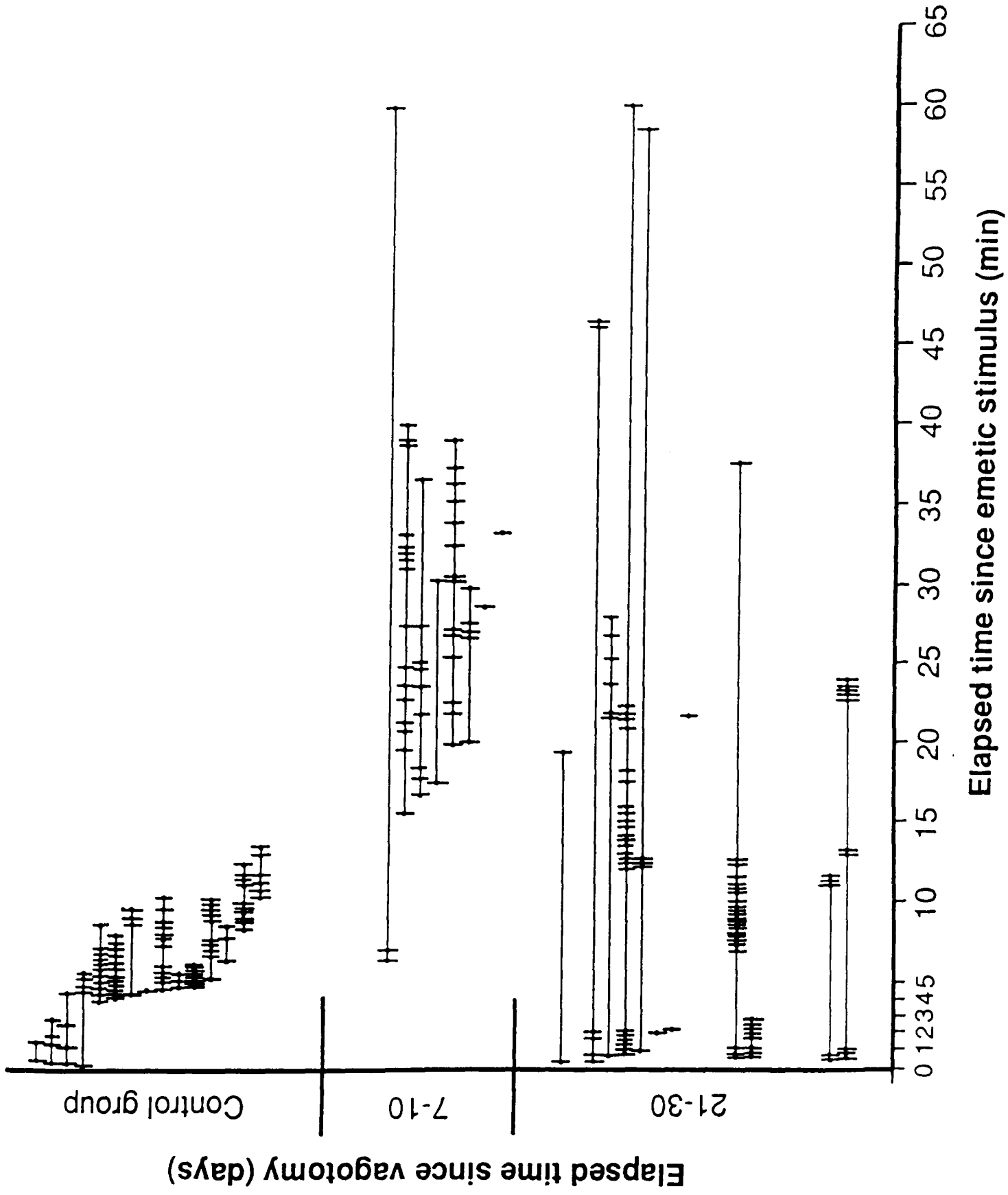


Figure 18 The Effect of Time Interval to Testing, after Abdominal Vagotomy, on the Vomiting Resp onse of the Ferret to Oral Sodium Chloride

Key: Vomiting episodes are indicated by the small vertical bars. Dose of Sodium Chloride was 30ml of 1M solution



combined lesion. Moreover, on examination of the response at 7 - 10 days post-lesion, the fact that neither vagotomy nor "splanchnectomy" abolished the emetic response whereas a combined lesion did, would imply that the emetic response can be driven by either the vagus or the greater splanchnic nerve, at this stage. The observation that a response to NaCl is present three weeks after a combined lesion indicates that another emetic mechanism has come into play or been induced.

Table 6 summarizes data on the incidence of vomiting in response to 40mg% CuSO4 after lesioning. From this it can be seen that the number of responders at 7 - 10 days is reduced by vagotomy. Although some 65% continued to respond suggesting that the vagus is not the sole mechanism involved in $CuSO_4$-induced emesis in this particular subgroup, we can say little about the animals who failed to respond. Examination of the response at 21 - 30 days post-lesioning, when the response rate rose again further supports the idea that the vagus is not the sole substrate of the emetic pathway for CuSO4. Nevertheless the observation that "splanchnectomy" has no effect on the incidence of emesis would suggest that the vagus does in fact take this role. However, a combined lesion, whilst reducing the incidence of vomiting still left 50 - 75% of animals responding, suggesting the existence of a pathway for gastrointestinal emetic stimuli other than via the vagus or greater splanchnic nerves. This conclusion is similar to that which we made for the analagous data from the NaCl experiments.

Whilst the position may appear confused it should be borne in mind that this analysis has only considered the incidence of emesis after various nerve lesions. Such a type of analysis has been used extensively by workers in the past (e.g. Wang and Borison, 1951) and may give rise to misleading interpretation of the effect of nerve lesions. In contrast when one examines the pattern of emesis produced by NaCl or CuS04 following nerve lesions one can see that relatively small changes in the incidence of emesis can mask marked changes occurring in the actual pattern of response. Therefore it may be unwise to examine the phenomena only in terms of incidence of emesis. For both NaCl and $CuSO_4$ the overall emetic response in control animals was confined to a tight band of latencies and durations (see Figs. 17 and 18). Following vagotomy at 7 - 10 days the latency usually increased and the episodes of emesis became more dispersed. In contrast, at the 21 - 30 day point, a high proportion of the animals responded with a latency shorter than that seen in control groups. For example 50% of animals tested responded in less than 45sec (n = 18) and we have termed this the ultra-short latency response. Following this very early burst of activity a subsequent bout of emesis was often observed to start at around +10min. These data contrast with the absolute incidence data above where although vagotomy per se appears to have little effect a representation of the pattern of vomiting clearly reveals that vagotomy has markedly altered that pattern of vomiting normally observed in control animals.

In contrast lesions of the greater splanchnic nerve did not affect the pattern of the emetic response at 7 - 10 days post lesioning.

We considered the number of animals in the combined lesion group to be too small to represent them graphically for these pattern recognition purposes. However, it is worth noting that in the 21 - 30 day post-lesion group, 4 out of the total of 5 animals which actually responded to NaCl or $CuSO_4$ did do with an ultrashort latency (i.e. less than 60sec).

3.1.8. Ipecacuanha (Ipecac)

Ipecacuanha is extensively used as an emetic for the treatment of accidental or intentional self-poisoning and has a therapeutic history going back many centuries (reviewed in a leader article of J. Am. Pharm. Assoc., 1971). It is an extract of a plant root (Cephaelis ipecacuanha or acuminata) containing a complex mixture of alkaloids of varying emetic potency (Manno and Manno 1977). Although the topic has been pursued on previous occasions it has never been conclusively proved where the principal site of emetic action is to be found. Moreover, Ipecac has, paradoxically, been responsible for its own morbidity and has occasionally resulted in fatalities, especially in young children (Miser and Robertson, 1978).

In view of our attempt to characterise the emetic profile of the ferret it was decided to establish an emetic dose of Ipecac' in the ferret, and investigate the effect of the various peripheral nerve lesions on the pattern of vomiting so established. An attempt was also made to identify which

constituent(s) of the Ipecac group formulation were responsible for the emetic action of Ipecac.

The results of Ipecac and Syrup B.P. administration to a series of ferrets is shown in Table 7.

The mean duration of emesis in ferrets given Ipecac Syrup (30ml) was 51.6 ± 0.2min (mean number of vomits 21 ± 5 and retches 87 ± 31). Vomiting continued in all three animals for over 2 hours, one animal died after one hour and diarrhoea was present in all animals. 30ml is the normal dose of Ipecac syrup used in the adult human, so on a weight for weight basis it exceeded the calculated dose appropriate for the ferret. The human equivalent dose was therefore also included in the experiment. Use of the Syrup B.P. vehicle appeared to markedly reduce latency and there was also a trend towards an increase in retching and vomiting. The effect of the vehicle, Syrup B.P., appeared to be important in these circumstances and subsequent administration of Syrup B.P. alone gave a vomiting response itself in 9/13 animals tested, with a mean latency of 7.5 ± 3.1min.

As a result of these investigations it was decided to compare the emetic effect of Syrup B.P. and weight-related doses of Ipecac syrup ($0.43 ml kg^{-1}$) delivered in Syrup B.P. or water in nine ferrets, rotating each ferret randomly through each of the treatments week by week using a Latin square. The results are seen in Table 8. On the basis of the incidence of vomiting after each treatment, no significant difference between the groups was detected ($\chi^2 = 0.791$ with 2 degrees of freedom). However as far as vomiting latency was concerned Syrup B.P. was significantly quicker than Ipecac in water ($p \leqslant 0.001$) but no

TABLE 7

The Response of the Ferret to Ipecacuanha Syrup (Ipecac') and Syrup B.P.

		Vomiting Response	Vomiting Latency (min)
Total human dose of Ipecac Syrup compared to syrup B.P. alone	Syrup B.P. (30ml)	9/13	7.5 ± 3.1
	Ipecac Syrup (30ml)	3/3	8.4 ± 0.2
Weight related dose of Ipecac based on human dose. Effect of vehicle compared to effect of Ipecacuanha Alkaloids.	Ipecac' Syrup[1] in 30ml of Syrup B.P.	14/14	12.1 ± 5.6***
	Ipecac' Syrup[1] in 30ml of water	7/13	24.2 ± 6.5

Footnote: 1. $0.43 mg kg^{-1}$ is the approximate human equivalent dose of Ipecac Syrup

TABLE 8

A Comparison of the Emetic Effect of Syrup B.P. and Weight Related Doses of Ipecacuanha Syrup Delivered in Syrup B.P. or Water

	Emetic Response	Vomiting Latency (min)	Number of Vomits	Number of Retches
Syrup B.P Alone	5/9	9.1 ± 3.4***	5 ± 6	29 ± 38
Ipecac' in Water	7/9	24.2 ± 6.5	6 ± 5	14 ± 15
Ipecac' in Syrup B.P.	8/9	13.1 ± 6.5	7 ± 4	41 ± 3

different to Ipecac in syrup. There was no difference in the number of vomits produced by each approach but a clear trend for the number of retches to be reduced with Ipecac and H_2O compared to Syrup B.P. or Ipecac and Syrup was demonstrated. Diarrhoea was present in 6/9 animals treated with Syrup alone and Ipecac in Syrup and in 3/9 treated with Ipecac in water.

The main emetic constituent of Ipecac on a weight for weight basis is emetine (Manno and Manno, 1977). The most potent 'emetogenic' constituent of Ipecac is cephaelin which is present in a very small quantity but on a weight for weight basis the major emetic constituent is emetine itself. As cephaelin could not be obtained for experimental use in the pure form it was decided to investigate the effect of emetine as an emetic per se, presented in solution in water or Syrup B.P. at a concentration equivalent to that present in the commonly available pharmaceutical preparation of Ipecac Syrup i.e., 150mg%. The results of administration of emetine in water and Syrup B.P. are recorded below -

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Emetine in water	3/4	40.7 ± 5.8	10 ± 12	37 ± 46
Emetine in Syrup B.P.	5/5	13.8 ± 5.0***	20 ± 7	149 ± 78

The average period of emesis in the emetine/water group lasted for about 20 minutes but all animals still showed

occasional retching and vomiting at 3 hours post-treatment. The animal that did not react did however display marked prodromata. Two animals displayed diarrhoea.

In the emetine/Syrup B.P. group all animals were still showing sporadic vomiting after 3 hours and all displayed very marked prodromata but only one showed marked diarrhoea.

3.1.8.1 Ipecacuanha and Peripheral Nerve Lesions

Preliminary studies were carried out on the effect of section of the abdominal vagus nerve on the emetic pattern resulting from treatment with various formulations of Ipecac and emetine.

Vagotomy completely abolished retching and vomiting in four animals tested with 30ml of undiluted Ipecacuanha syrup 7 - 10 days post-lesioning. Prodromata were present from 15min onwards and seizures supervened in all animals at 45min leading to death or the requirement to sacrifice the animals humanely. The results of challenge with emetine in water on animals with a previous vagotomy are summarized below:-

	Vomiting Response	Vomiting Latency	Mean Number of Vomits	Mean Number of Retches
Control Group	3/4	40.7 ± 5.8	10 ± 12	37 ± 46
Post Vagotomy Group	3/4	51.9 ± 26.5	8 ± 8	72 ± 25

Mucoid diarrhoea was present in all animals and marked prodromata were displayed. Subsequent to treatment 2 of the 4 animals tested died.

3.2 Systemic Emetic Stimuli

3.2.1 Controls

Appropriate volumes of saline (154mM NaCl) injected intravenously, intraperitoneally and subcutaneously as controls for the administration of drugs produced no change in behaviour or any evidence of prodromata or retching and vomiting.

3.2.2. Apomorphine

Apomorphine has been used extensively as an emetic test substance for many years (see Meester, 1980); a standard against which the efficacy of various anti-emetic drugs has been measured (Proctor et al., 1978).

It was appropriate therefore to characterise thoroughly its action as an emetic in the ferret to establish a base-line of data in this new animal model.

3.2.2.1 Subcutaneous Administration

Groups of ferrets were tested with a range of doses from $10\mu gkg^{-1}$ to $500\mu gkg^{-1}$ for their emetic response. The results are illustrated in Fig. 19 and Table 9.

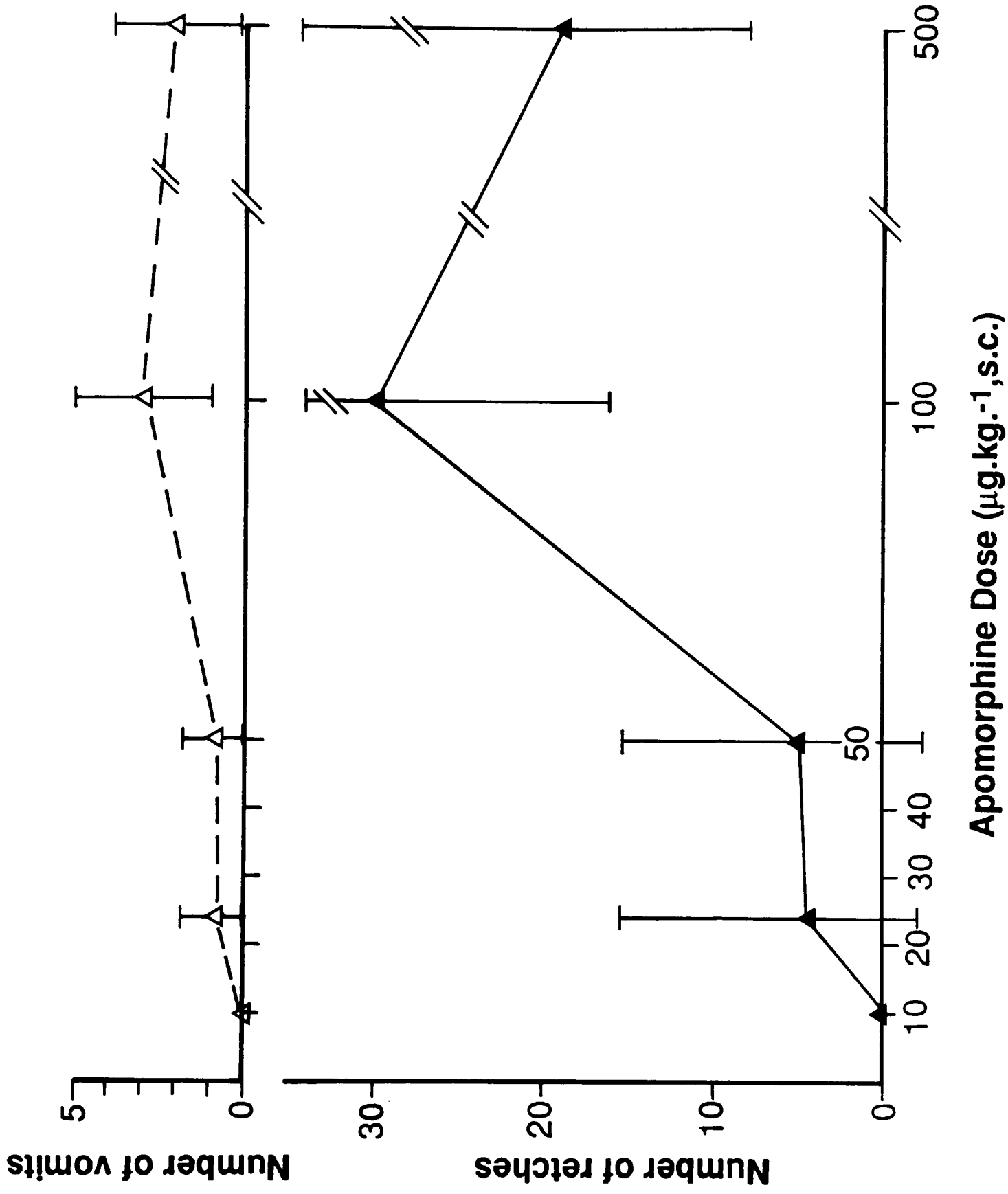
From the emetic response data (the total number of retches and vomits) it appears that a "bell-shaped" response curve governs the reaction of the ferret to this range of doses via the subcutaneous route. At very much higher doses ($5mgkg^{-1}$) no vomiting or retching or prodromata were observed (see also Florczyk et al., 1982). The ED_{100} for vomiting to apomorphine in the ferret by the s.c. route is $100\mu gkg^{-1}$; a dose which

TABLE 9

The Emetic Effect of Subcutaneously Administered Apomorphine on the Ferret

Dose (μgkg^{-1} s.c.)	Emetic Response	Vomiting Latency (min)	Number of Vomits	Number of Retches
10	0/4	-	0	0
25	2/9	3.9 ± 3.0	1 ± 1	5 ± 11
50	10/26	4.2 ± 3.1	1 ± 1	6 ± 10
100	10/10	5.2 ± 1.3	3 ± 2	30 ± 14
500	2/4	12.1 ± 15.6	2 ± 2	19 ± 15

Figure 19 The Emetic Dose-response Curve for Subcutaneously Administered Apomorphine in the Ferret
(n = 4 - 10 animals)



also produced the greatest response in terms of emetic episodes. The pattern of retching and vomiting differed markedly from animal to animal but in all cases vomiting had ceased 10 minutes after administration.

3.2.2.2 Intravenous Administration

Similar groups of ferrets were tested with apomorphine administered i.v. at doses ranging from 10 - 500μgkg^{-1}. The result of these tests is illustrated in Table 10. No response was elicited until the dose reached 100μgkg^{-1} although there was some retching at 50μgkg^{-1}. However, an ED_{100} for vomiting could not be reached, the best response being at 100μgkg^{-1} when $^{3}/8$ animals vomited (prodromata were observed in the non-responders). Overall the emetic responses to a range of doses of apomorphine given i.v. compared with those from the same range of doses given s.c were considerably reduced, being difficult to elicit and less reliable. A comparison of the effectiveness of the s.c. and i.v. routes of administration is given below -

Apomorphine ($100\mu gkg^{-1}$)	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
s.c. route	10/10	5.2 ± 1.3	3 ± 2	30 ± 14
i.v. route	3/8	4.8 ± 3.6	1 ± 1	6 ± 11

3.2.2.3 Anti-emetics and Apomorphine

Domperidone abolished retching, vomiting and prodromata in two groups of 4 animals tested with 50 and 100μgkg^{-1} of apomorphine given via the subcutaneous route.

TABLE 10

The Emetic Effect of Intravenously Administered Apomorphine on the Ferret

Dose (μgkg^{-1} i.v.)	Emetic Response	Vomiting Latency (min)	Number of Vomits	Number of Retches
10	0/5	-	0	0
25	0/4	-	0	0
50	0/8	-	0	1 ± 11
100	3/8	5 ± 4	1 ± 1.5	6 ± 11
500	1/4	[13.3]	1 ± 1	9 ± 19

Two similar groups of animals (n = 5 in each case) were also pre-treated with metoclopramide and then challenged with 50 and 100μgkg^{-1} apomorphine s.c.. No retching or vomiting was recorded during the 30min observation period although prodromata were noted in animals from both groups.

3.2.2.4 Apomorphine and Peripheral Nerve Lesions

Apomorphine-induced vomiting (100μgkg^{-1} s.c.) was not significantly affected by vagotomy at one and eight weeks after lesioning. Similarly vagotomy combined with greater splanchnic nerve section also did not significantly affect the vomiting response of the ferret to 100μgkg^{-1} of apomorphine s.c. Thus:-

Apomorphine (100μgkg^{-1})	Mean Number of Vomits	Mean Number of Retches
Control Group (n = 10)	3 ± 1	30 ± 14
Post vagotomy (n = 16)	5 ± 3	21 ± 9
Post combined (n = 15)	2 ± 3	14 ± 9

Lesion

3.2.3. Cytotoxic Drugs

3.2.3.1 Cycloheximide

Cycloheximide produced profound prodromata preceding the onset of retching and vomiting, along with persistent severe diarrhoea. The diarrhoea, prodromata, and emesis continued for well in excess of 2 hours.

Following a dose of cycloheximide (20mgkg^{-1} i.p.), vomiting began after 17.2 ± 5.4min (n = 9) and continued in bursts throughout the observation period of 120min. An

example of the pattern of vomiting and retching from a typical animal is illustrated in Fig. 20 and is summarised for the group in Fig. 21. During the observation period of 120min following the administration of the cycloheximide 20mgkg^{-1} i.p., the mean number of retches was 110 ± 38 and vomits was 16 ± 9.

Effect of Vagotomy

In the presence of an abdominal vagotomy only one animal vomited out of 5 tested, and then only on one occasion. All animals retched and diarrhoea was present. The effect of vagotomy is summarized thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	9/9	17.2 ± 5.4	16 ± 9	110 ± 38
Vagotomy Group	1/5	[96.7]	[1]	43 ± 39

Effect of Metoclopramide

Pre-treatment with metoclopramide did not significantly influence the vomiting latency. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	9/9	17.2 ± 5.4	16 ± 9	110 ± 38
MCP Group	6/6	22.2 ± 4.9	14 ± 9	150 ± 58

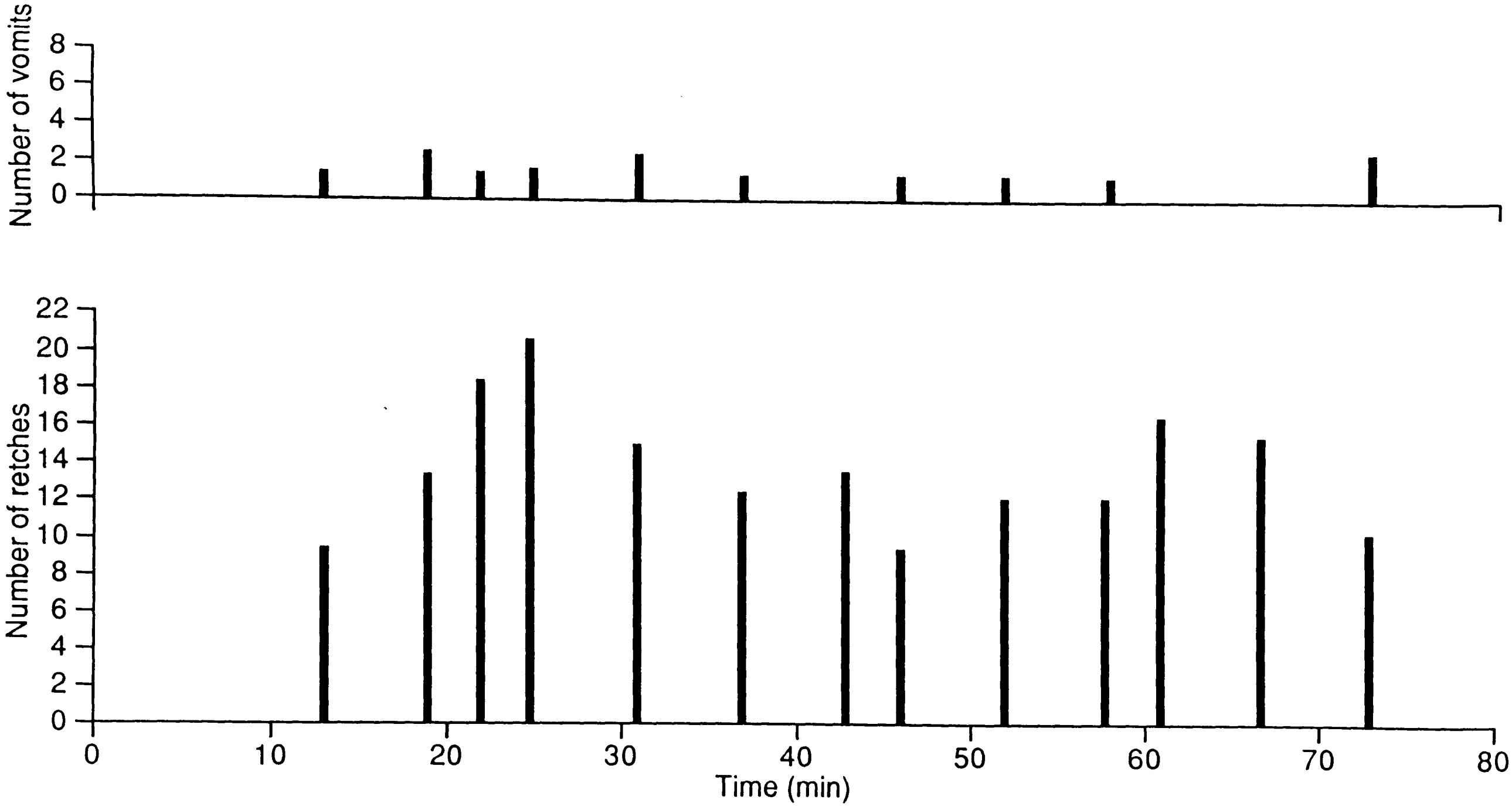


Figure 20 The Emetic Response of a Single Ferret to Intraperitoneally Administered Cycloheximide (20mgkg^{-1})

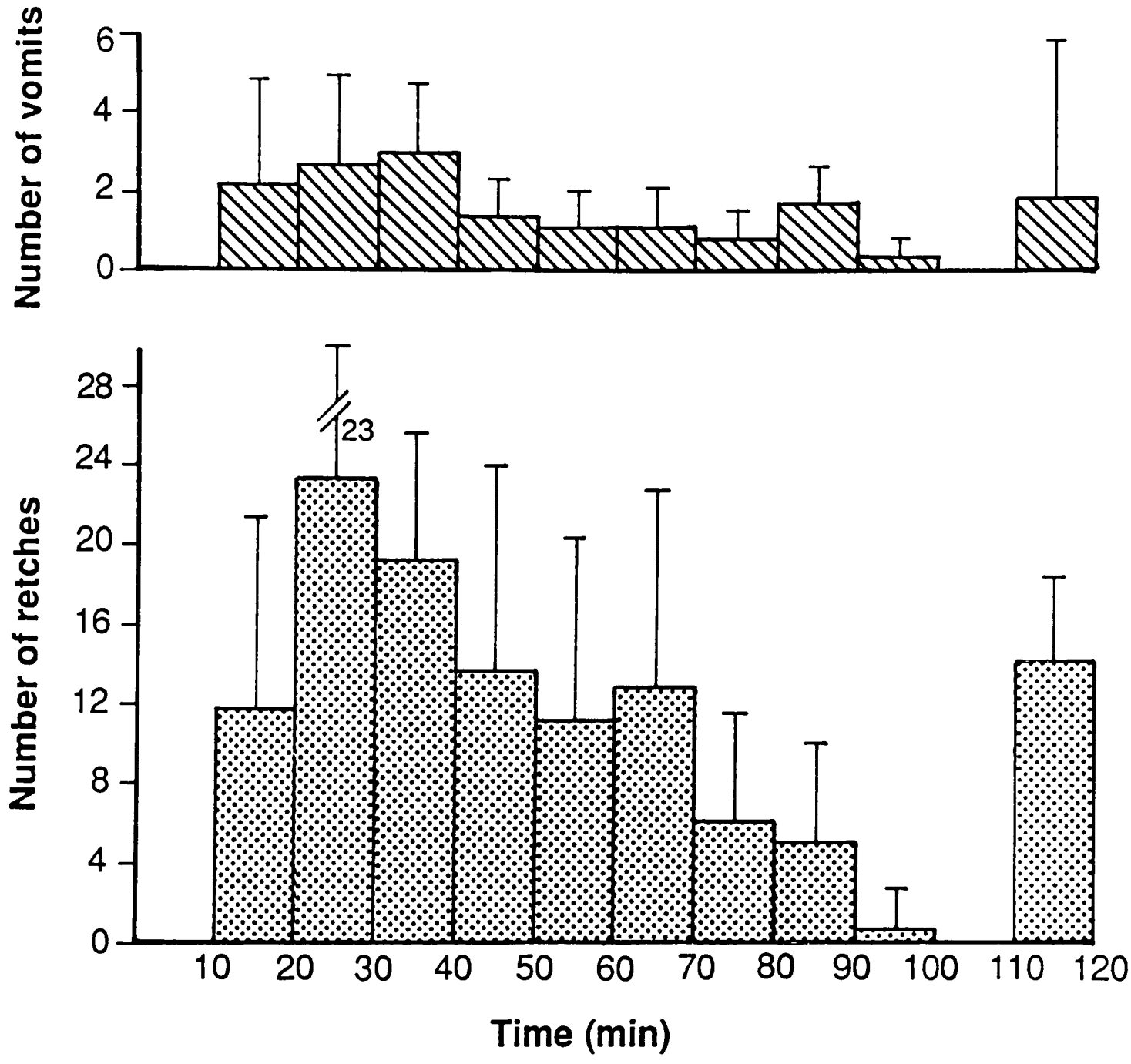


Figure 21 The Emetic Response of a Group of Ferrets to Intraperitoneally Administered Cyclohexmide (20mgkg^{-1})

The pattern of retching and vomiting is resolved into 10min time periods after injection of cycloheximide at time zero (n = 9 animals)

Retching and vomiting frequency was broadly comparable to that observed in the control group and continued for up to 3½ hours post-treatment as did diarrhoea was still present.

Effect of BRL 24924

Four ferrets pre-treated with this compound, (a derivative of the benzamide drug metoclopramide with greater specificity for the $5HT_3$ receptor), vomited to cycloheximide but with a greatly increased latency. Prodromata and diarrhoea were still present after treatment despite the reduction in emetic effect of cycloheximide.

The effect of BRL24924 is summarized thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	9/9	17.2 ± 5.4	16 ± 9	110 ± 38
BRL24924 Group	4/4	96.3 ± 12.1***	3 ± 1**	30 ± 29**

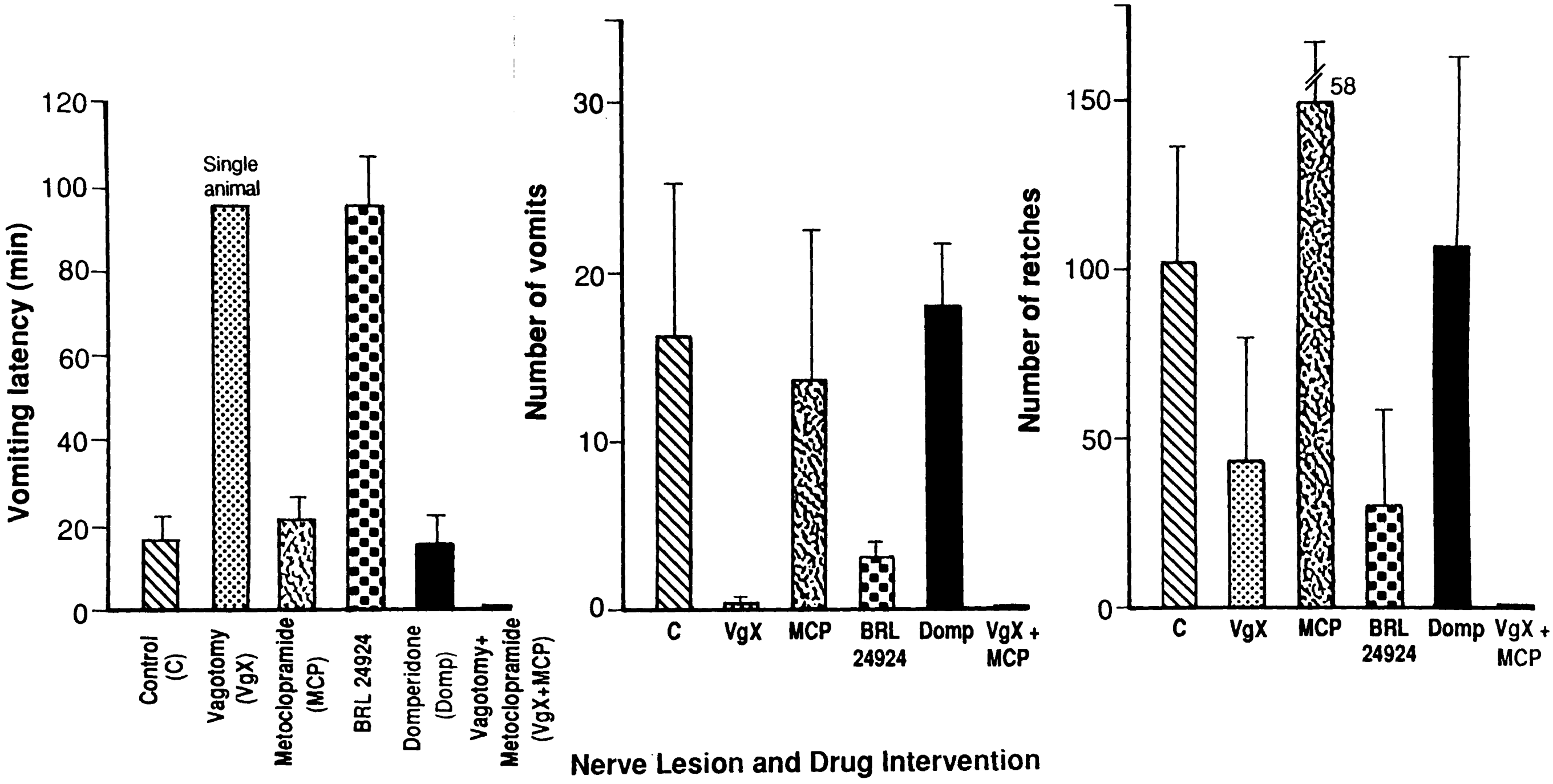
Effect of Domperidone

Three out of three ferrets pre-treated with domperidone vomited to cycloheximide. There was no significant effect on vomiting latency or emetic potential and prodromata and diarrhoea were still present. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	9/9	17.2 ± 5.4	16 ± 9	110 ± 38
Domperidone Group	3/3	17.5 ± 6.0	18 ± 5	106 ± 57

Figure 22 The Effect of Nerve Lesions (Abdominal Vagotomy) and Drug Treatments (Metoclopramide, BRL 24924 and Domperidone) on Cycloheximide-Induced Emesis in the Ferret

Total number of retches and vomits to the 120min observation period. Doses of drugs were; MCP-5mgkg^{-1} s.c., BRL 24924-5mgkg^{-1} s.c. Domp.-500µgkg^{-1} i.m. (n = 3 - 9 animals)


Vomiting latency (min)
120
100
80
60
40
20
0
Single animal
Control (C)
Vagotomy (VgX)
Metoclopramide (MCP)
BRL 24924
Domperidone (Domp)
Vagotomy+ Metoclopramide (VgX+MCP)
Number of vomits
30
20
10
0
C
VgX
MCP
BRL 24924
Domp
VgX + MCP
Number of retches
150
100
50
0
58
Nerve Lesion and Drug Intervention

The effect of nerve lesions and the influence of drug treatments on cycloheximide emesis are summarized in Fig. 22.

3.2.3.2 Diacetoxyscirpinol (DAS)

Following a dose of 1.5mgkg^{-1} i.p. of DAS, vomiting began in 22.0 ± 8.5min (n = 5) and continued in bouts throughout 100min of the observation period of 120min. An example of the pattern of retching and vomiting from one animal is illustrated in Fig. 23 and is summarized for the group in Fig. 24. In the 120min following the injection of DAS the mean number of retches was 72 ± 29 and vomits was 6 ± 2.

Effect of Vagotomy

Following abdominal vagotomy only animal one vomited, three retched and all displayed prodromata. Erythema of the skin and mild ataxia were observed. Retching and vomiting were significantly reduced. Thus:-

	Vomiting Response	Mean Number of Vomits	Mean Number of Retches
Control Group	5/5	6 ± 2	72 ± 29
Vgx Group	1/4	1 ± 2	5 ± 6**

Effect of Metoclopramide

Pre-treatment with metoclopramide did not prevent vomiting in any of the 5 animals tested. There was a tendency for the vomiting latency to be increased by metoclopramide but this was balanced by a contrary tendency for the mean numbers of vomits and retches to increase, with retching still continuing to occur at 2½ - 3h post treatment.

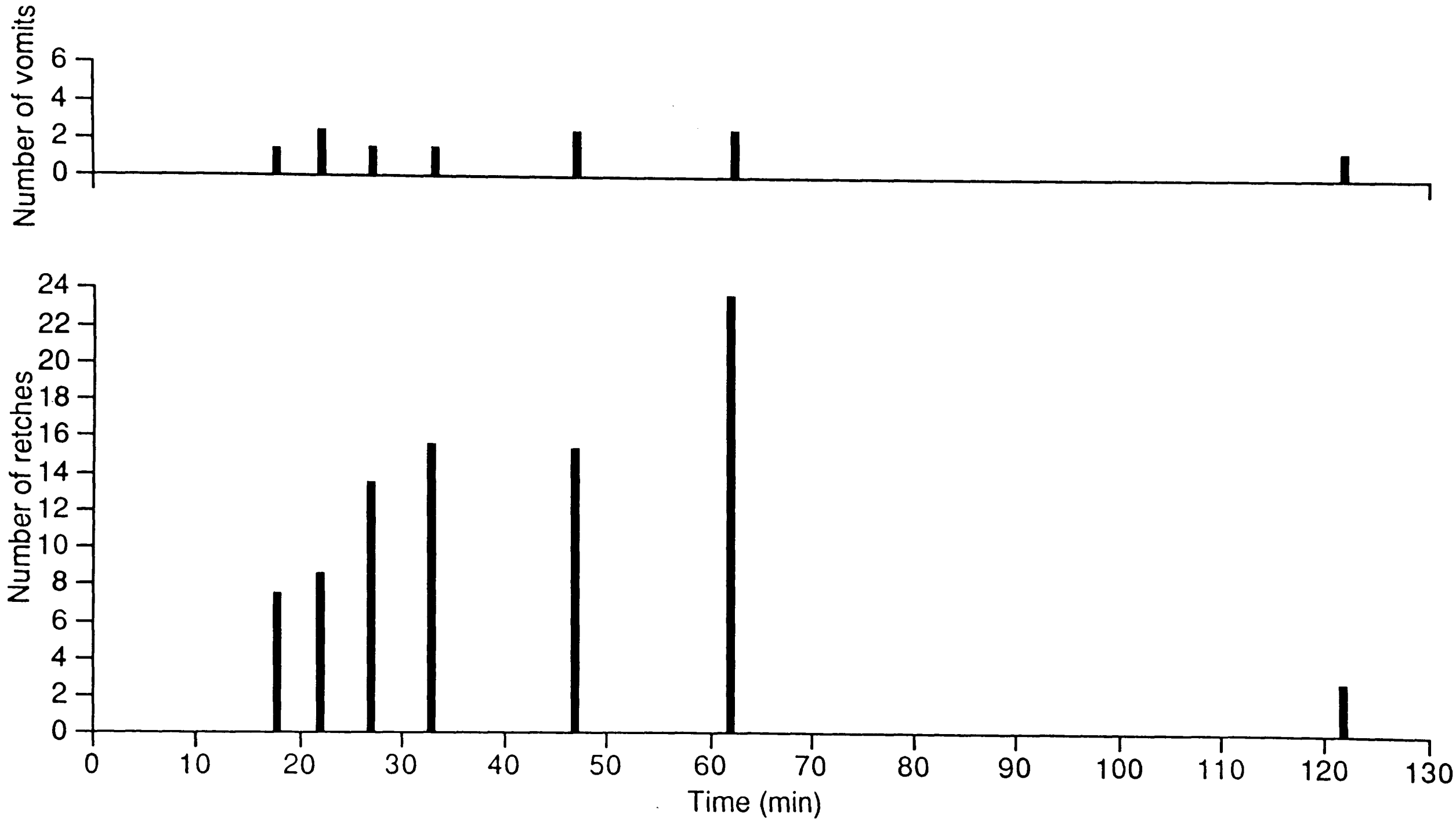


Figure 23 The Emetic Response of a Single Ferret to Intraperitoneally Administered Diacetoxyscirpinol ($1.5 mg kg^{-1}$)

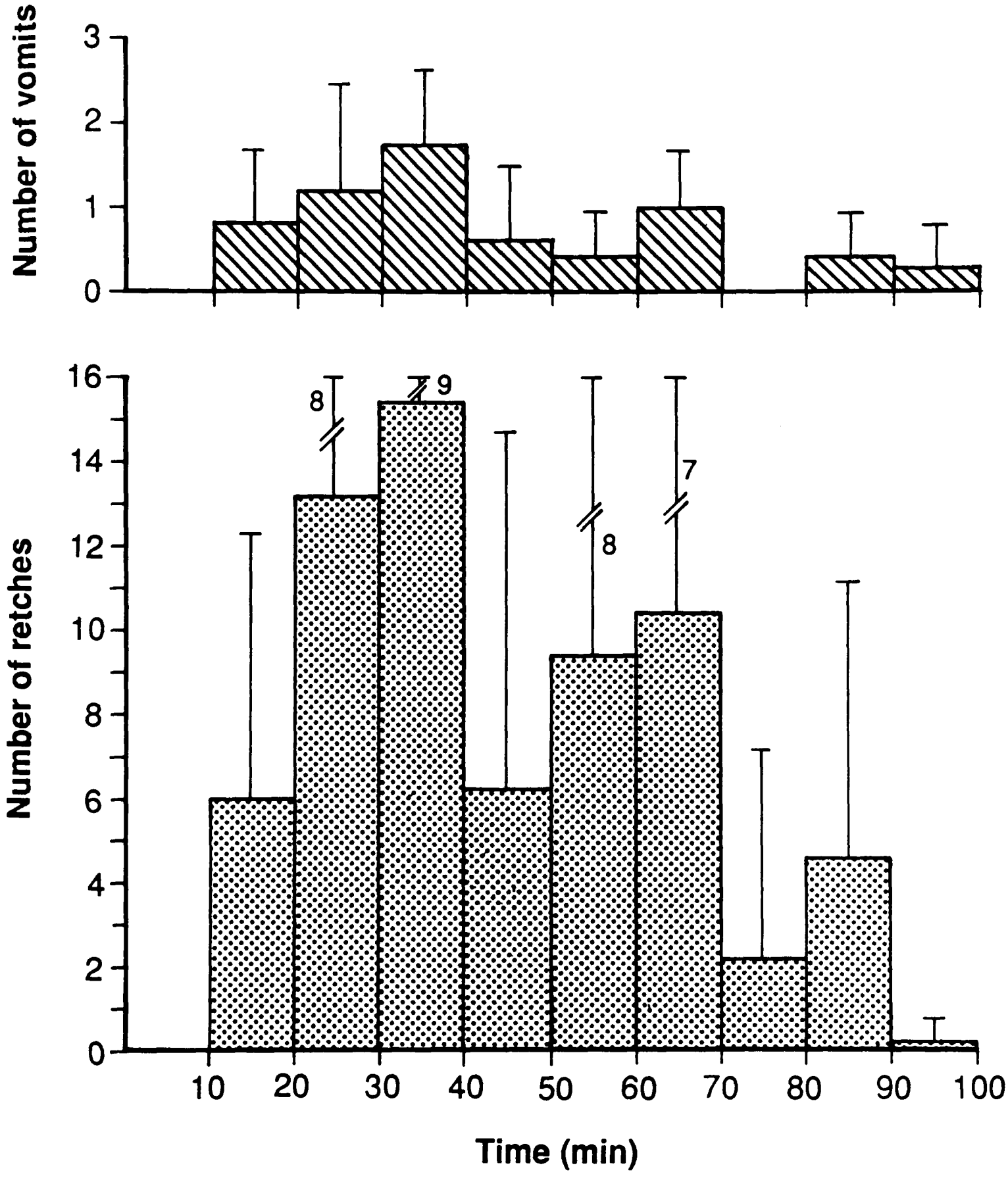


Figure 24 The Emetic Response of a Group of Ferrets to Intraperitoneally Administered Diacetoxyscirpinol ($1.5 mg kg^{-1}$)

The pattern of retching and vomiting is resolved into 10min time periods after injection of diacetoxyscirpinol at time zero (n = 5 animals)

	Vomiting Response	Mean Number of Vomits	Mean Number of Retches
Control Group	5/5	6 ± 2	72 ± 29
MCP Group	5/5	15 ± 8	99 ± 53

Effect of BRL 24924

Animals pre-treated with BRL 24924 all continued to vomit after DAS but the latency was significantly increased. However emetic potential remained much the same. Emetic prodromata were displayed by all individuals and it was noted that the skin of the animals was hot and erythematous after the experiment. The effect of the drug is summarized thus:-

	Vomiting Response	Mean Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	5/5	22.0 ± 8.5	6 ± 2	72 ± 29
BRL24924 Group	5/5	82.4 ± 15.7***	5 ± 2	41 ± 17

Effect of Domperidone

Domperidone pre-treatment did not prevent vomiting to DAS. There was an apparent and paradoxical shortening of the latency which did not reach statistical significance associated with a

tendency for the number of retches and vomits to increase. Thus:-

	Vomiting Response	Mean Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	5/5	22.0 ± 8.5	6 ± 2	72 ± 29
Domperidone Group	5/5	13.9 ± 2.9	18 ± 5**	16 ± 49**

The influence of the various interventions on DAS-induced emesis are summarized in Fig. 25.

3.2.3.3 Emetine

Animals challenged with 20mgkg^{-1} i.p. of emetine suffered considerable prodromata and vomiting and in addition all animals (n = 5) suffered profound diarrhoea, throughout the observation period of 90min.

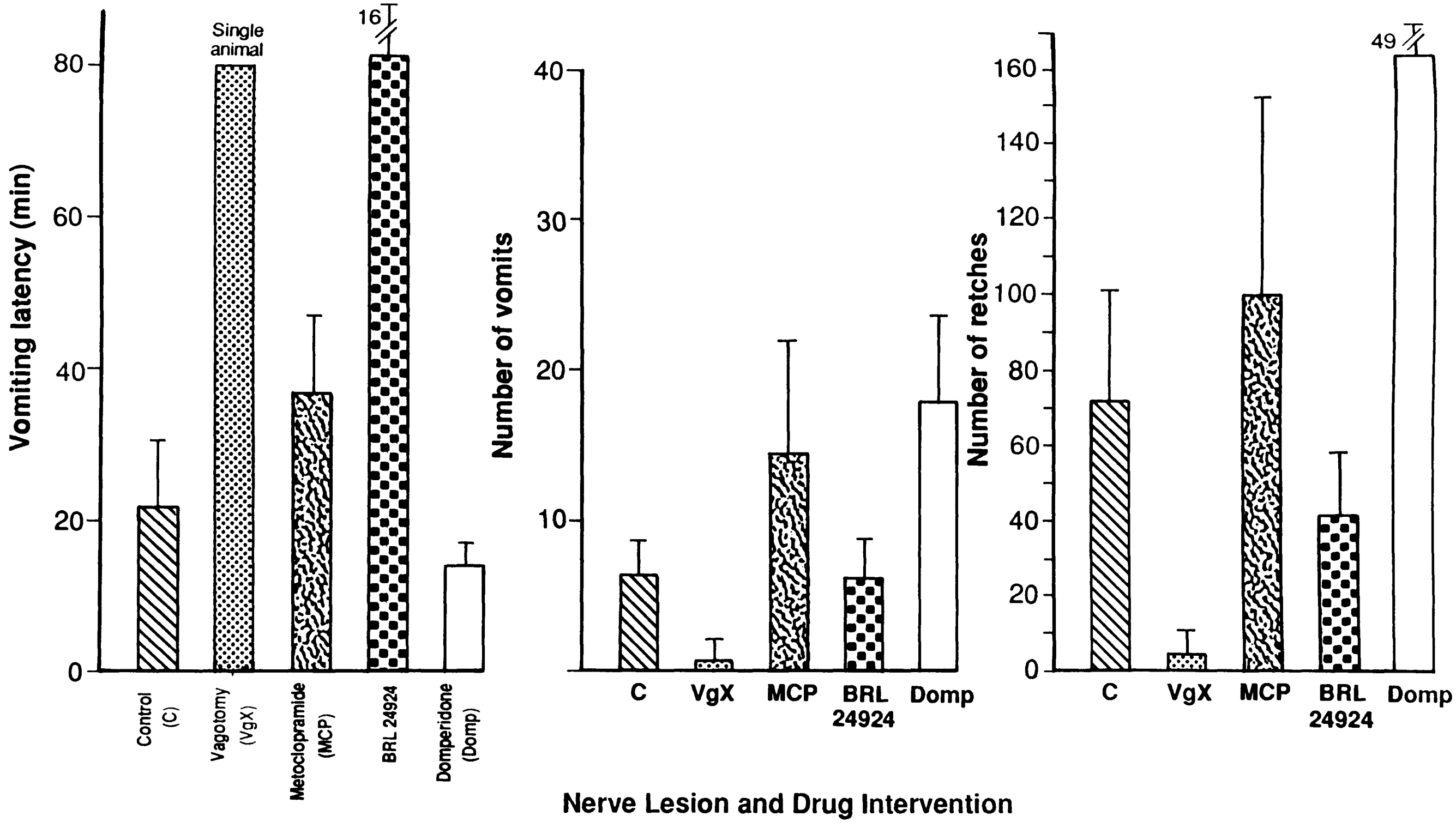
Following a dose of 20mgkg^{-1} i.p. vomiting began after 35.9 ± 7.2min and continued for up to 2 hours after injection although detailed observation ceased after 90min. An example of the pattern of retching and vomiting from a typical animal is illustrated in Fig. 26 and is summarised for the group in Fig. 27. In the 90min observation period the mean number of retches was 84 ± 62 and vomits, 16 ± 8.

Effect of Vagotomy or Combined Vagotomy and Greater Splanchnic Nerve Section

Abdominal vagotomy prevented vomiting in 3/4 animals tested although $^{3}/4$ retched and continued to do so for over 2 hours.

Figure 25 The Effect of Nerve Lesions (Abdominal Vagotomy) and Drug Treatments (Metoclopramide, BRL 24924, and Domperidone) on Diacetoxyscirpinol-Induced Emesis in the Ferret

Total minutes of retches and vomits refer to the 120min observation period. Doses of drugs were; MCP-5mgkg^{-1} sc, BRL24924-5mgkg^{-1} sc, Domp.-500μgkg^{-1} im. (n = 4 - 5 animals)


Vomiting latency (min)
80
60
40
20
0
Single animal
16
Control (C)
Vagotomy (VgX)
Metoclopramide (MCP)
BRL 24924
Domperidone (Domp)
Number of vomits
40
30
20
10
C
VgX
MCP
BRL 24924
Domp
Number of retches
160
140
120
100
80
60
40
20
0
49
C
VgX
MCP
BRL 24924
Domp
Nerve Lesion and Drug Intervention

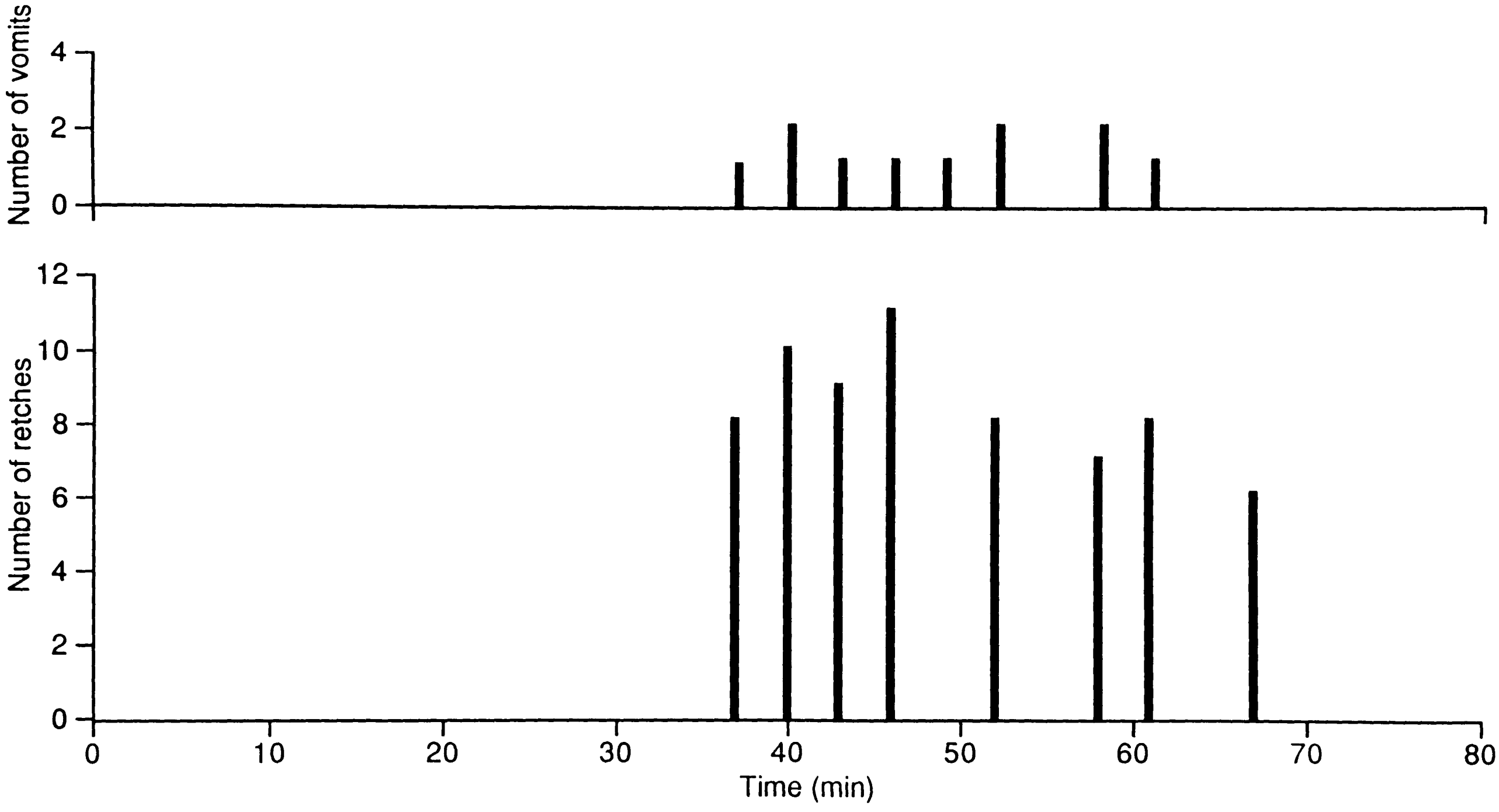


Figure 26 The Emetic Response of a Single Ferret to Intraperitonealy Administered Emetine ($20mgkg^{-1}$)

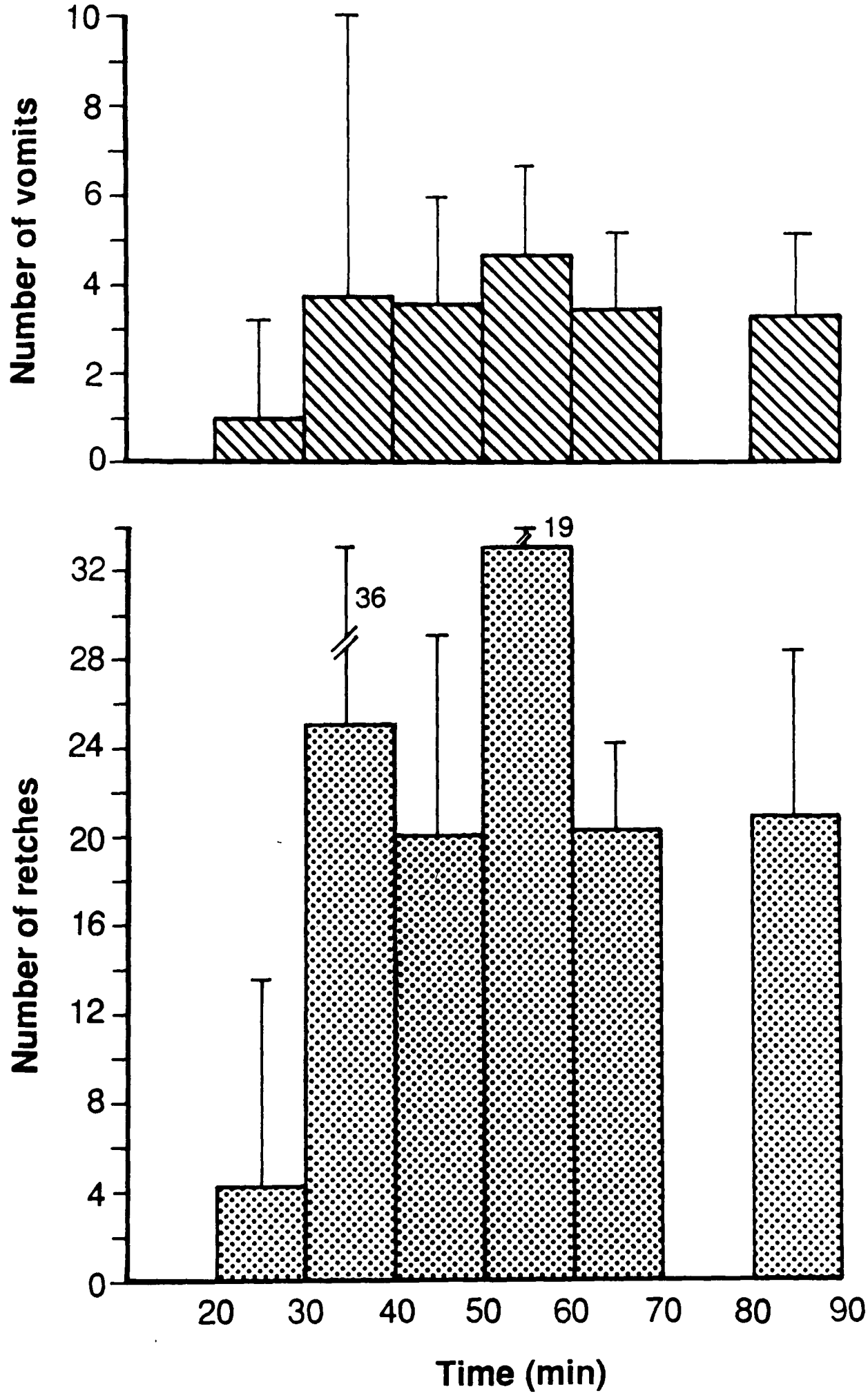


Figure 27 The Emetic Response of a Group of Ferrets to Intraperitoneally Administered Emetine ($20mgkg^{-1}$)

The pattern of retching and vomiting is resolved into 10min time periods after injection of emetine at time zero (n = 5 animals)

Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	5/5	35.8 ± 7.2	16 ± 8	84 ± 62
Vagotomy Group	1/4	[75]	1 ± 1***	42 ± 49

Combined vagotomy and greater splanchnic nerve section prevented vomiting in $^{3}/_{4}$ animals tested and only one animal retched. Diarrhoea and displays of prodromata were however noted in all animals. Sporadic retching and vomiting were noted after the 90min observation period expired.

Effect of Metoclopramide

Pre-treatment with metoclopramide did not prevent vomiting in the four animals tested and emetic response was not affected significantly either. Half of the animals displayed diarrhoea. The effect of metoclopramide (MCP) on vomiting is summarized thus:-

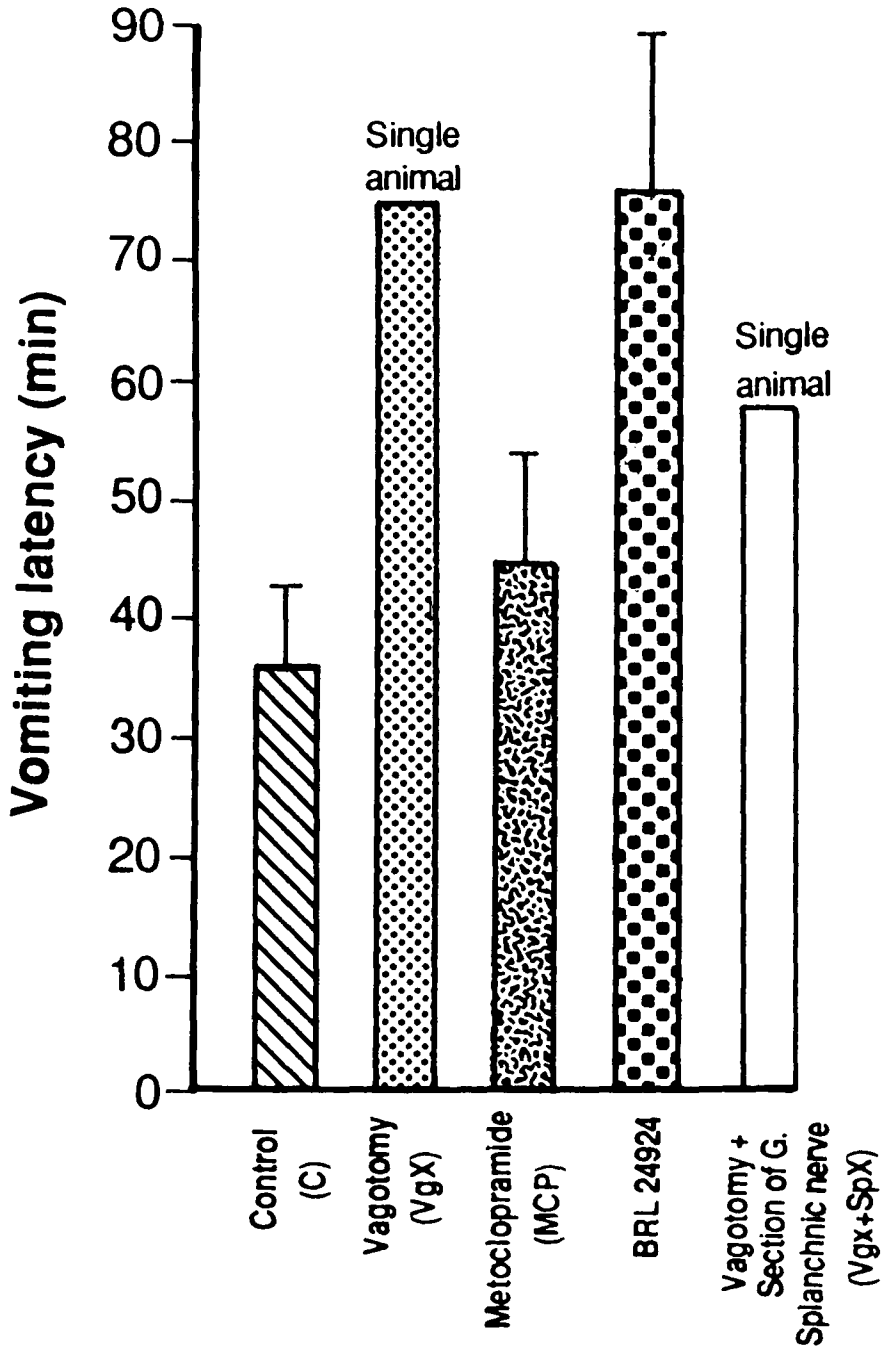
	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	4/4	35. 0 ± 7.2	16 ± 8	84 ± 62
MCP Group	5/5	44. 6 ± 10.2	11 ± 7	75 ± 60

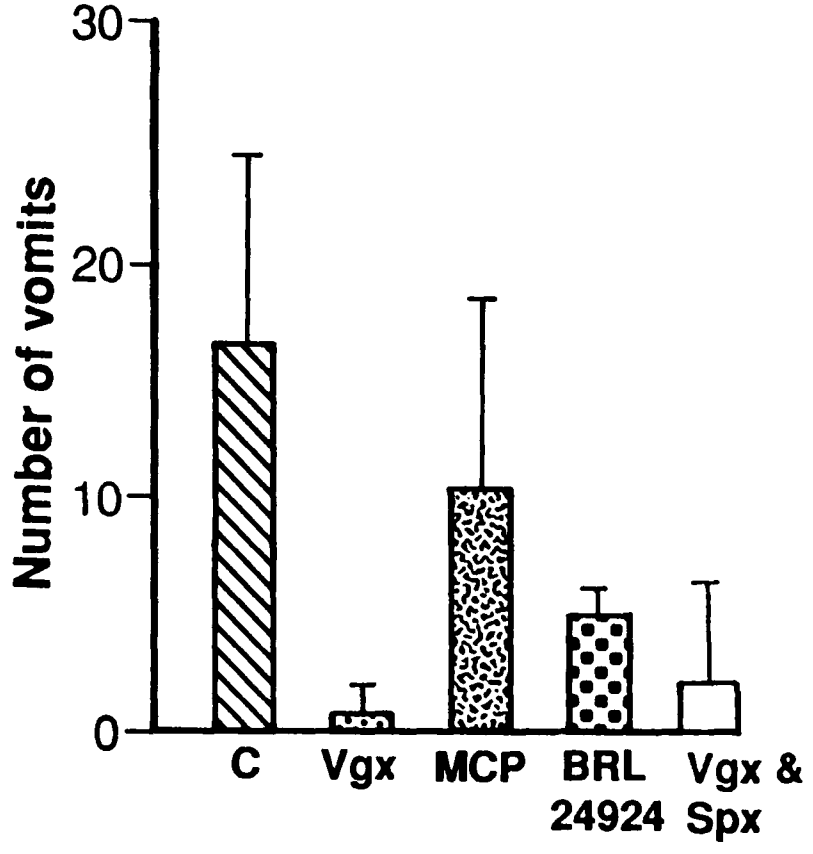
Effect of BRL 24924

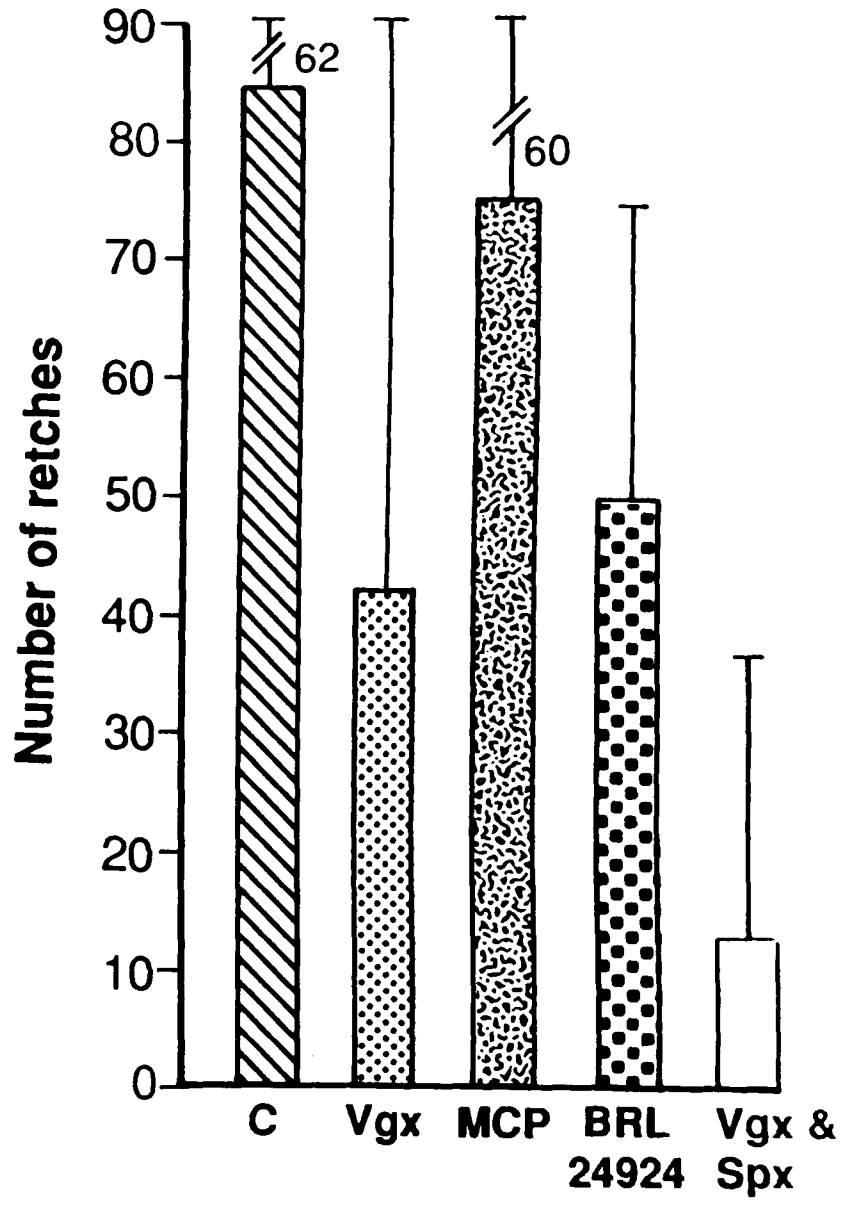
Pre-treatment with BRL 24924 did not prevent vomiting in the five animals challenged with emetine but vomiting latency was significantly lengthened.

Figure 28 The Effect of Nerve Lesions (Abdominal Vagotomy and Combined Vagotomy with Section of the Greater Splanchnic Nerves) and Drug Treatments (Metoclopramide and BRL 24924) on Emetine-Induced Emesis in the Ferret

Total numbers of retches and vomits refer to the 90min observation period. Doses of drugs were; MCP-5mgkg^{-1} s.c., BRL24924-5mgkg^{-1} s.c.) (n = 4 - 5 animals)







Nerve Lesion and Drug Intervention

Prodromata and diarrhoea were still present in this group but there was a tendency for the emetic potential to be reduced. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	5/5	35.0 ± 7.2	16 ± 8	84 ± 62
BRL24924	5/5	75.7 ± 16.3***	5 ± 1**	49 ± 25

Effect of Domperidone

The effect of domperidone pre-treatment on emetine induced vomiting was demonstrated in only one animal. It reacted with a latency of 49.8min, vomiting 8 times and retching 111 times during the observation period. It continued to produce emetic episodes for 2 hours and was in every way indistinguishable from the animals with emetine alone.

The effect of the various nerve lesion and drug interventions on the emetic profile of emetine is summarized in Fig. 28.

3.2.3.4 Cisplatin

Conflicting information on the response of the ferret to cisplatin (Schurig, personal communication) led to the necessity of constructing a dose response relationship the results of which are illustrated in Fig. 29. The minimum effective dose causing vomiting in all animals tested was $10mgkg^{-1}$ i.v. $(n = 4)$ with a mean latency of 74.9 ± 28.9min (mean numbers of retches 37 ± 24 and mean number of vomits 5 ± 4). The earliest manifestation of treatment with cisplatin was profound diarrhoea which always preceded the onset of vomiting. Peculiar to the ferret's

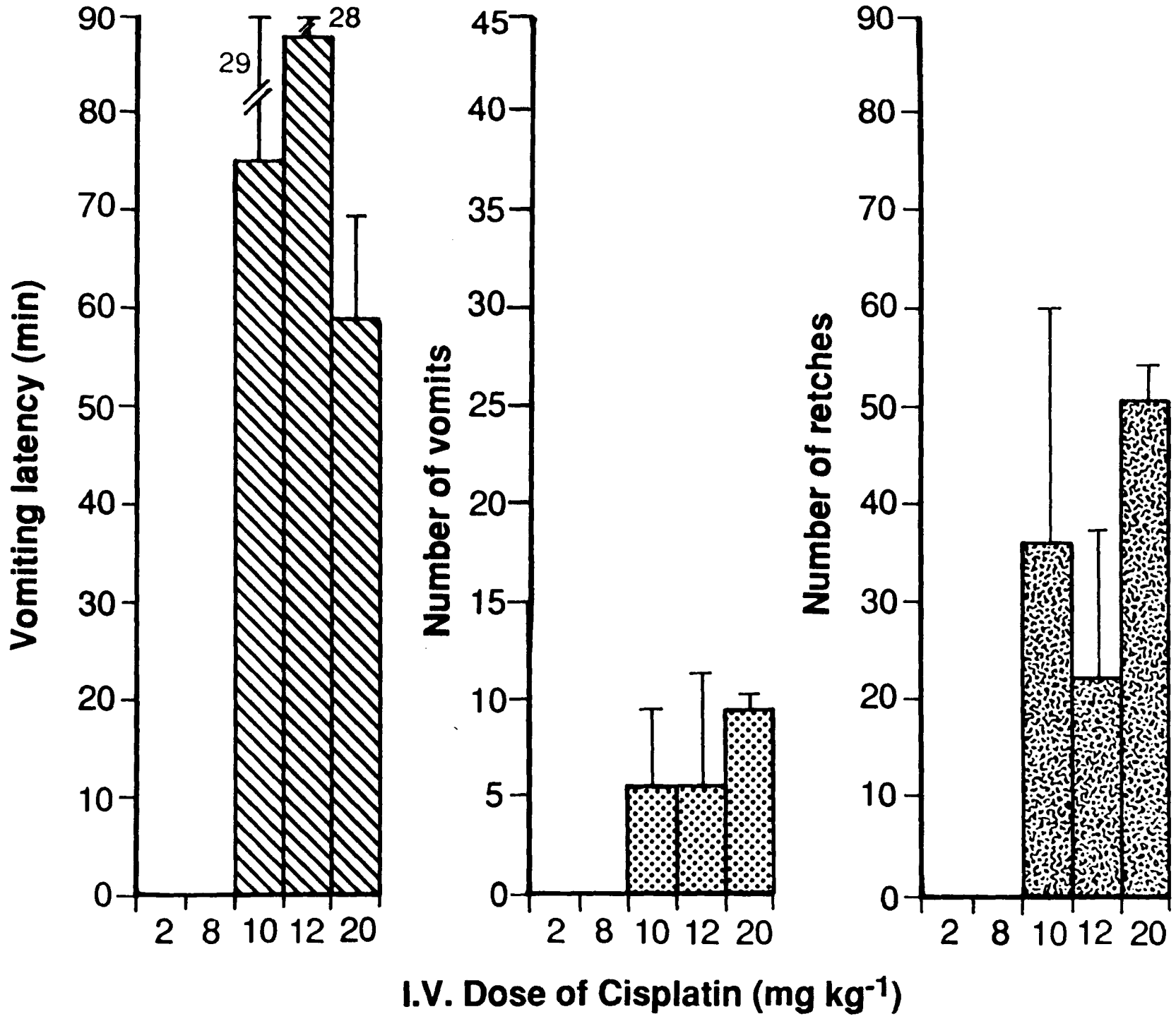


Figure 29 The Emetic Dose-Response Relationship for Intravenously Administered Cisplatin in the Ferret (n = 4 animals)

response to cisplatin administration at doses which cause emesis is the onset after some 20 - 30 minutes of "flop-down" behaviour in which the animal appears to collapse in a "cataplectic-like" state for 15 - 90 seconds with the chin resting on the floor and the hind legs splayed.

An example of the pattern of retching and vomiting in one animal is illustrated in Fig. 30 and is summarised for the group in Fig. 31.

At lower doses which do not produce retching or vomiting (2 and 8mgkg^{-1}) mild prodromata were evident. In the case of all doses at which retching and vomiting did occur, emesis persisted, in excess of the observation period of 120 minutes, for up to 5 hours in some cases.

In contrast to the reliable response that 20mgkg^{-1} elicits from the ferret when administered intravenously, the same dose when given intraperitoneally to 6 ferrets produced no emesis and only minor prodromata, although the animals remained responsive to apomorphine 100μgkg^{-1} s.c. three hours after challenge with cisplatin.

Effect of Vagotomy

In the presence of an abdominal vagotomy performed 7 - 10 days prior to challenge, emesis was abolished in all three animals tested with 10mgkg^{-1} of cisplatin i.v.. No prodromata were noted in these animals during the observation period of 120 minutes.

Effect of Metoclopramide

Metoclopramide (5mgkg^{-1} sc) pretreatment prevented vomiting in 3 out of 4 animals challenged with 10mgkg^{-1} of cisplatin i.v.

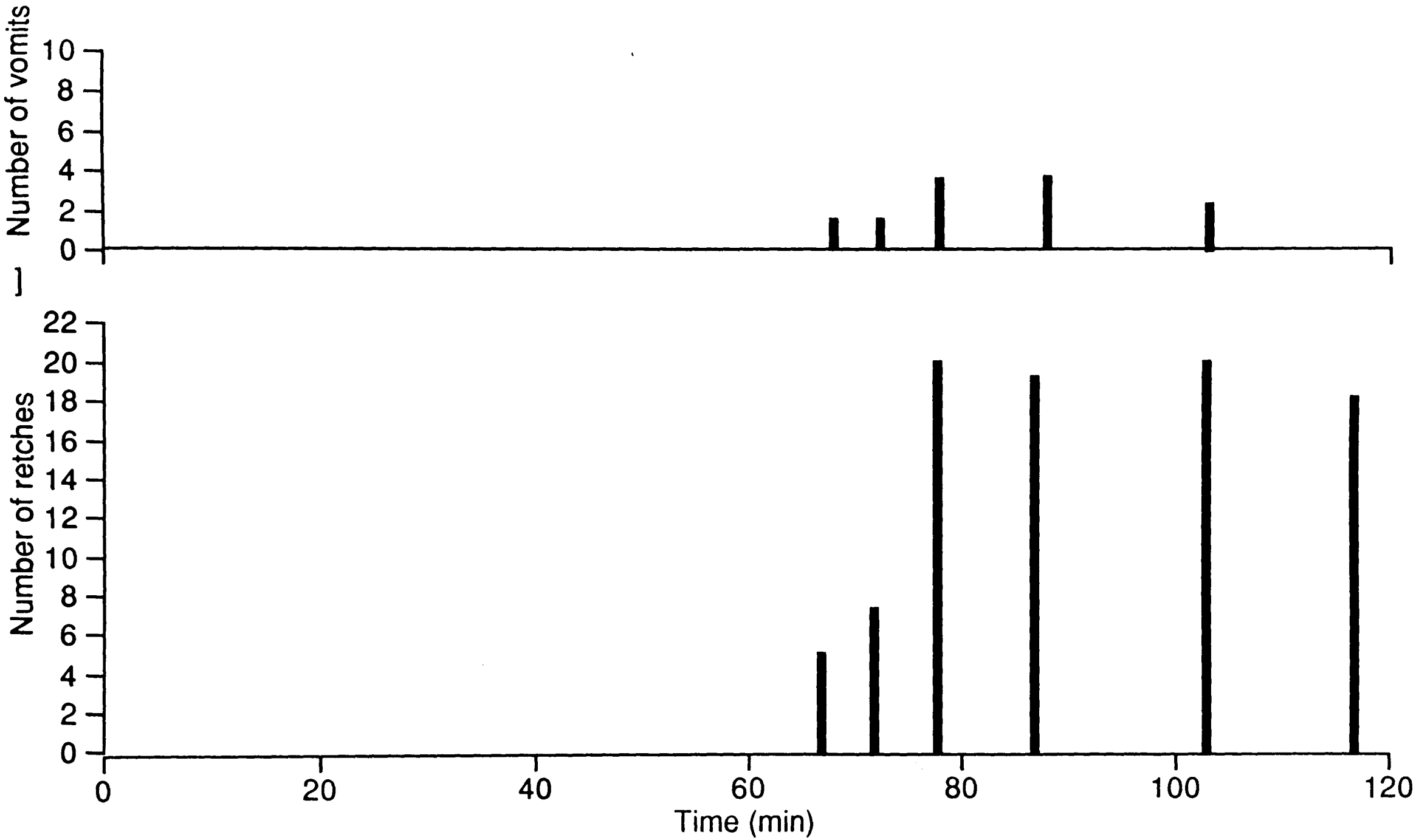


Figure 30 The Emetic Response of a Single Ferret to Intravenously Administered Cisplatin ($10 mg kg^{-1}$)

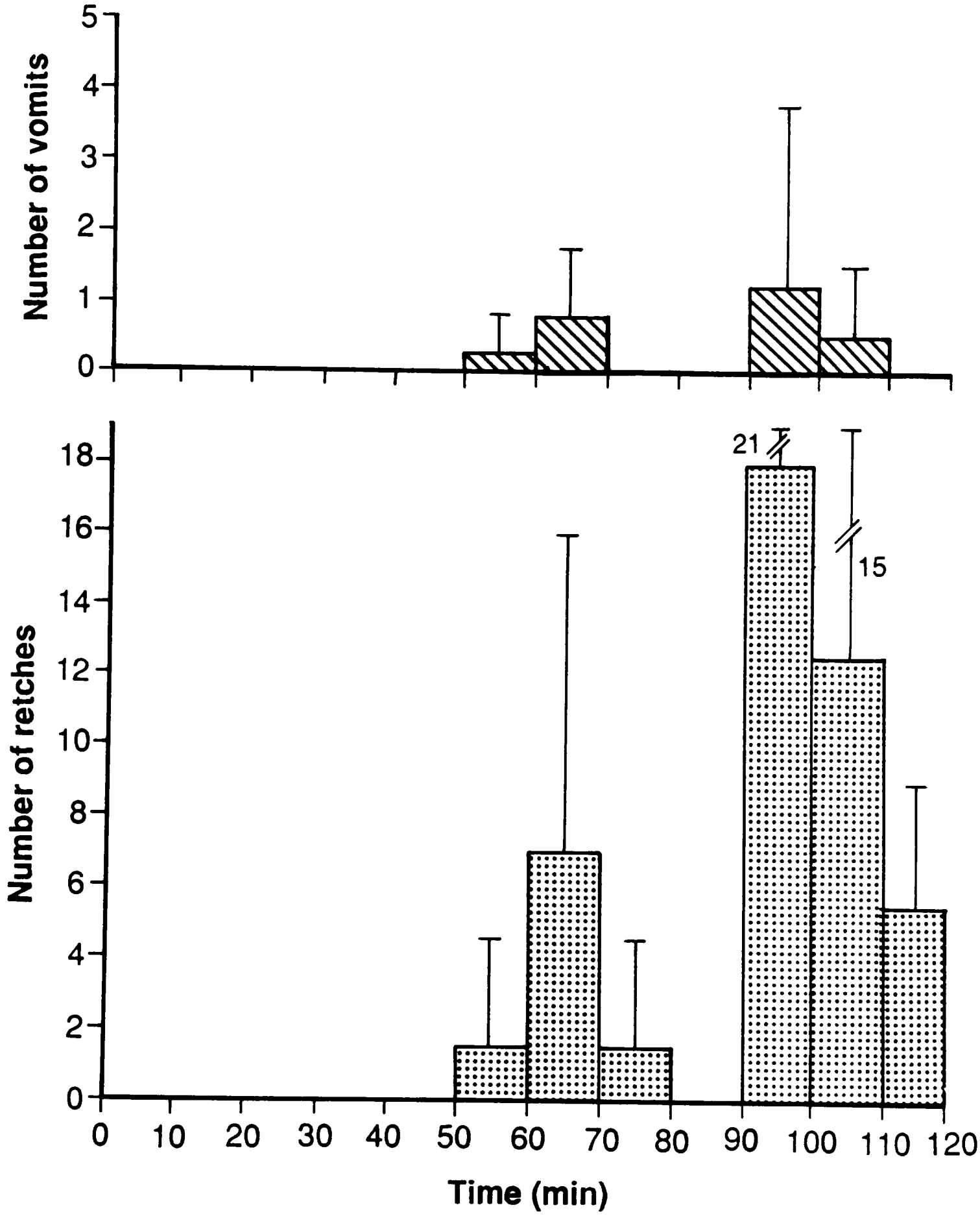


Figure 31 The Emetic Response of a Group of Ferrets to Intravenously Administered Cisplatin ($10mgkg^{-1}$)

The pattern of retching and vomiting is resolved into 10min time periods after injection of cisplatin at time zero (n =4 animals)

The single animal that vomited did so with a latency (91min) not significantly different from the control group but emetic potential showed a marked tendency to reduction compared to controls. All animals displayed prodromata.

Effect of BRL 24924

BRL 24924 (5mgkg^{-1} s.c.) prevented vomiting in three out of four animals challenged with 10mgkg^{-1} cisplatin. In the animal that did vomit the vomiting latency did not differ from the control group but the emetic potential showed some tendency to reduction. All four animals displayed prodromata.

Effect of Abdominal Vagotomy combined with Metoclopramide pre-treatment

Four animals previously given an abdominal vagotomy were pre-treated with metoclopramide then challenged with 10mgkg^{-1} of cisplatin i.v.. Although all four animals displayed definite signs of prodromata there was no emesis during the initial observation period of 120mins. Further observation for 6 hours revealed only one minor bout of retching in one animal.

The various parameters studied and the influence of the treatments and interventions on cisplatin induced vomiting are summarized in Fig. 32.

3.2.3.5 Mustine

An initial challenge of three ferrets with an equivalent total dose of mustine regularly used in cytotoxic chemotherapy in man (400μgkg^{-1} i.v.) failed to produce any emetic episodes although prodromata were displayed by all three. However following a dose of 1200μgkg^{-1} i.v. vomiting began after 28.1 ± 7.2 minutes (n = 4). Severe diarrhoea was present in all

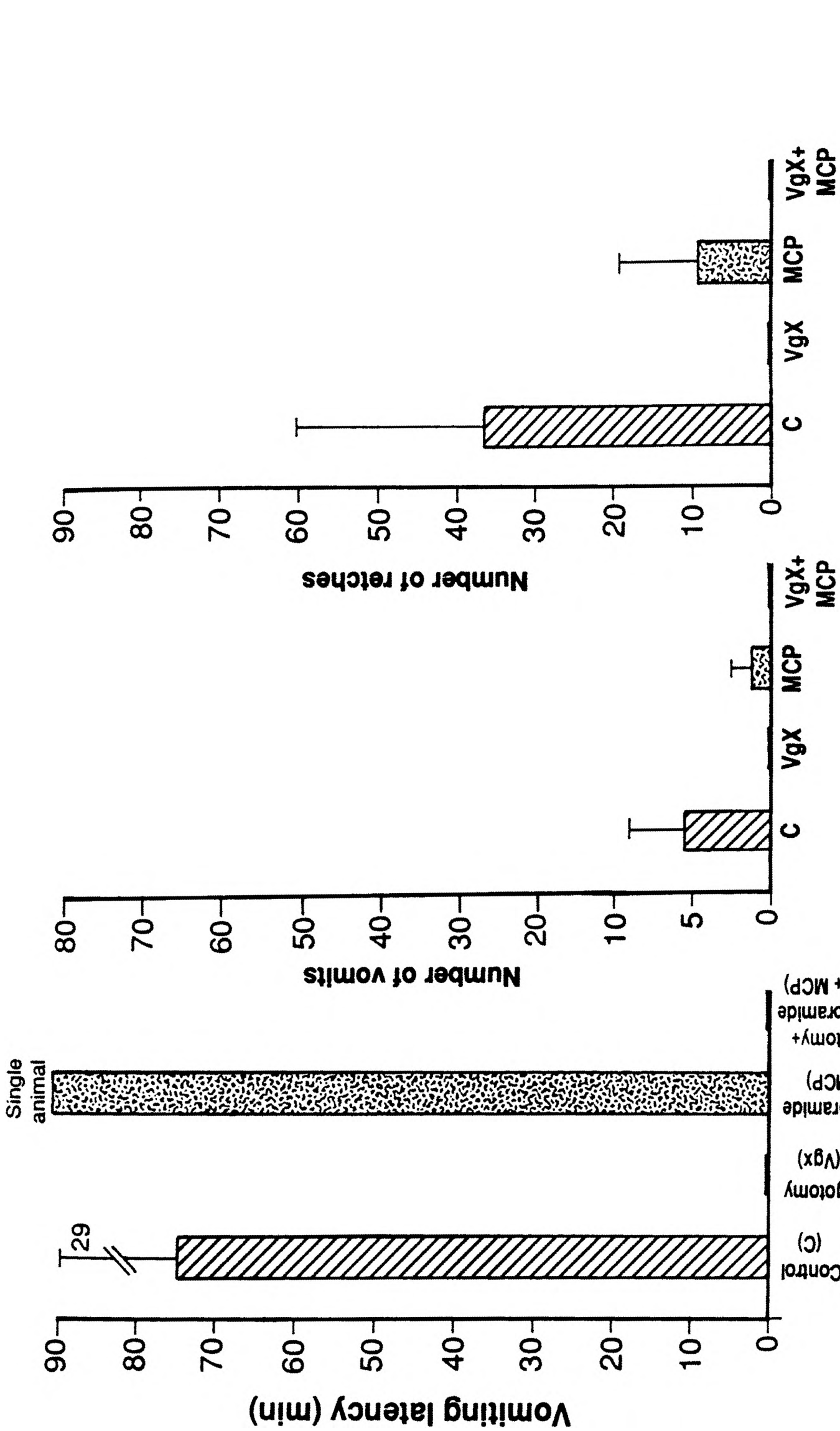


Figure 32 The Effects of Nerve Lesions (Abdominal Vagotomy) and Drug Treatments (Metoclopramide) on Cisplatin-Induced Emesis in the Ferret

Total numbers of retches and vomits refer to the 120min observation period. Dose of drug MCP was 5mgkg^{-1} sc. (n = 3 - 4 animals)

animals and marked prodromata preceded emesis and was interspersed between bouts of vomiting. In the 120 minutes of the observation period the mean number of retches was 47 ± 23 and vomits 8 ± 2. An example of the pattern of retching and vomiting from one typical animal is illustrated in Fig. 33 and is summarized for the group in Fig. 34.

Some animals were followed for three hours after challenge with this dose of mustine and all died within that period.

Effect of Vagotomy

In the presence of an abdominal vagotomy (vgx) only 1/4 animals vomited (displaying 5 vomits and 57 retches in total with a vomiting latency of 110.9 minutes and a duration of 5 minutes) There was little evidence of prodromata and the ferrets displayed a normal activity pattern. Thus:

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	4/4	28.1 ± 7.2	8 ± 2	47 ± 23
VgX Group	1/4	[110.9]	1 ± 2	14 ± 28

Effect of Metoclopramide

Only 1/5 animals pre-treated with metoclopramide vomited (with 45 retches and 7 vomits, a vomiting latency of 107.0mins and a duration of 13 minutes). The whole pattern of response was very similar to that obtained after testing animals with a previous abdominal vagotomy. Those animals that did not vomit,

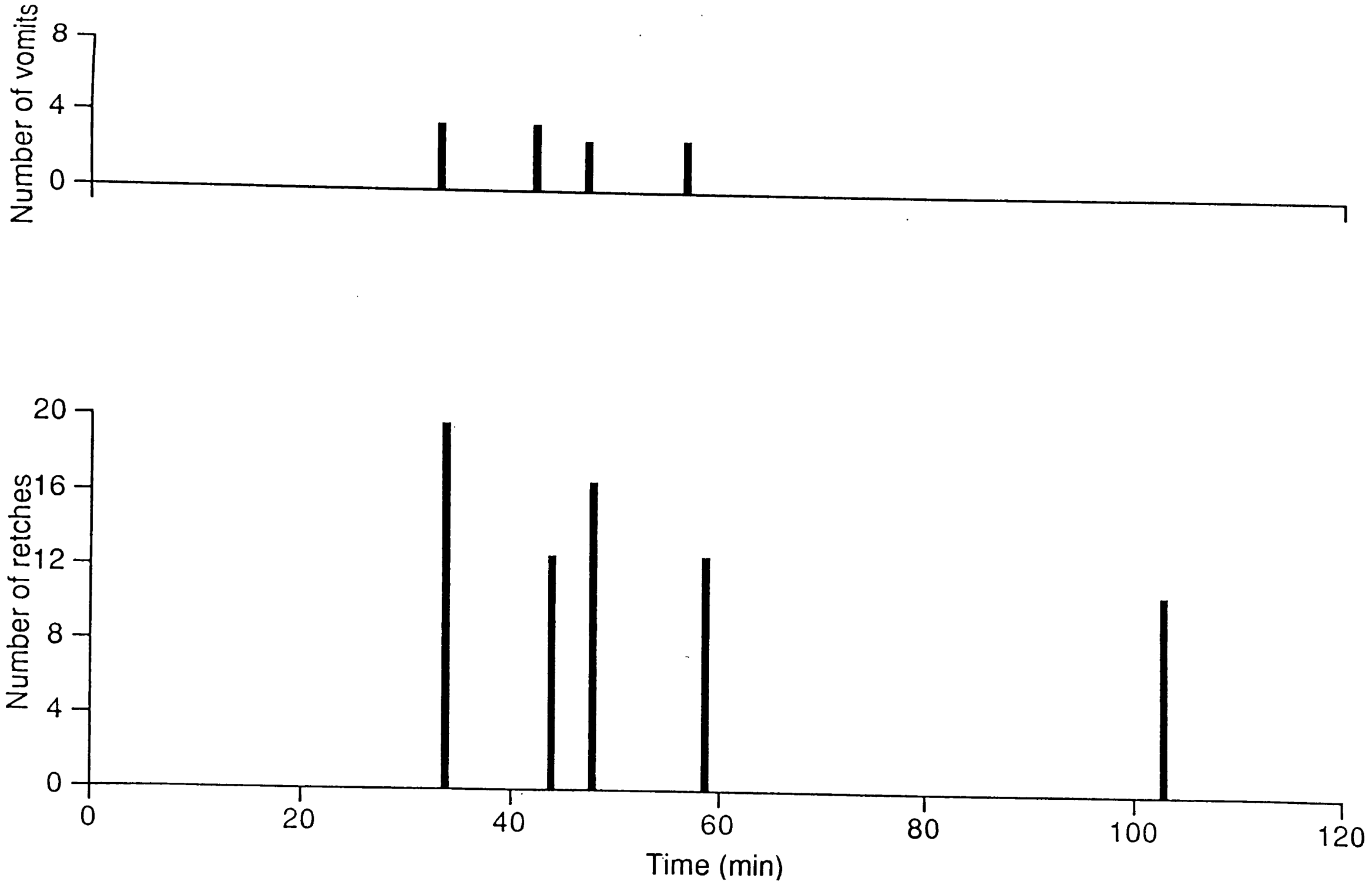


Figure 33 The Emetic Response of a Single Ferret to Intravenously Administered Mustine ($1200\mu gkg^{-1}$)

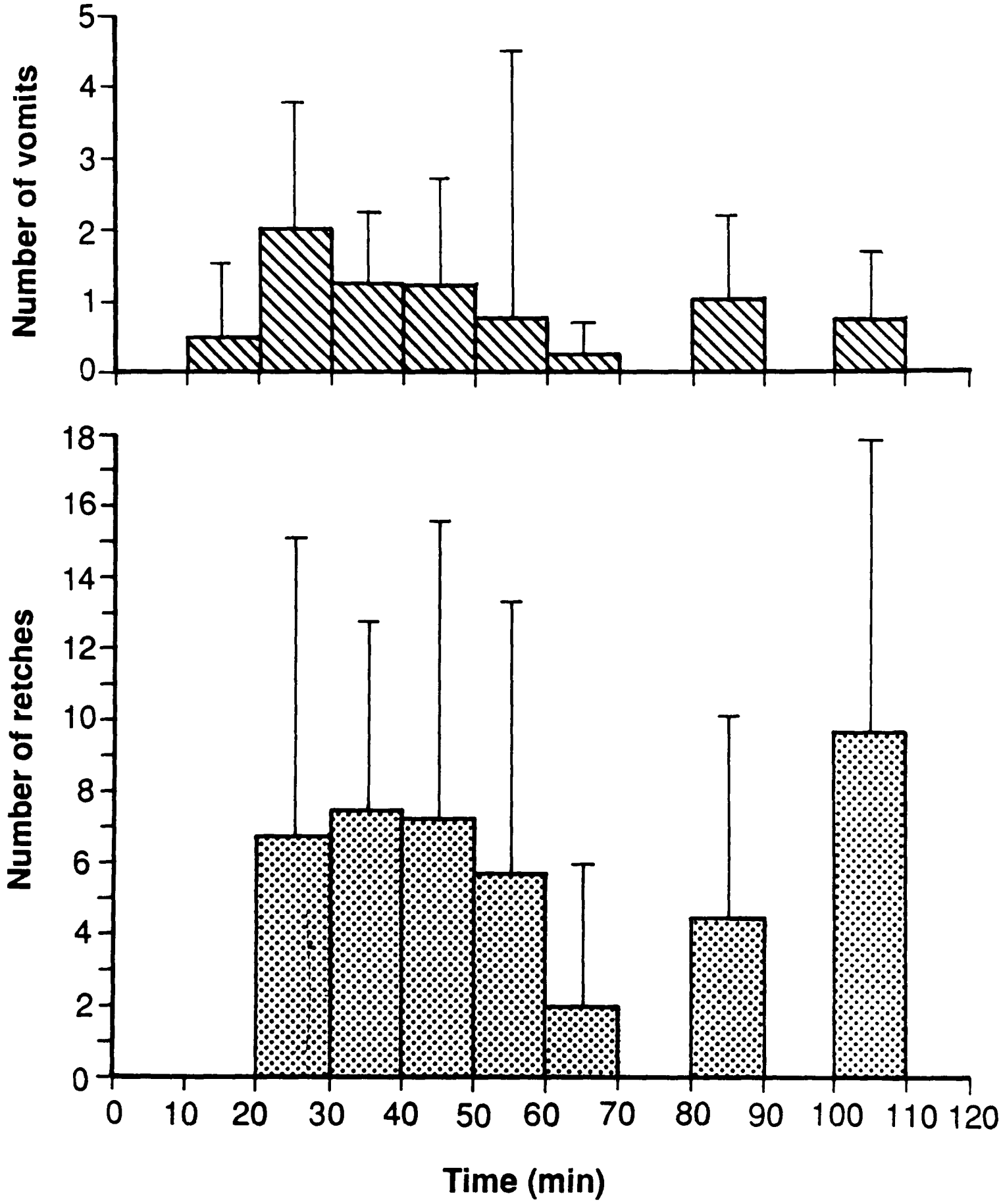


Figure 34 The Emetic Response of a Group of Ferrets to Intravenously Administered Mustine

The pattern of retching and vomiting is resolved into 10min time periods after injection of mustine at time zero (n = 4 animals)

curled up and attempted to sleep, a reaction commonly seen in association with outright displays of emetic prodromal behaviour. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Groups	4/4	28.1 ± 7.2	8 ± 2	47 ± 23
MCP	1/5	[107]	2 ± 3	9 ± 20

The results of these interventions are summarised in Fig.35.

3.2.4 Peptide YY

On the basis that this peptide has been implicated as the "emetic peptide" (Harding et al., 1984) involved in some way in the control of radiation-induced vomiting in the dog it was decided to perform a limited investigation of its actions in the ferret. A range of doses ($1\mu gkg^{-1}$ - $10\mu gkg^{-1}$) were administered intravenously to four groups of ferrets (n = 4 - n = 6). At the lowest dose no animal vomited and only one retched (x3 at 1.7mins). However all four animals in the group displayed prodromata.

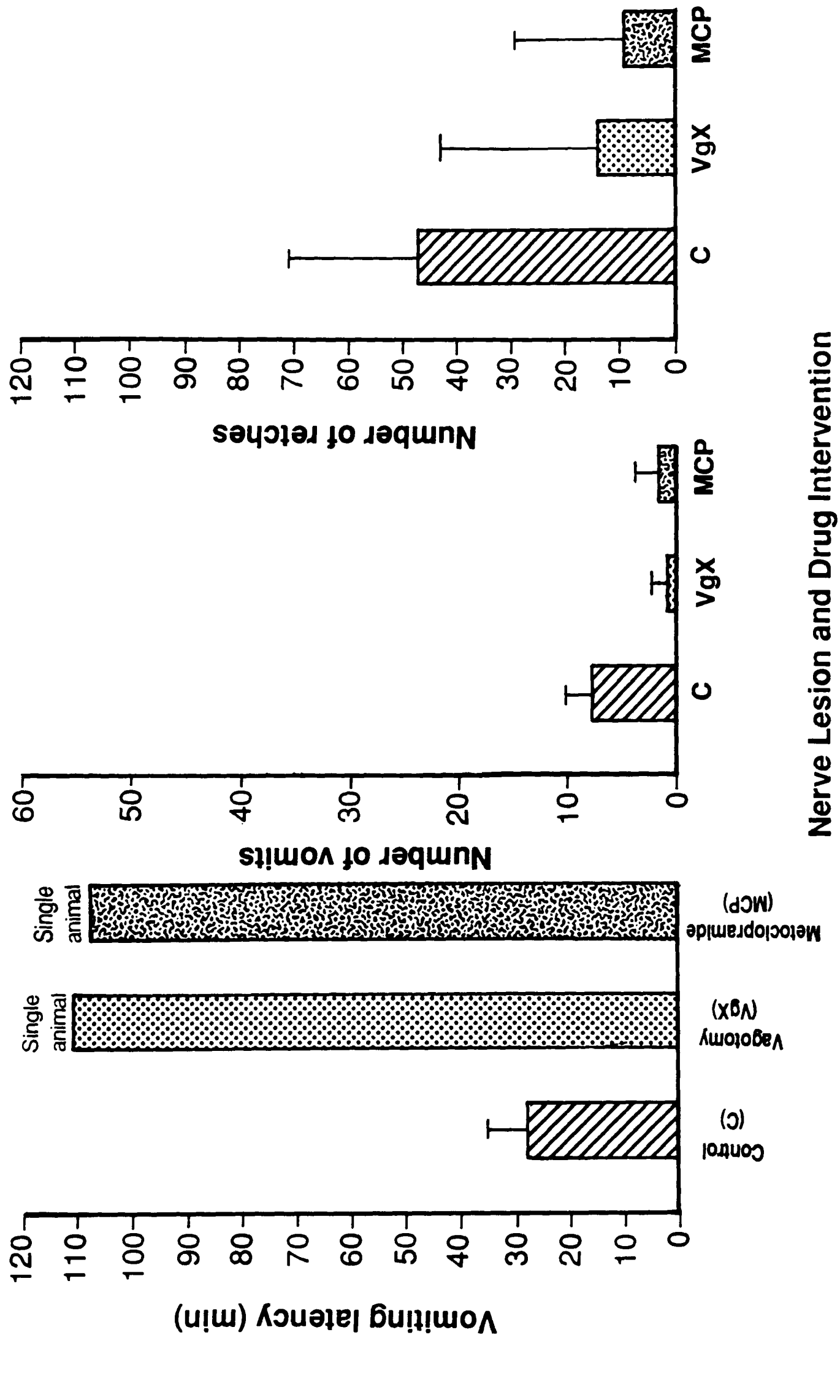
At a dose of $2\mu gkg^{-1}$ there were no emetic episodes during the 30 minute observation period but once again all animals displayed prodromata.

At $5\mu gkg^{-1}$ one animal vomited with a latency of 2.7 minutes (3 vomits and 14 retches) and a duration of 1.6 minutes. 3/5 animals retched and all animals showed prodromata.

Six animals were challenged with $10\mu gkg^{-1}$. There was still only one animal which vomited (latency, 2.2min) although 2/6

Figure 35 The Effect of Nerve Lesions (Abdominal Vagotomy) and Drug Administrations (Metoclopramide) on Mustine-Induced Emesis in the Ferret

Total numbers of retches and vomits refer to the 120min observation period. Dose of drug MCP was 5mgkg^{-1} s.c. (n = 4 - 5 animals)



retched. Prodromata were very mild although displayed by all animals in this group. The reaction of ferrets to a variety of doses of PYY is summarized below:-

Dose of Peptide YY (μgkg^{-1} i.v.)	Vomiting Response	Retching Response	Mean Number of Vomits	Mean Number of Retches
1	0/4	1/4	-	1 ± 2
2	0/4	0/4	-	-
5	1/5	3/5	1 ± 1	5 ± 6
10	1/6	2/6	1 ± 1	2 ± 3

3.3 X-RADIATION

Prior to the start of this work no published record existed describing the emetic reaction of the ferret to irradiation with hard X-rays. It was thus necessary to investigate the relationship between the dose of X-rays and subsequent behaviour of the ferret with special regard for its tendency to display emetic prodromata and frank retching and vomiting.

Throughout all experiments in this series a 90min basic observation period was used and all calculations are based upon this.

3.3.1 Dose-response Curve for X-ray Induced Vomiting in the Ferret

Eleven doses between 50cGy and 1600cGy were given to 60 ferrets divided into groups, where n = 3 to n = 8. The first dose to produce vomiting in all animals tested (ED_{100}) was 125cGy (mean latency 31.6 ± 8.0min, mean retches 34 ± 2, mean vomits 5 ± 3, n = 5). The dose-response curve relating X-rays to emesis in the ferret is illustrated in Figs. 36 and 37. The

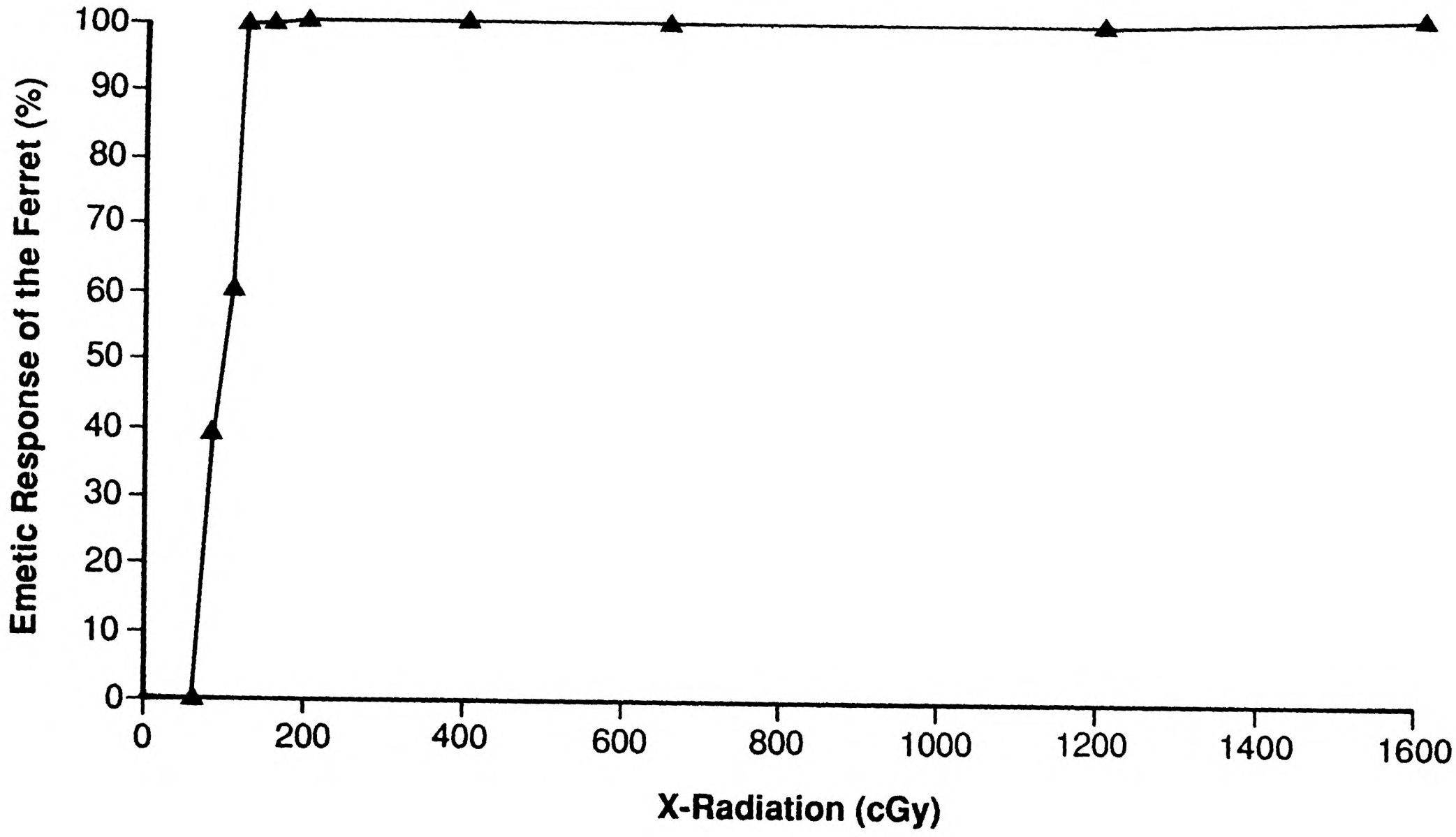
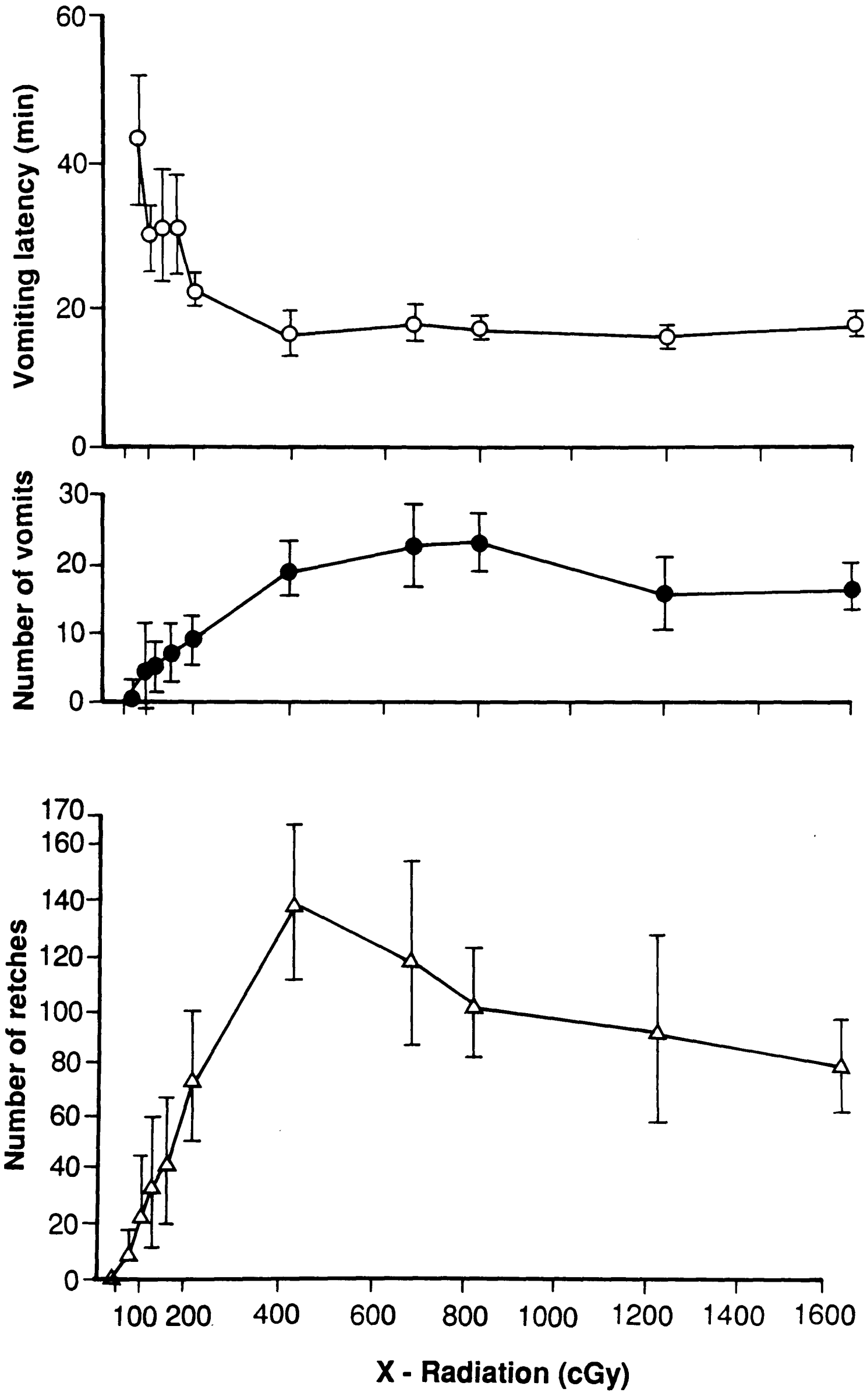


Figure 36 The Emetic Dose-Response Curve for X-radiation in the Ferret

The response to varying doses of whole-body X-radiation (50 - 1600cGy) is visualised as the percentage of treated animals which vomited during the observation period of 90min. (n = 3 - 9 animals)

Figure 37 The Emetic Dose-Response Relationship for X-radiation in the Ferret

The response to varying doses of whole-body X-radiation (50-1600cGy) illustrated in terms of latency from initiation of stimulus to first vomiting episode and mean total numbers of retches and vomits during the observation period of 90min (n = 3 - 9 animals)


Vomiting latency (min)
60
40
20
0
Number of vomits
30
20
10
0
Number of retches
170
160
140
120
100
80
60
40
20
0
100 200
400
600
800
1000
1200
1400
1600
X - Radiation (cGy)

response is quantified in terms of numbers of animals responding, total numbers of retches and vomits and vomiting latency.

At the lowest dose tested (50cGy) none of the 6 animals vomited during the 3 hour observation period but some displayed prodromata. Mild diarrhoea was also present in most animals. 75cGy was the first dose at which vomiting was noted to occur. Prodromata were seen in all animals in this group accompanied by diarrhoea.

Above 125cGy of X-rays all animals tested, vomited. It was apparent however, that throughout the dose range the emetic response and vomiting latency of the ferret varied with the dose of X-rays. Vomiting latency fell from 43.6 ± 9.5 minutes at 75cGy to 16.0 ± 2.6 minutes at 400cGy. Thereafter a plateau was reached and no significant change in latency was produced even though the dose was increased to 1600cGy (latency 16.8 ± 1.8min).

A complimentary pattern was seen when the incidence of retching and vomiting was examined, with retches peaking at 400cGy (140 ± 28) having risen from 7 ± 10 at 75cGy. Similarly the vomiting response rose from 8 ± 3 vomits at 75cGy to 22 ± 6 at 660cGy with a plateau at 800cGy and above.

Diarrhoea was universally present throughout the dose range, becoming steadily worse as the dose rose. At 800cGy profound diarrhoea was present, being mucoid or watery in character, becoming bloody as time went by and peaking in intensity at around 60 minutes after irradiation. These animals were followed for 5 hours, by which time some recovery had taken place.

At doses above 800cGy the animals became rather lethargic sedated, and ataxic but still displayed profound mucoid diarrhoea. These doses coincide with the plateau of the vomiting latency. Some decline in emetic response was seen particularly in numbers of retches recorded. After 2 hours the animals showed peripheral vasoconstriction, being cold to touch and were curled asleep. Some improvement was noted after 6 hours just prior to sacrifice.

At doses between 400 and 1600cGy the duration of emesis remained between 45 - 60min although at the doses of 400cGy which caused the greatest emetic response, sporadic retching and vomiting occurred up to 2 hours following irradiation.

Two doses were chosen from the range used, against which to test a variety of drugs and nerve lesions; firstly, 200cGy was used as a "low" but 100% effective dose with a duration of action mostly confined to the first 30min following irradiation. Secondly, 800cGy was used as the "high-dose" challenge, still 100% effective and without obvious evidence of sedation or ataxia, but with a duration of action that continues throughout the first and second 30min periods and into the third 30min period of observation. Moreover, it is also the dose which was previously used to challenge animals used for models of vomiting, e.g. dog and primate (e.g. Mattsson and Yochmowitz, 1980 and Chinn and Wang, 1954).

Examples of the pattern of retching of vomiting from typical animals irradiated with 200cGy and 800cGy are shown in Fig. 38. The group reactions at 200 and 800cGy are summarized in Fig. 39.

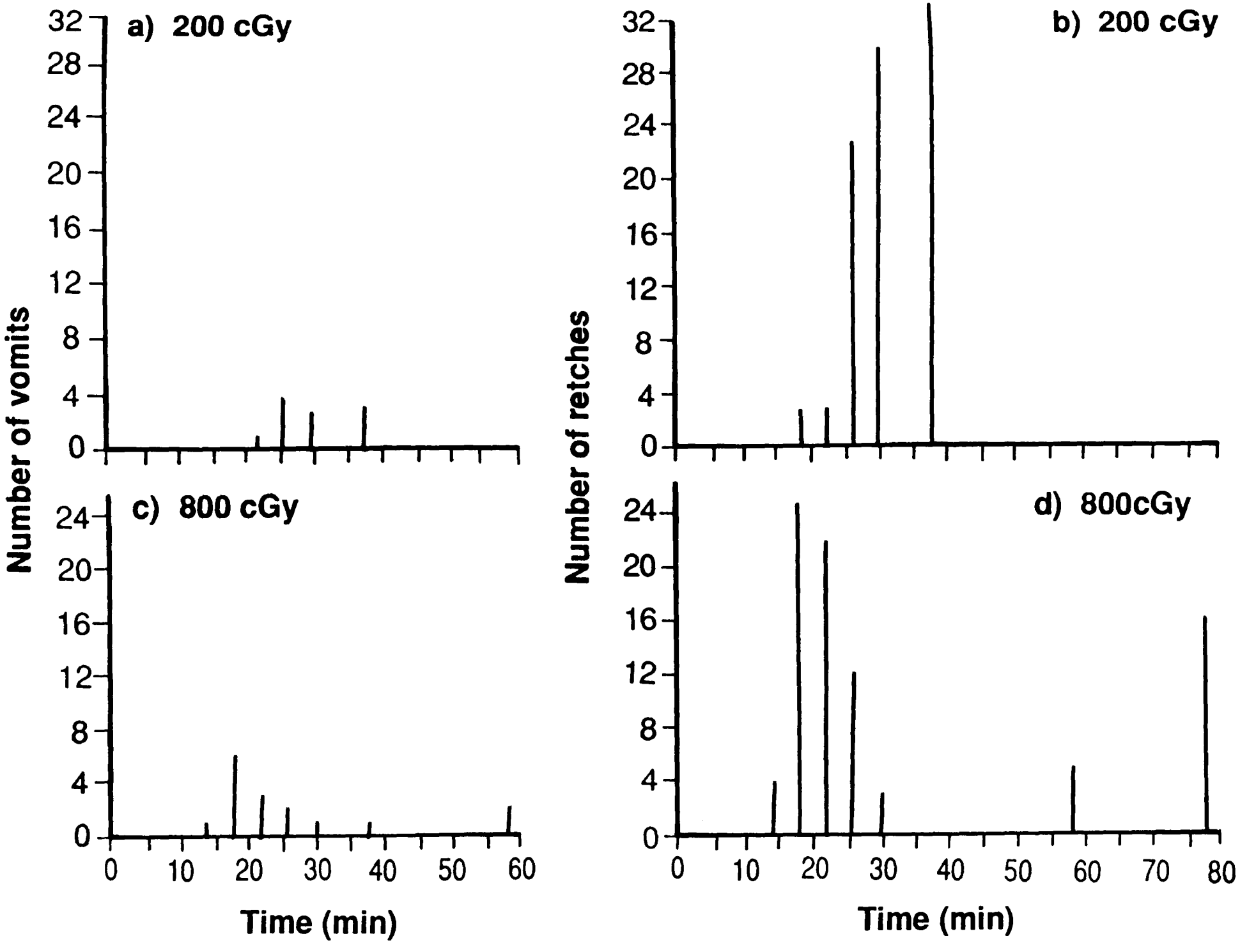
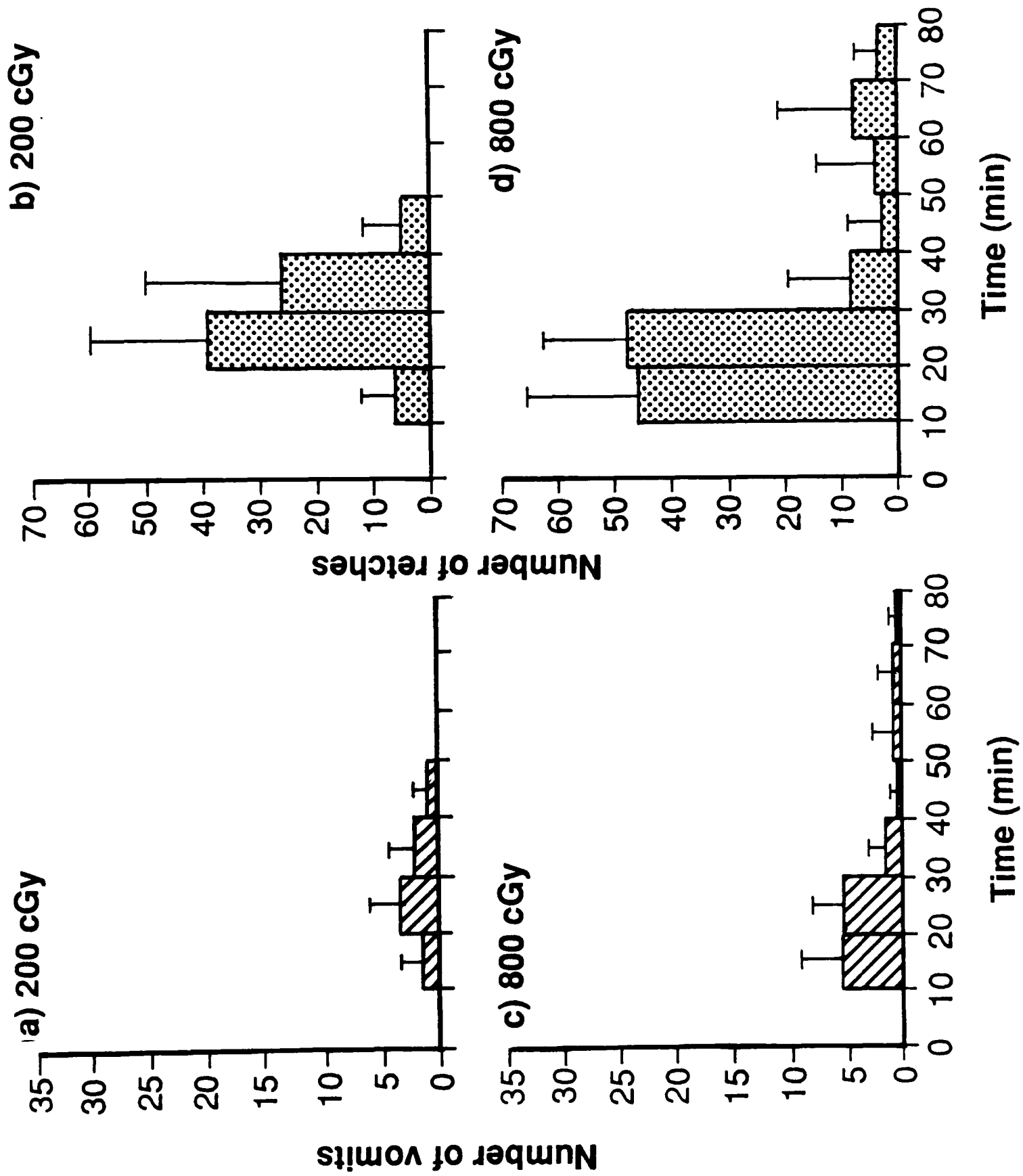


Figure 38 The Emetic Response of a Single Ferret to X-irradiation (200 and 800cGy)

Figure 39 The Emetic Response of a Group of Ferrets to X-irradiation (20 and 800cGy)

The pattern of retching and vomiting to 200cGy and 800cGy is resolved into 10min time periods after irradiation at time zero. (n = 6 and 8 animals) are compared, to illustrate the differences in depth and duration of emetic response



3.3.2 The Effect of Nerve Lesions, Antiemetics and other Drugs on X-ray induced Emesis

3.3.2.1 Effect of Abdominal Vagotomy and Combined Vagotomy and Greater Splanchnic Nerve Section

Abdominal vagotomy completely abolished the emetic response of 6 animals exposed to 200cGy radiation at 10 days post-vagotomy leaving only residual displays of prodromata and some diarrhoea (see Figs. 40 and 44). All animals in this group were observed for 6 hours during which no delayed vomiting was noted. Two animals with abdominal vagotomies were kept for a further 6 months after surgery and when challenged with 200cGy showed only prodromata but no sign of retching or vomiting.

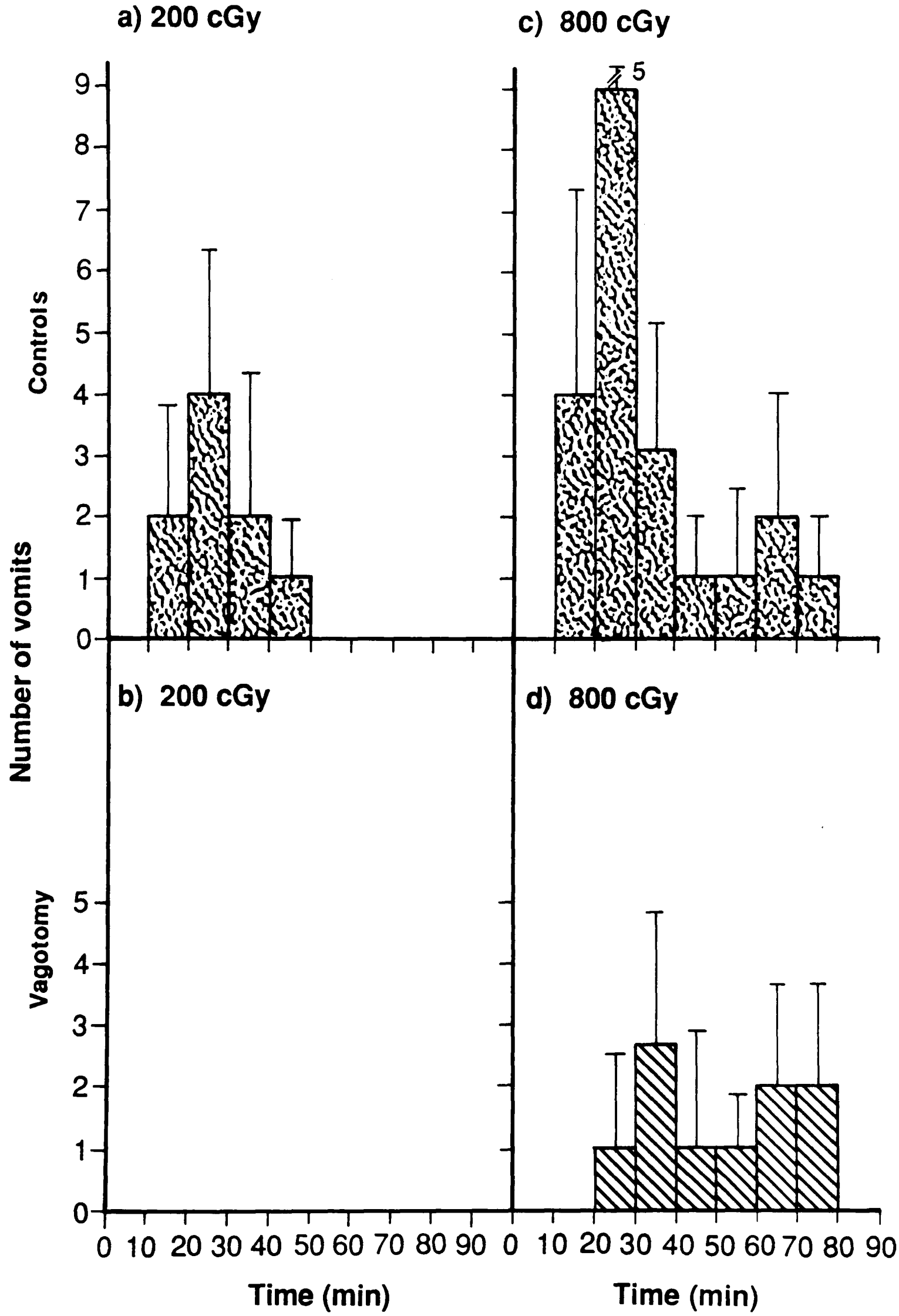
At 800cGy all of the animals tested after abdominal vagotomy vomited but with a significantly increased latency and the total number of vomits in the 90min observation period was significantly reduced (see also Fig. 45). Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	8/8	16.8 ± 3.0	22 ± 4	104 ± 21
VgX Group	6/6	30.5 ± 4.0***	11 ± 3***	63 ± 23**

Fig. 40 plots the mean number of retches and vomits from the Control and vagotomy groups in 10min time periods and reveals that the main effect of vagotomy was to reduce the total numbers of retches and vomits in the first 30min after

Figure 40 The Effect of Abdominal Vagotomy on Vomiting Induced by 200 and 800cGy of X-radiation in the Ferret

The pattern of the vomiting response is resolved into 10min time periods over the 90min observation period. Note in particular the total abolition of vomiting by vagotomy at 200cGy. (n = 6 and 8 animals)


a) 200 cGy
c) 800 cGy
b) 200 cGy
d) 800 cGy
Number of vomits
Controls
Vagotomy
0
1
2
3
4
5
6
7
8
9
5
0 10 20 30 40 50 60 70 80 90
0 10 20 30 40 50 60 70 80 90
Time (min)
Time (min)

irradiation leaving the remainder of the emetic response unaffected (see also Fig. 41). This illustrates that at high doses of X-radiation the emetic response can be resolved into a vagally dependent and a vagally independent component. The presence of prodromata and the occurrence of diarrhoea in these animals was apparently not altered by vagotomy.

A further group of animals (n = 5) in which a combined abdominal vagotomy and greater splanchnic nerve section was performed were left for six months before exposure to 800cGy. All five animals retched and 3/5 vomited but with a latency not significantly different from those animals with a vagotomy alone tested at 10 days. Diarrhoea and prodromata were present in all animals in this group. The effect of vagotomy alone tested at 10 days post irradiation and a combined lesion tested after 6 months is summarized thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Vagotomy alone (tested at 10 days)	6/6	30.5 ± 4.0	11 ± 3	63 ± 23
Vagotomy and Splanchnectomy (tested at 6 months)	3/5	32.5 ± 1.3	3 ± 4	41 ± 36

3.3.2.2 Effect of Greater Splanchnic Nerve Section

Three out of three animals with sections of the greater splanchnic nerve (SpX) performed 10 days previously vomited to 800cGy of X-rays with a latency not significantly different from the control group (16.8 ± 3.0min). Mean numbers of vomits did not differ significantly from the control and similarly mean numbers of retches were unaffected by the procedure. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	8/8	16.8 ± 3.0	22 ± 4	104 ± 21
SpX Group	3/3	17.4 ± 2.6	21 ± 9	110 ± 38

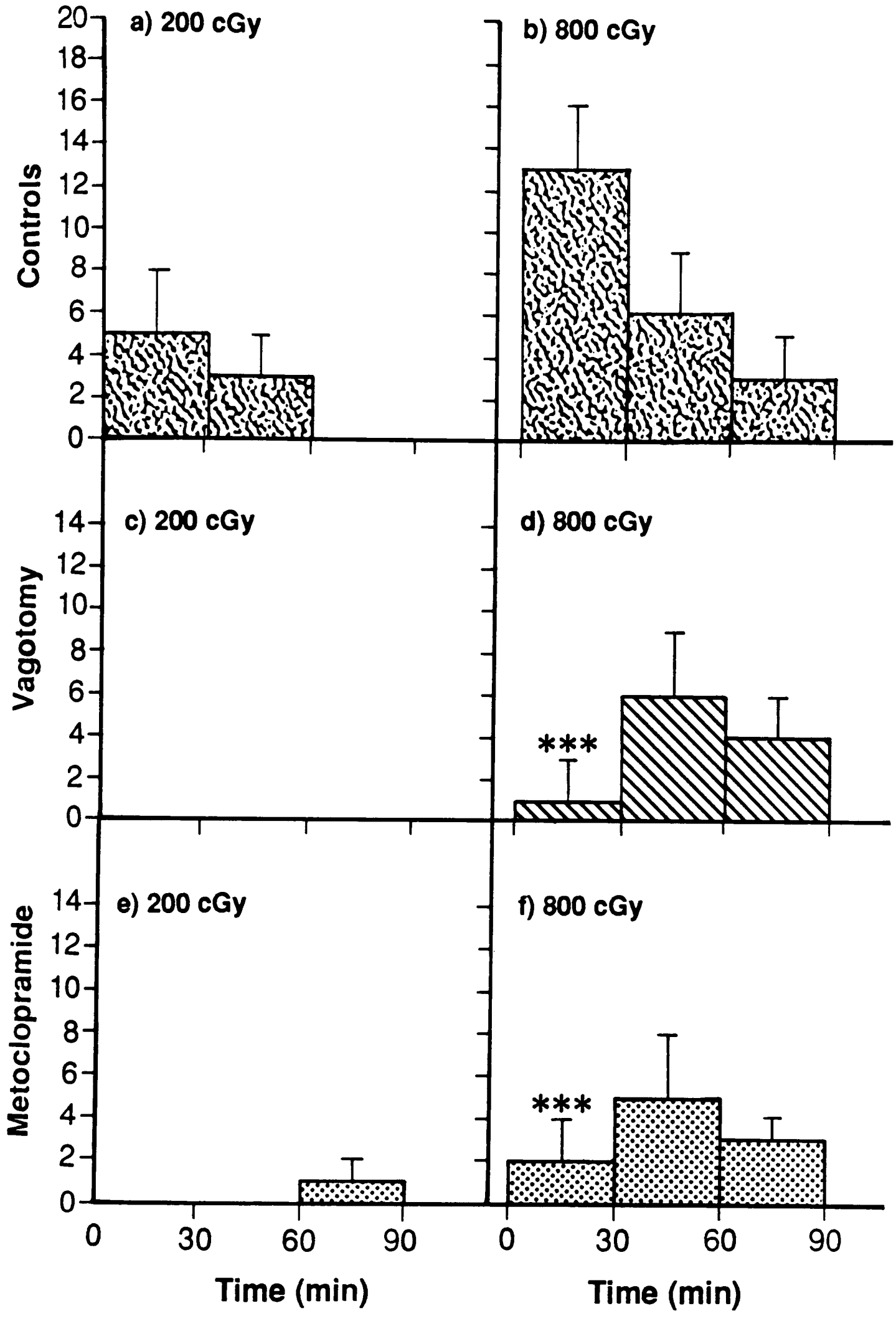
As no difference was detected between animals with and without greater splanchnic nerve section challenged with 800cGy (see also Fig. 45), including degree of prodromata and diarrhoea, the experiment was not repeated at the lower dose of 200cGy.

3.3.2.3 Effect of Metoclopramide

Pre-treatment with metoclopramide (5mgkg^{-1} s.c.) reduced the vomiting response to 1/5 in a group of animals challenged with 200cGy of X-rays and mean total episodes of retching and vomiting were consequently significantly reduced with no emesis occurring during the first 30min period of observation. Moreover, prodromata were virtually absent in the metoclopramide pre-treated group and only sporadic episodes of diarrhoea were noted. In summary the effect of metoclopramide

Figure 41 A Comparison of the Effect of Abdominal Vagotomy and Metoclopramide Administration on Vomiting Induced by 200 and 800cGy of X-radiation in the Ferret

The pattern of vomiting is resolved into 30min time periods over the 90min observation period. Dose of the drug MCP was 5mgkg^{-1} s.c.. Note how MCP mimics the effect of vagotomy at both doses of radiation (n = 6 - 8 animals)


a) 200 cGy
b) 800 cGy
c) 200 cGy
d) 800 cGy
e) 200 cGy
f) 800 cGy
Controls
Vagotomy
Metoclopramide
***
***
0
2
4
6
8
10
12
14
16
18
20
0
30
60
90
Time (min)
Time (min)

on irradiation with 200cGy is:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	6/6	22.1 ± 1.9	9 ± 3	75 ± 26
MCP	1/5	[66.3]	1 ± 1***	2 ± 5***

At 800cGy there was a similar attenuation of the emetic response to ferrets pre-tested with metoclopramide although all 5 animals that were tested did vomit. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	8/8	16.8 ± 3.0	22 ± 4	104 ± 21
MCP Group	5/5	31.7 ± 10.6***	10 ± 3***	34 ± 22***

The vomiting latency was significantly increased. The pattern of emesis resolved into 30min time periods in animals treated with Metoclopramide and exposed to 200cGy and 800cGy is shown in Fig. 41 and reveals that Metoclopramide produces a similar effect to vagotomy with its most pronounced effect being in the first 30min after irradiation. Diarrhoea was still present but not in all animals of the group.

3.3.2.4 Effect of BRL 24924

Pre-treatment with BRL 24924 (5mgkg^{-1} s.c.) prevented vomiting in 3/5 animals irradiated with 200cGy and significantly increased the vomiting latency along with total numbers of retches and vomits. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	6/6	22.1 ± 1.9	9 ± 3	75 ± 26
BRL24924 Group	3/5	60.3 ± 4.2	1 ± 1***	6 ± 8***

The main part of this effect is seen during the first 30min of the observation period when vomiting and retching were completely abolished (see Figs. 42 and 43). Prodromata and diarrhoea however persisted in the treated group.

At 800cGy the pattern of response after pre-treatment with BRL 24924 was broadly similar to that observed at 200cGy (see Figs. 42 and 43). Only 2/5 animals tested, actually vomited and only then after a significantly increased latency; emetic response was significantly reduced accordingly. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	8/8	16.8 ± 3.0	22 ± 4	104 ± 21
BRL24924 Group	2/5	39.7 ± 14.9***	2 ± 4	11 ± 20***

Figure 42 The Effect of Vagotomy and Drug Administrations on Vomiting Induced by 200and 800cGy of X-radiation in the Ferret

The pattern of vomiting is resolved into 30min time periods over the 90min observation period. Doses of drugs were; MCP-5mgkg^{-1} s.c., BRL 24924-5mgkg^{-1} s.c., BRL 43694-5mgkg^{-1} s.c. CIS-2mgkg^{-1} s.c. (n = 5 - 8 animals). Note that at 200cGy all drugs mimic the effect of vagotomy whereas at 800cGy this mimicry only extends to the effect of MCP, whilst treatment with the other agents have a more pronounced effect

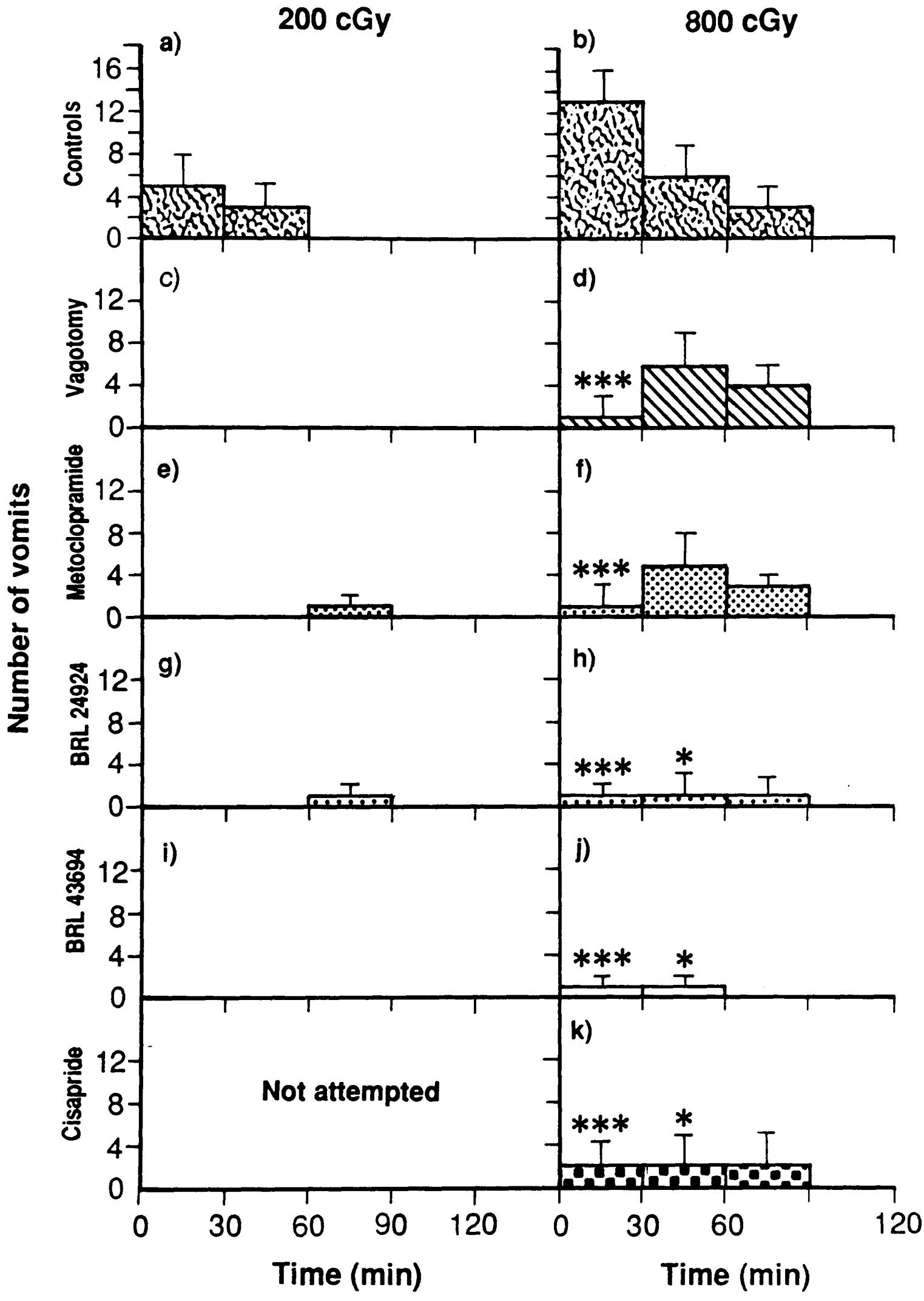
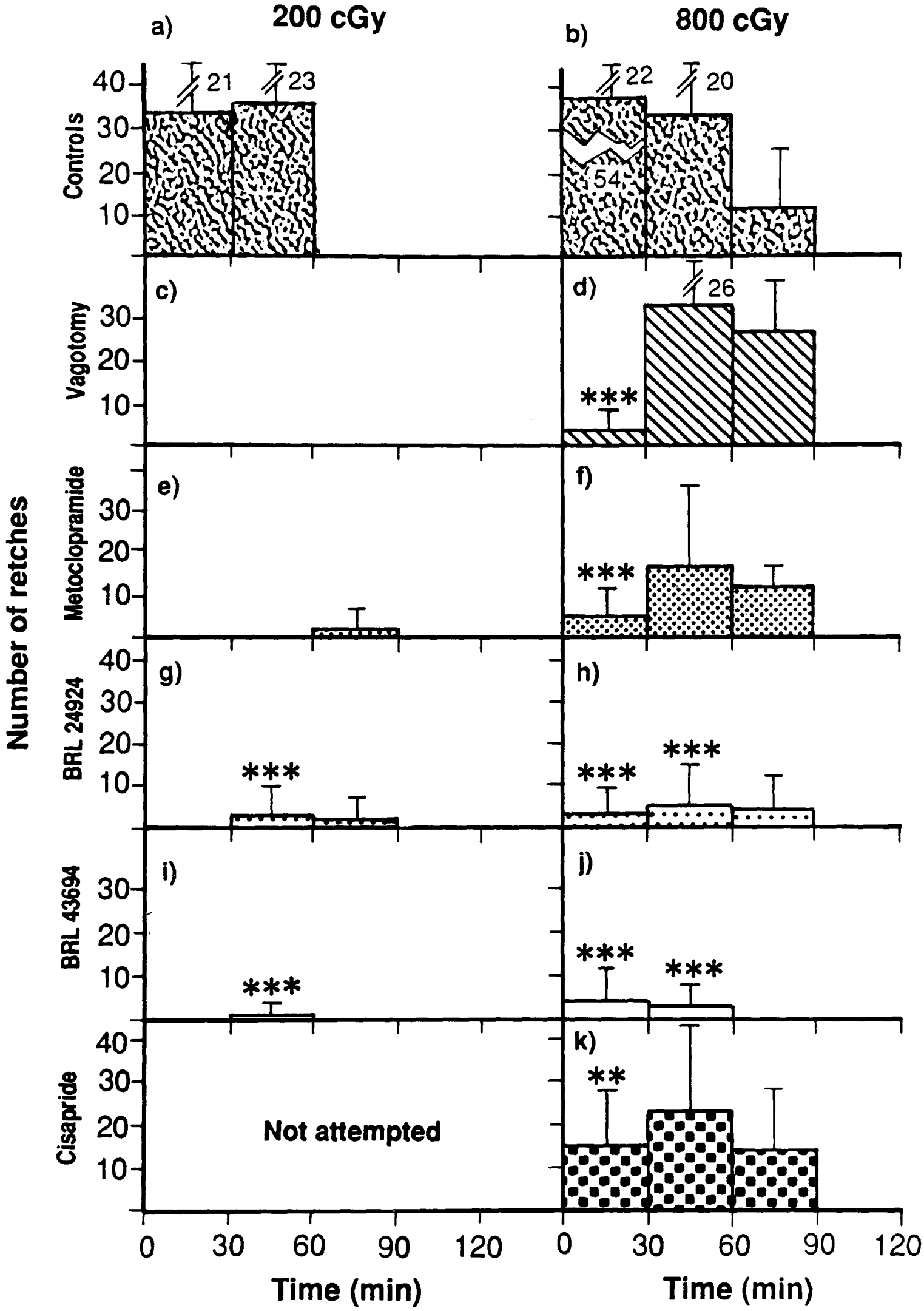

200 cGy
800 cGy
a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
k)
Controls
Vagotomy
Metoclopramide
BRL 24924
BRL 43694
Cisapride
Number of vomits
0
4
8
12
16
***
*
Not attempted
0
30
60
90
120
Time (min)

Figure 43 The Effect of Vagotomy and Drug Administrations on Retching Induced by 200 and 800cGy of X-radiation in the Ferret

The pattern of retching is resolved into 30min time periods over the 90min observation period. Doses of drugs as in Fig. 42 (n = 5 - 8 animals).
Note that similar patterns as those revealed in Fig. 42 are illustrated here as an effect on the retching response. BRL 24924 and 43694 have an extensive effect on the pattern at 800cGy whereas MCP closely parallels the effect of vagotomy


a)
200 cGy
b)
800 cGy
Controls
21
23
22
20
54
c)
d)
26
Vagotomy
***
e)
f)
Metoclopramide
***
g)
h)
BRL 24924
***
***
***
i)
j)
BRL 43694
***
***
***
k)
Cisapride
**
Not attempted
Number of retches
40
30
20
10
0
30
60
90
120
Time (min)

As at 200cGy, there was no reduction in the degree of mucoid diarrhoea noted or for that matter in prodromata. No vomiting was seen in any animal, after that shown, for up to three hours after irradiation.

3.3.2.5 BRL 43694

At 800cGy 2/5 animals vomited after pre-treatment ($5mgkg^{-1}$ s.c.) with a markedly increased mean latency. There was no vomiting or retching at all in three members of the group, although mucoid diarrhoea was present and prodromata was displayed by all animals. Considering the whole observation period in total the emetic response was greatly reduced (see Figs. 42 and 43). Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	8/8	16.8 ± 3.0	22 ± 4	104 ± 21
BRL43694 Group	2/5	32.3 ± 4.1***	1 ± 1***	7 ± 10***

At 200cGy vomiting was completely abolished throughout a group of animals pre-treated with BRL 43694 ($5mgkg^{-1}$ s.c.) leaving only one residual bout of 6 retches in a single animal after approximately 60min (see Figs. 42 and 43). All animals however displayed emetic prodromata but diarrhoea was, unusually, absent.

3.3.2.6 Effect of Domperidone

A group of four animals was pre-treated with domperidone ($500\mu gkg^{-1}$ s.c.) and then exposed to 200cGy of X-rays. All four vomited with a mean latency of 25.0 ± 4.2min, not significantly different from the control group (22.1 ± 1.9min).

The vomiting potential of 200cGy was not reduced and indeed there was even an indication that the incidence of retching actually rose after domperidone (see Fig. 44). Overall Domperidone appeared to have no beneficial effect on the ferrets tested with 200cGy and it was therefore decided not to proceed with a challenge of 800cGy. Prodromata and diarrhoea were displayed by the majority of animals in this group.

3.3.2.7 Effect of Cisapride

Five animals pre-treated with cisapride (2mgkg^{-1} s.c.) and exposed to 800cGy all vomited with a marginally increased mean latency. Mucoid diarrhoea and the prodromata of vomiting were present, but the overall emetic response was significantly reduced. Thus:-

	Vomiting Response	Vomiting Latency (min)	Mean Number of Vomits	Mean Number of Retches
Control Group	8/8	16.8 ± 3.0	22 ± 4	104 ± 21
Cisapride	5/5	25.2 ± 5.6	7 ± 4***	52 ± 46*

However, the principal target of the effect is in the first 30min period after irradiation as can be seen in Figs. 42 and 43.

3.3.2.8 Summary (Figs 44 and 45)

The similarities and differences between the effects of various drug administrations and nerve lesions upon radioemesis in the ferret is illustrated in figures 44 and 45. The parameters compared are the usual ones of vomiting latency, total vomits and total retches (for the standard observation period); Figure 44 shows the effect at 200cGy and Figure 45 that at 800cGy.

Figure 44 A Summary of the Effect of Nerve Lesions and Drug Administrations on Emesis in the Ferret Induced by 200cGy of X-radiation

The emetic response is characterised in terms of latency to first vomit after irradiation and mean total numbers of retches and vomits in the observation period of 90min.
Doses of drugs were: Domp-500μgkg^{-1} i.m. MCP-5mgkg^{-1} s.c., BRL 24924-5mgkg^{-1} s.c. BRL 43694-5mgkg^{-1} s.c. (n = 4 - 6 animals)

200 cGy

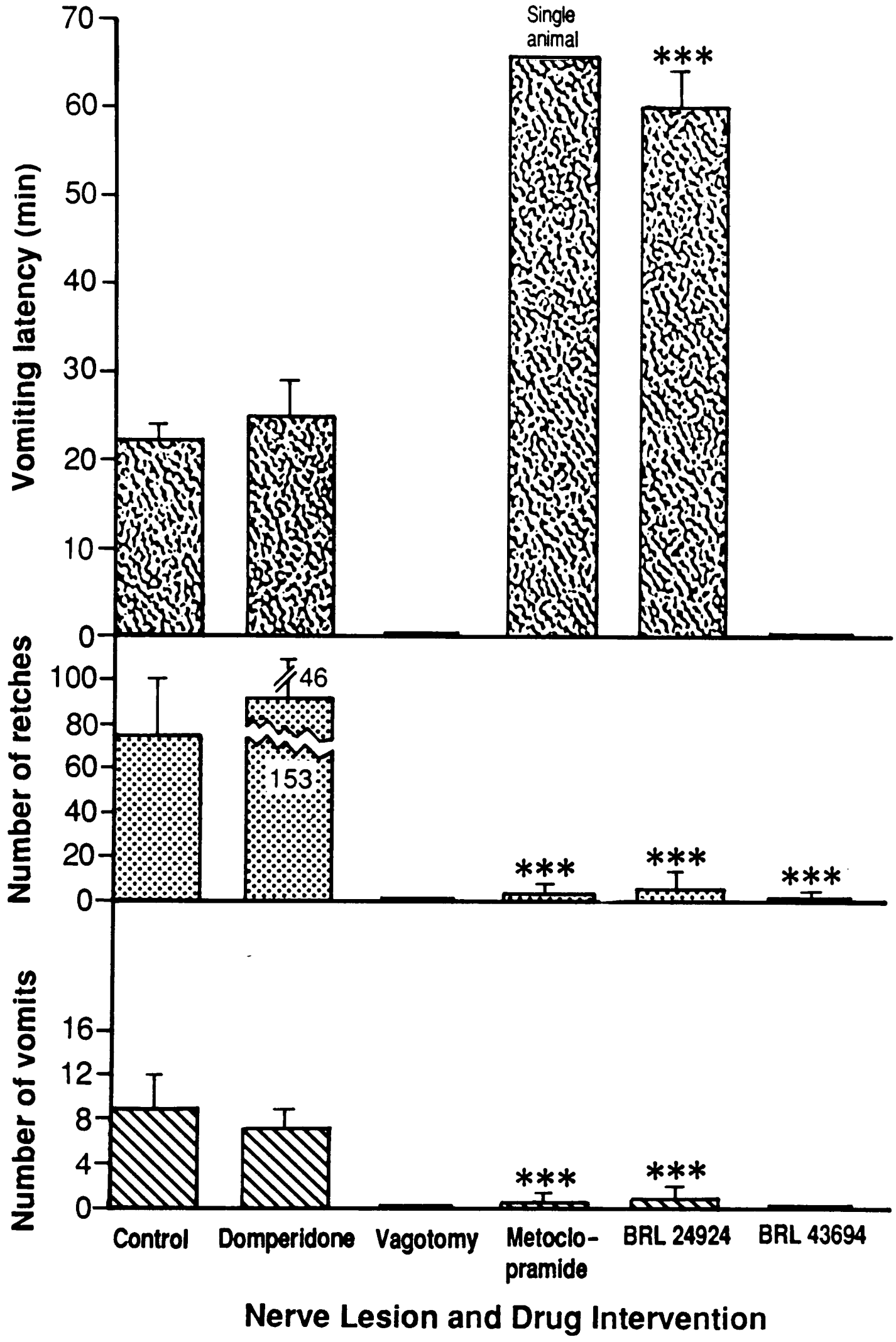
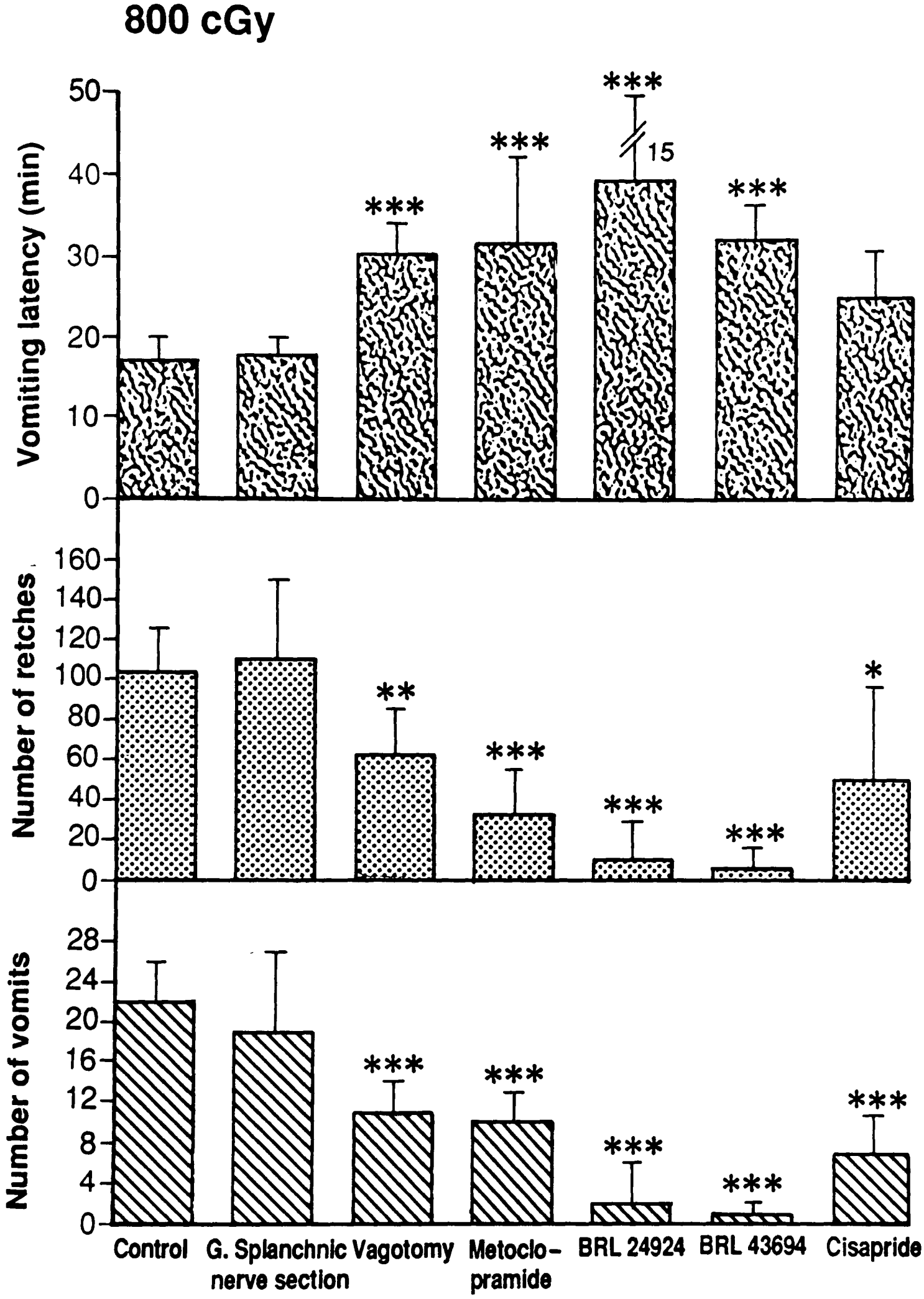

Vomiting latency (min)
70
60
50
40
30
20
10
0
Single animal
***
Number of retches
100
80
60
40
20
0
46
153
***
***
***
Number of vomits
16
12
8
4
0
***
***
Control
Domperidone
Vagotomy
Metoclo-pramide
BRL 24924
BRL 43694
Nerve Lesion and Drug Intervention

Figure 45 A Summary of the Effect of Nerve Lesions and Drug Administrations on Emesis in the Ferret Induced by 800cGy of X-radiation

The emetic response is characterised in exactly the same way as for Fig. 44. Doses of drugs were: MCP-5mgkg^{-1} s.c., BRL 24924-5mgkg^{-1} s.c. BRL 43694-5mgkg^{-1} s.c., CIS-2mgkg^{-1} s.c. (n = 3 - 8)


800 cGy
Vomiting latency (min)
50
40
30
20
10
0
***
***
***
15
***
Number of retches
160
140
120
100
80
60
40
20
0
**
***
***
***
*
Number of vomits
28
24
20
16
12
8
4
0
***
***
***
***
***
Control
G. Splanchnic nerve section
Vagotomy
Metoclo-pramide
BRL 24924
BRL 43694
Cisapride
Nerve Lesion and Drug Intervention

CHAPTER 4

EXPERIMENTAL RESULTS; 2-DEOXYGLUCOSE STUDIES OF EMETIC CHALLENGE

"A week without an experiment is ageing without grace"

Professor Jonathan Magnes 1912 - 1980

From the dedication by Rami Rahamimoff in "Metabolic Probes of Central Nervous System Activity in Experimental Animals and Man" by Louis Sokoloff, 1984

CHAPTER 4: EXPERIMENTAL RESULTS - 2-DEOXYGLUCOSE STUDIES OF EMETIC CHALLENGE

4.1. INTRODUCTION AND NEUROANATOMY OF THE FERRET BRAIN STEM

When this study commenced only one other paper had appeared in the scientific literature applying the 2-DG technique to the investigation of the neuronal activity of the brain stem during vomiting; utilising the squirrel monkey (Brizzee and Dunlap 1983).

It was therefore necessary to proceed by analogy; both from the point of view of targeting neuroanatomical sites of interest (since no brain atlas of the ferret existed at that time see Andrews and Lawes 1984, Bosley et al., 1983) and in assuming that in an animal capable of vomiting like the ferret, the information required to achieve an emetic response would involve similar pathways and brain nuclei as had been previously shown to be involved by other techniques in other animal models (Odekunle and Bower 1985). Fortunately ferrets although from the Mustelida family are related to the dog and cat, respectively from the Canidae and Felidae, through membership of the same order, the Carnivora, and the three animals resemble each other in many respects (Wen et al.,1985).

An attempt will now be made to outline briefly the principal neuroanatomical features of interest in the brain stem of the ferret with relevance to the vomiting response so far as they have been elucidated (see also Lawes and Andrews, 1988). Thus:-

(a) Special visceral efferents from the Nucleus Ambiguus pass longitudinally and ventrolaterally in the reticular formation ultimately to supply striated muscle of the pharynx, larynx and upper oesophagus.

(b) General visceral efferents arise in the DMVN, a spindle shaped longitudinal nucleus dorsolateral to the hypoglossal cell column in the rostral medulla. They supply parasympathetic fibres to larynx, trachea, lungs, GIT from oesopagus to left colic flexures and associated visceral glands and ducts.

(c) General somatic afferents from the superior vagal ganglia project to the Trigeminal Nuclei. Fibres relay sensory impulses from the skin of the back of the external auditory meatus and concha of the ear.

(d) Special visceral afferents: taste fibres in the vagus nerve transmit impulses from the taste buds on the epiglottis and the vallecula. They constitute part of the peripheral processes of the autonomic ganglial cells localised in the inferior vagal (or nodose) ganglia. The central process of these ganglial cells project to the gustatory subnucleus of the NTS.

(e) General visceral afferents have their localised origin in the inferior vagal ganglion. The peripheral processes of the ganglial cells convey general visceral sensory information from:-

(i) Pharynx, lower respiratory tract, lungs, post-pharyngeal wall, GIT up to the left colic flexure, associated organs and ducts

(ii) Pressure receptors of aortic arch, atrium and heart ventricles and the pulmonary tree

(iii) Chemoreceptors in aortic bodies and major thoracic arteries

Central processes from the vagal trunk enter the medulla and run in the Solitary Tract before terminating in the various subnuclei of the NTS.

The central division of the vagus consists of several bunches of fibres in two groups, afferent and efferent. The afferents project in the Solitary Tract, a longitudinal bundle of fibres lateral to the DMVN extending throughout the medulla oblongata. From there the afferents and collaterals project to the various subgroups of the NTS.

The efferent bundles comprise two groups; fibres from the anterior vagal nuclei i.e. the Nucleus Ambiguus, Nucleus Retroambiguus, Nucleus Retrofacialis and fibres from the dorsally placed nuclei i.e. the DMVN and Solitary Nucleus.

The DMVN itself consists of a bilaterally symmetrical column of cells which is buried in the floor of the inferior part of the fourth ventricle and the closed part of the medulla oblongata. The rostral part is in the grey matter of the floor of the IV ventricle dorsolateral to the Hypoglossal column and medial to the Solitary Tract and the NTS. The caudal part is situated in the grey matter of the closed part of the medulla lateral to the central canal and dorsal to the Hypoglossal Nucleus. The mean rostro-caudal extent of DMVN is from 2.36mm rostral to 1.62mm caudal, to the Obex. The mean medio-lateral extent is 1.29mm.

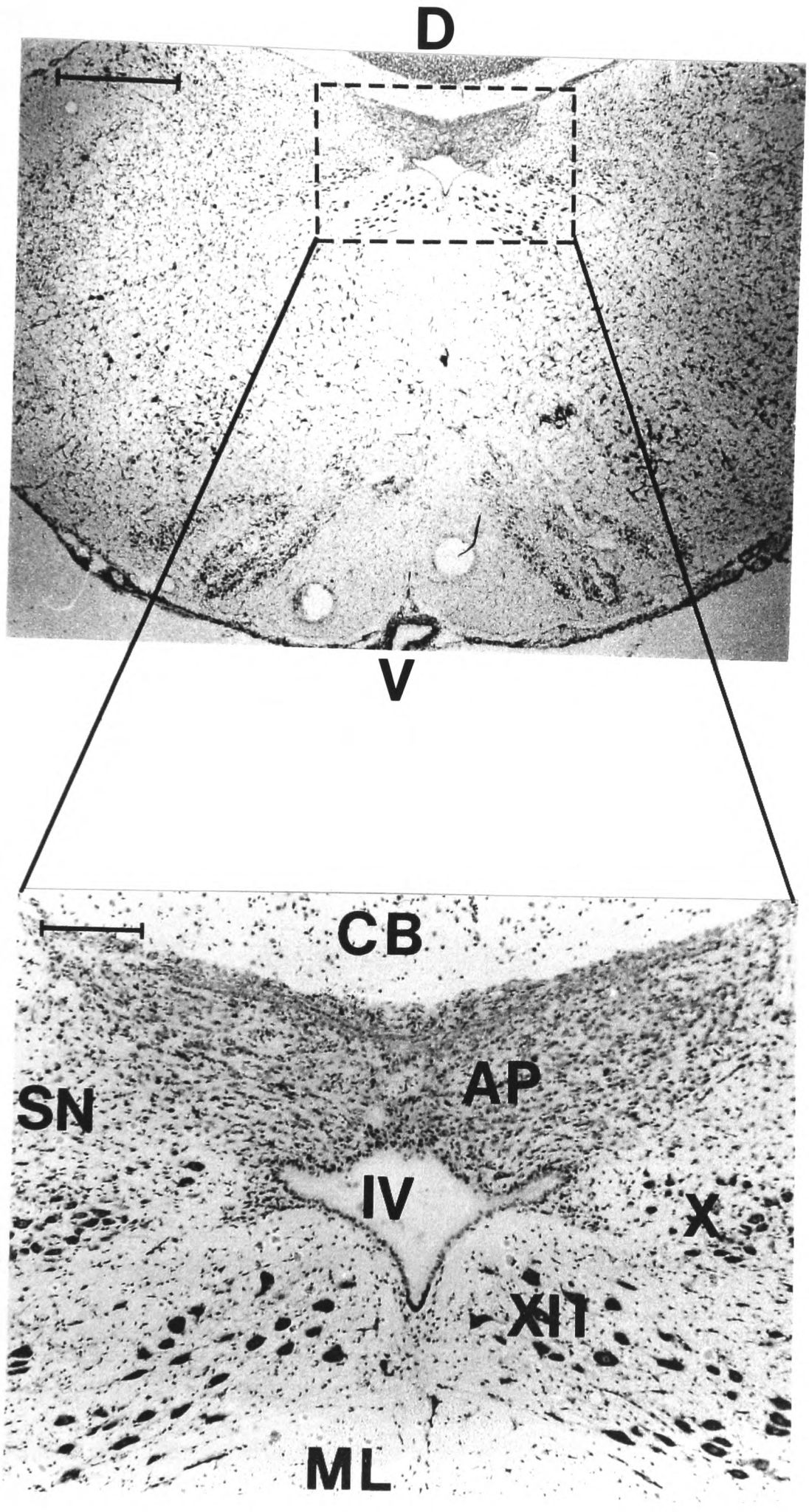
The Solitary Complex is very difficult to localise throughout its extent. The Solitary Tract itself can be identified near to the AP at the Obex. Throughout the extent of the AP from the Obex, the Solitary Tract is a bilaterally symmetrical longitudinal column situated dorsolateral to the

Figure 46 Histology of the Medulla of the Ferret at the Level of the Area Postrema

A 20μm section of ferret brain stem at the level of the Area Postrema, stained with 0.5% aqueous Cresyl Fast Violet, to show cytoarchitectural detail.

Upper panel: Low power (X17) view of medulla with area containing nuclei of particular interest outlined with broken black border Scale Bar = 1mm. Key: D = Dorsal Surface V = Ventral Surface

Lower panel: High power (x 70) view of the area of the Dorsal Vagal Complex,
Scale Bar = 200μm
Key: CB = Cerebellum, AP = Area Postrema
SN = Solitary Nucleus, IV = Fourth Ventricle
X = Dorsal Motor Nucleus of the Vagus
XII = Hypoglossal Nucleus ML = Medial Lemnisci


D
V
CB
SN
AP
IV
X
XII
ML

DMVN. Medially and ventrolaterally are clustered two groups of cells, the medial and lateral subgroups of the NTS. There is a third cluster dorso-laterally. The mean rostrocaudal extent of the Solitary Tract is 2.94mm rostral and 0.72 caudal to the Obex.

The AP is a clearly demarcated zone separated from the medial subnucleus of the Solitary Tract on either side of the midline by a sparsely populated zone. The AP can be recognised from the Obex to 0.73mm rostral to the Obex. Caudally it seems to merge with the Commissural Nucleus of Cajal as it crosses the midline below the Obex. Within the AP can also be seen many fibres (Odekunli and Bower 1985).

The relative position of these nuclei and tracts in the brain-stem of the ferret is illustrated in Fig. 46 by reference to a representative section through the medulla approximately in the region where the AP attains its greatest cross-sectional area.

4.2 LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING ELECTRICAL STIMULATION OF THE ABDOMINAL VAGUS OR APOMORPHINE ADMINISTRATION IN THE ANAESTHETISED FERRET

Electrical stimulation of the abdominal vagus in the anesthetised ferret produced a retching and licking response as described previously (Andrews et al., 1985) and indeed all three animals in the experimental group actually vomited at least once, despite the constraints of gastric intubation.

Apomorphine administration on the other hand was not sufficiently provocative in the anaesthetised ferret to produce frank vomiting although evidence of its physiological activity in this preparation was shown by the brief transient fall in

the systolic B.P. with a quick recovery to normal levels, and the reflex relaxation of the stomach, which usually occurs prior to the initiation of vomiting (Lefebvre et al., 1981). Occasional weak retching was noted in the two of the three experimental animals.

In the case of vagal stimulation the optical density or O.D. ratio or relative metabolic activity was increased significantly in the AP (26.0%) NTS (33.3%) and DMVN (32.1%) (Table 11). These changes are represented graphically in Fig. 47. An example of the corresponding autoradiograms from which these measurements were made are illustrated in Figs. 48 and 49.

Apomorphine stimulation only produced significant detectable changes under these circumstances in the AP (16%). The effect of apomorphine administration is shown in Table 11 and the percentage changes in metabolic activity are illustrated in Fig. 47.

4.3 BLOOD GLUCOSE VARIATION IN THE FERRET UNDER VARYING CONDITIONS OF ANAESTHESIA AND EMETIC STRESS

4.3.1. Variation in Blood Glucose Concentration in the Normal Conscious Ferret

Experience of the first set of 2-DG experiments carried out in the anaesthetised ferret showed that apparent intra-experimental hypoglycaemia (see Section 4.3.2) was causing high residual levels of 2-DG to be present at the moment of sacrifice. It is highly likely that this factor was contributing to the diminished sensitivity and discrimination of changes being achieved in experiments in anaesthetised ferrets. It was therefore decided to investigate briefly the

TABLE 11

Local Cerebral Glucose Utilization in the Brain Stem following Vagal Stimulation and Apomorphine Administration in the Anaesthetized Ferret using [3H]-2-DG

	OPTICAL DENSITY RATIOS	
	Control Group (n=4)	Vagal Stimulation (n=3)
AP	2.97 ± 0.20	3.73 ± 0.26**
NTS	2.55 ± 0.27	3.40 ± 0.23**
DMVN	2.52 ± 0.31	3.33 ± 0.06**
XII	2.29 ± 0.30	2.70 ± 0.17

	Control Group (n=3)	Apomorphine Administration (n=3)
AP	2.87 ± 0.05	3.33 ± 0.22*
NTS	2.53 ± 0.32	2.37 ± 0.14
DMVN	2.51 ± 0.37	2.37 ± 0.21
XII	2.29 ± 0.36	2.15 ± 0.16

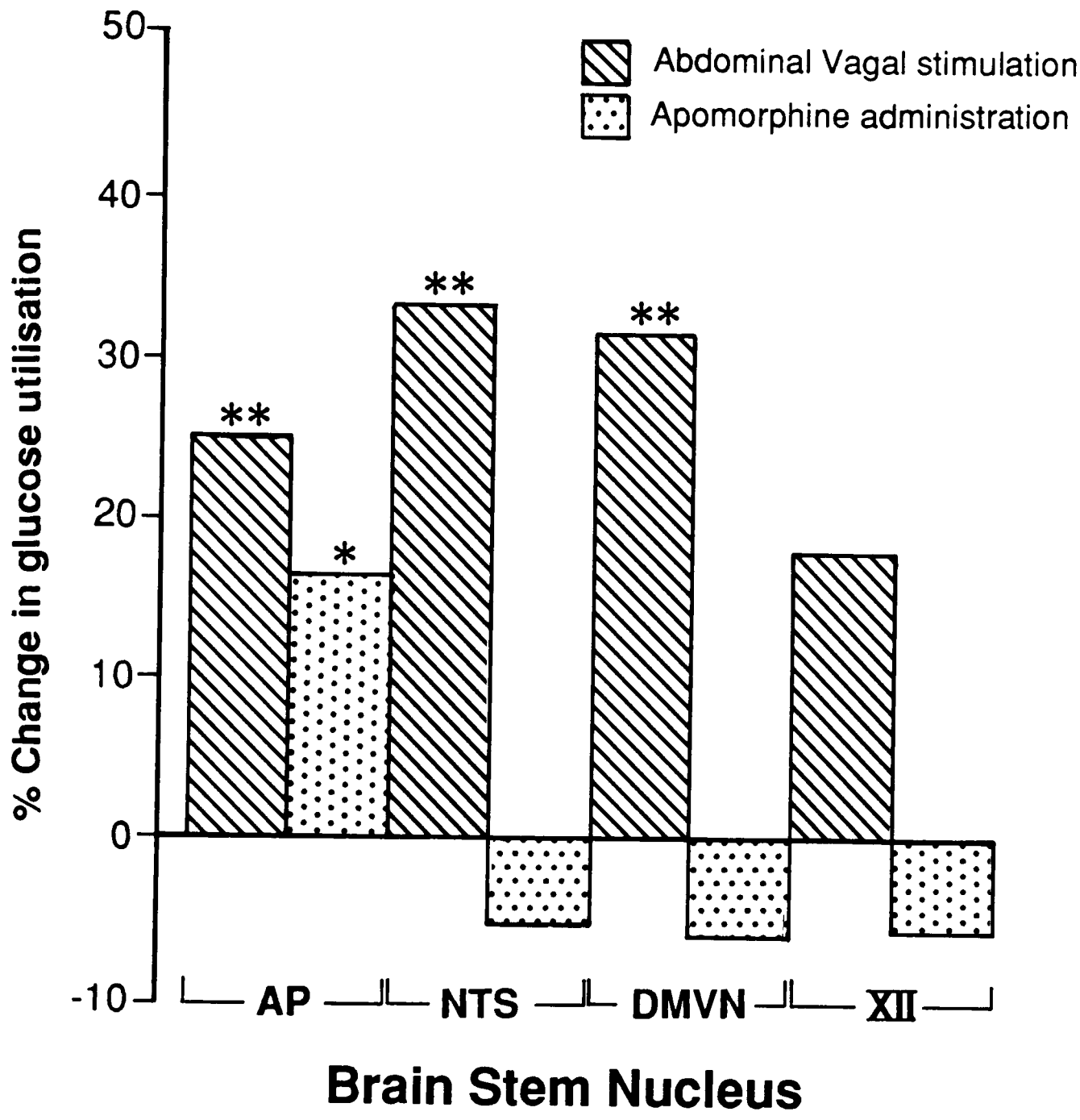
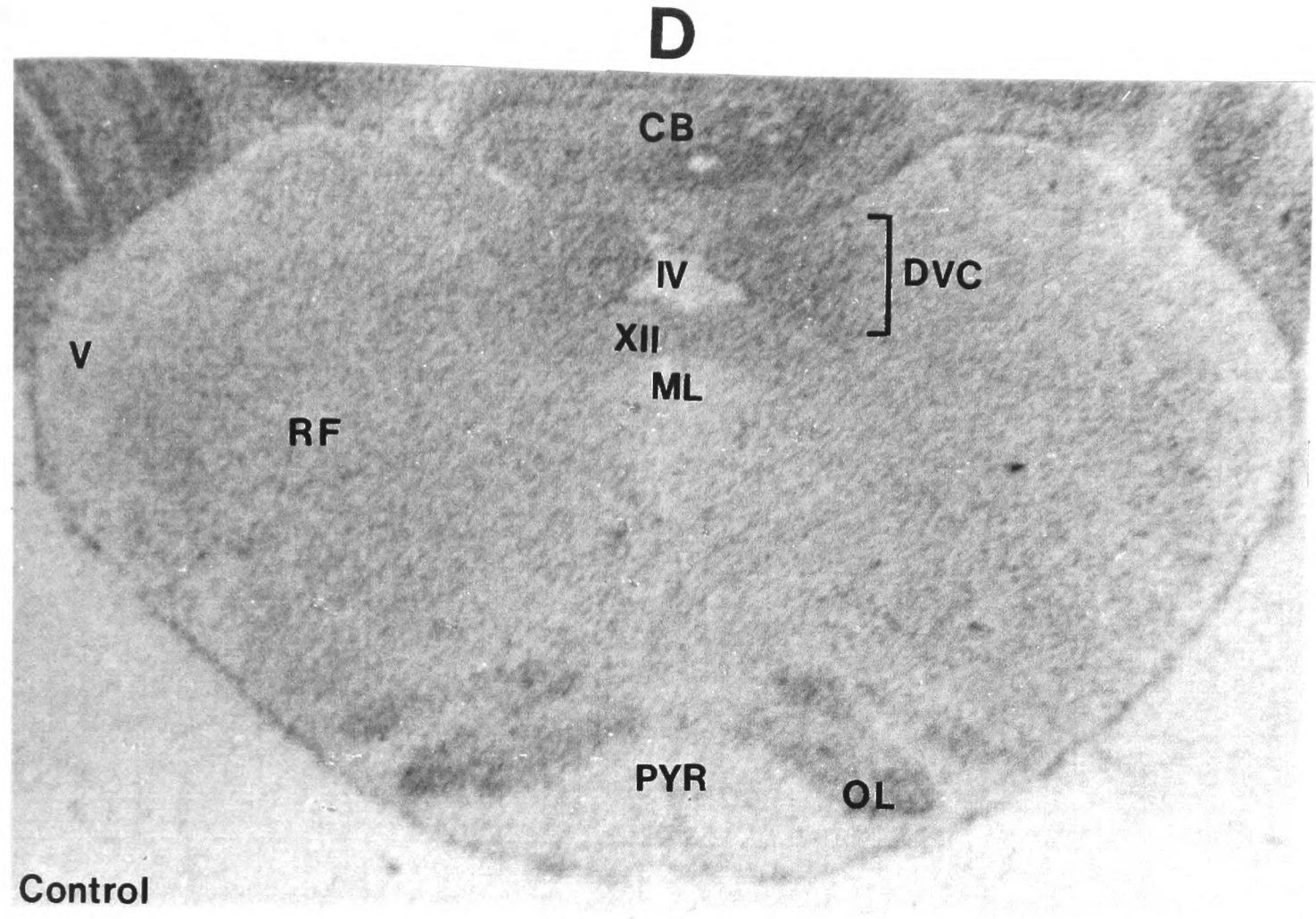


Figure 47 Percentage Change in Glucose Utilization in the Brain Stem Nuclei of the Urethane-Anaesthetised Ferret in Response to Electrical Stimulation of the Abdominal Vagus (30Hz, 20v, 0.5ms) or Apomorphine Administration ($50\mu gkg^{-1}$ i.v.)

The ^{3}H-2-DG isotope was used. (n = 3 - 4 animals). Key: AP = Area Postrema, NTS = Nucleus Tractus Solitarii DMVN = Dorsal Motor Nucleus of the Vagus XII = Hypoglossal Nucleus

Figure 48 Autoradiogram following Abdominal Vagal Stimulation in the Anaesthetised Ferret

^{3}H-2-DG autoradiograms of ferret brain stem transverse sections at the level of the area postrema in two urethane anaesthetised animals. The upper picture is from a control animal. The lower picture is from an animal in which the cut central end of the abdominal vagus was stimulated (30 Hz, 20v, 0.5ms). Note the increased density of labelling in the DVC (NTS, DMVN and AP) (see also Leslie, 1985) of the stimulated animals.
Key: D = Dorsal Surface, V = Ventral Surface
CB = Cerebellum, DVC = Dorsal Vagal Complex
V = Spinal Tract of the Trigeminal Nucleus,
XII = Hypoglossal Nuclei, IV = Fourth Ventricle
PYR = Pyramidal tracts, OL = Inferior Olivary Nuclei, RF = Reticular Formation, ML = Medial Lemnisci, AP = Area Postrema,NTS = Nucleus Tractus Solitarii, X = Dorsal Motor Nucleus of the Vagus


D
CB
IV
DVC
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PYR
OL
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V

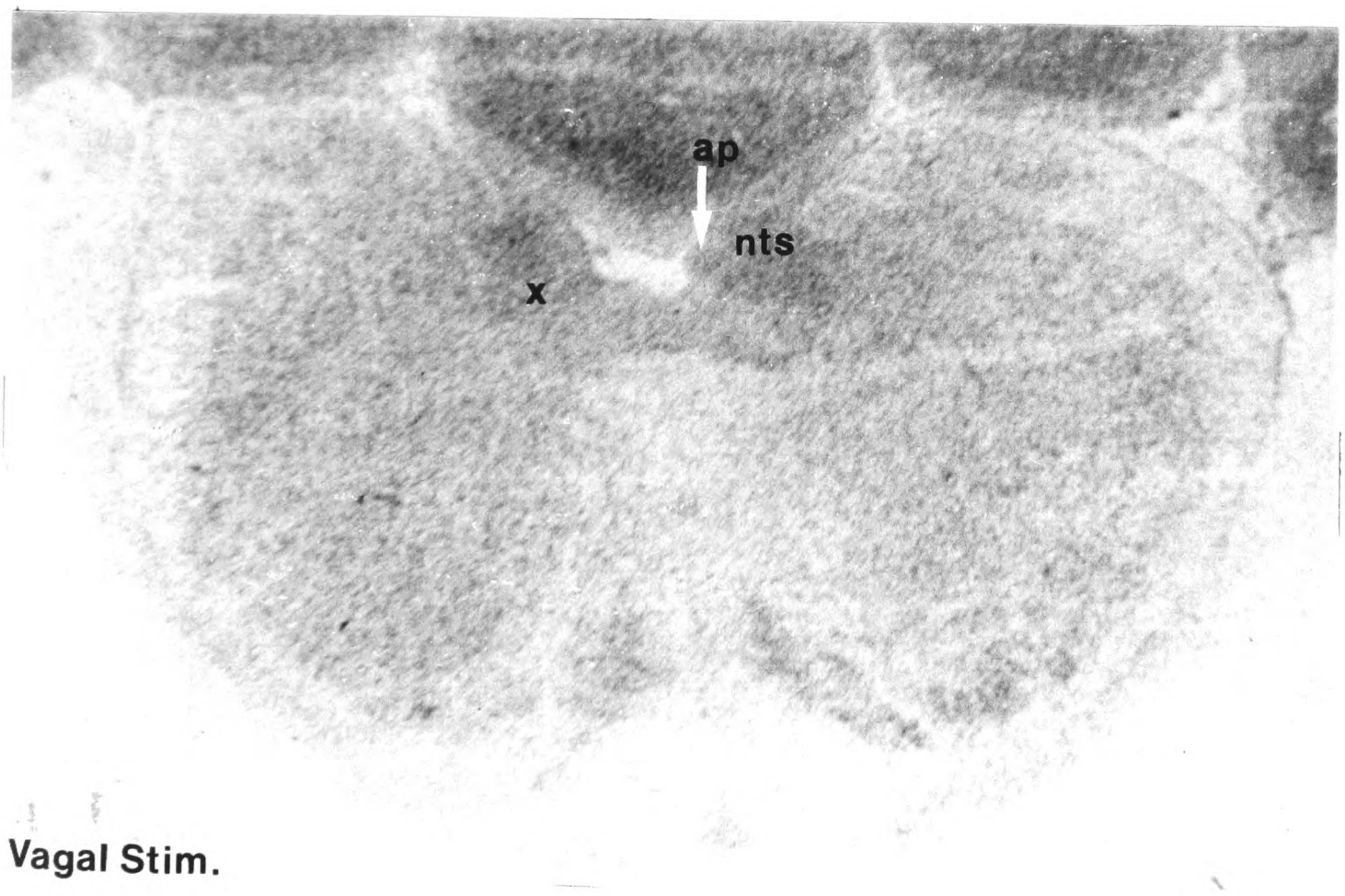

ap
nts
x
Vagal Stim.

Figure 49 Pseudo-colour Coded Autoradiogram following Abdominal Vagal Stimulation

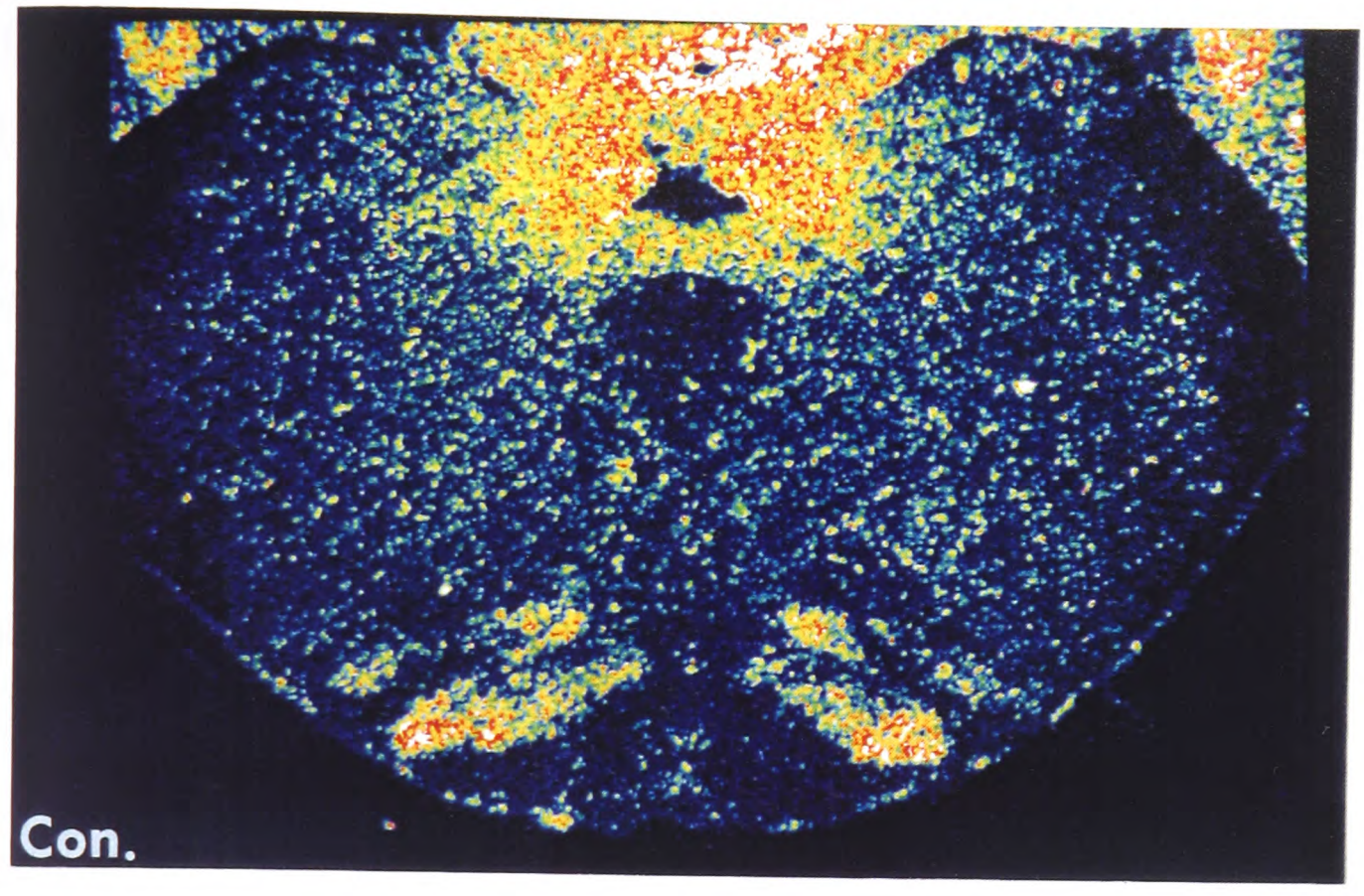
Pseudocolour representation of the autoradiograms in Fig. 48. Rank order of relative metabolic activity is represented thus:-

White > Red > Yellow > Green > Blue > Purple > Black (background Density)

Most Active Areas ⟵⟶ Least Active Areas

Such a transformation from Grey Levels to Colour Spectrum is not of course absolute. However, providing the same transformation table is applied to both autoradiograms to be directly compared under the same conditions of illumination then the two results may be compared as we have done here.

Note that increased relative metabolic activity can be distinguished in comparison with the control, in the AP, NTS and X nuclei.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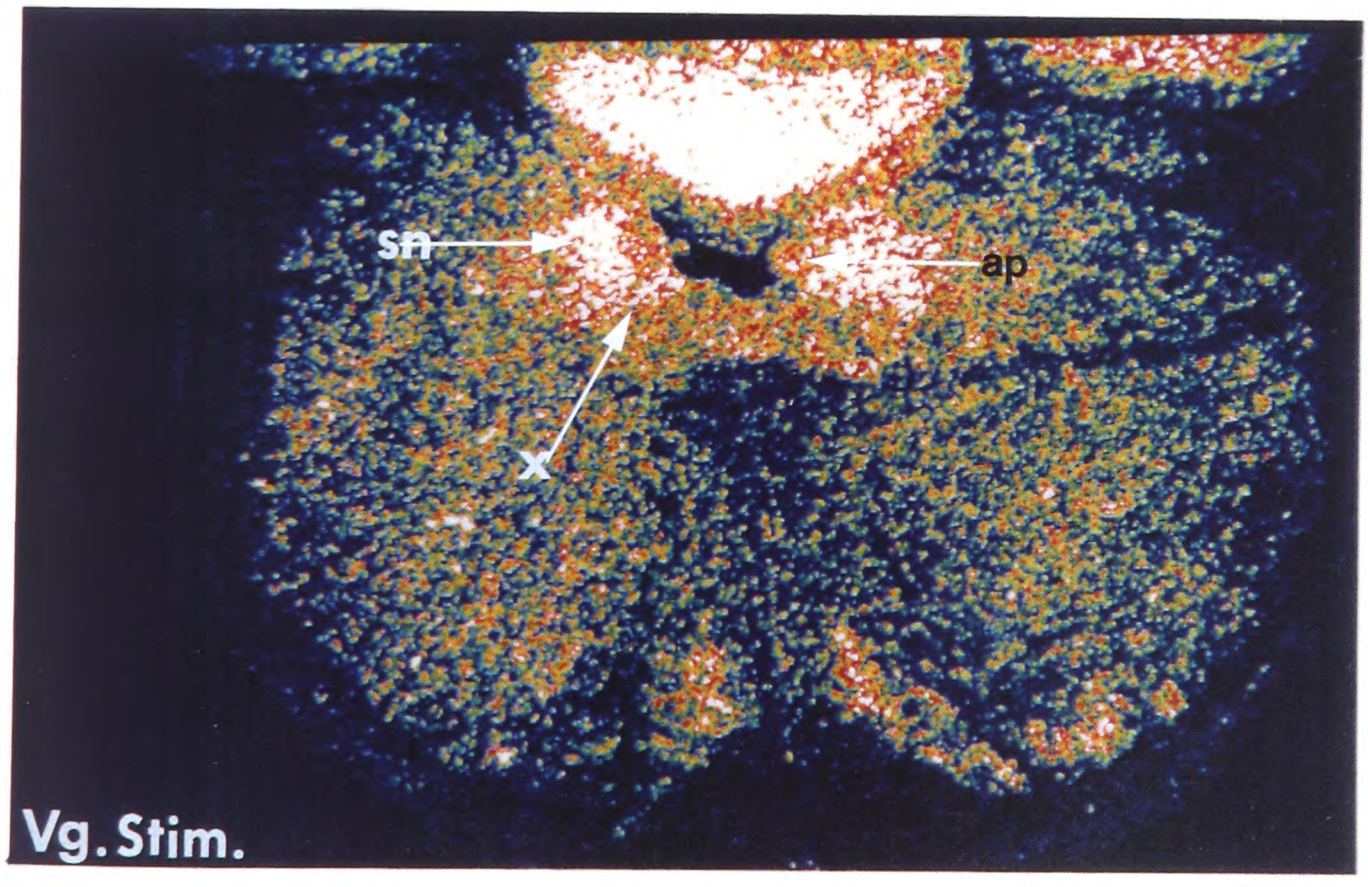

sn
ap
x
Vg. Stim.

blood glucose concentration on a population of ferrets to establish a mean and range of fasting levels in the arterial blood of conscious ferrets and to see the effect of feeding on these levels. It was originally intended to carry out this work using arterial blood samples but this proved technically very difficult and only one blood sample of this type was obtained from the conscious ferret. Indeed even regular routine blood sampling from the central venous cannulae in the conscious animal proved problematical for reasons that subsequent investigation did not reveal. This limited the quantity of data that became available from the experiment in which the blood glucose profile was followed before and after feeding so that the eventual number was restricted to three. However the aim was to provide us with a comparator against which we could assess the glycaemic state of animals in future experiments and in this respect the investigation was successful.

Twenty ferrets were taken at random without regard to age, sex, type and weight and venous blood samples of 1ml were withdrawn at varying times between 09.00h and 18.00h at various points between 12 and 24 hours after the previous meal. The resulting calculated Mean glucose concentration was 7.35 ± 1.24mmol. l^{-1} with a Range of 5.50 - 10.50mmol l^{-1}. The one available 24 hour fasting glucose obtained from an arterial sample was 6.70mmol. l^{-1}.

To investigate the effect of food intake on the blood glucose concentration following a 24 hour fast three ferrets with indwelling central venous cannulae had food withdrawn for

24 hours (whilst still allowed free access to water). Blood samples were then taken at 60min and 15min prior to allowing the animals free access to 100ml of cows' milk. Milk is a favourite food of the ferret and is drunk at maximum rate at all times and was therefore for this purpose the food stimulus that could be administered in the minimum time to allow rapid post-prandial sampling. In each case approximately 65mls of milk was drunk in the first ten minutes allowing the first blood sample to be taken at +15min after initiation of feeding. Further samples were then taken at +30, +60, +135 and +225min after initiation of feeding. The results are presented in Fig. 5D.

Although expected variations did occur, with a mean peak glucose concentration of 8.10 ± 2.40mmol l^{-1} after 15min (n = 3), at no time during this 4 hour observation period did blood glucose concentration move outside the range of fasting levels established for the ferret group as a whole i.e. 5.00 - 10.50mmol l^{-1} with a mean of 7.34 ± 1.24mmol l^{-1} (n = 20).

4.3.2 Variations in Blood Glucose Concentration in the Ferret undergoing Emetic Stimulation in the Anaesthetised and Conscious State

Single intracardiac samples of mixed venous/arterial blood were obtained at point of sacrifice (either 45 or 60min post administration of 2-DG) from each of the animals involved in 2-DG experiments. For comparison the end-stage blood plasma glucose levels for all 10 experimental groups are summarised in Table 12. As implied earlier the impetus to investigate the

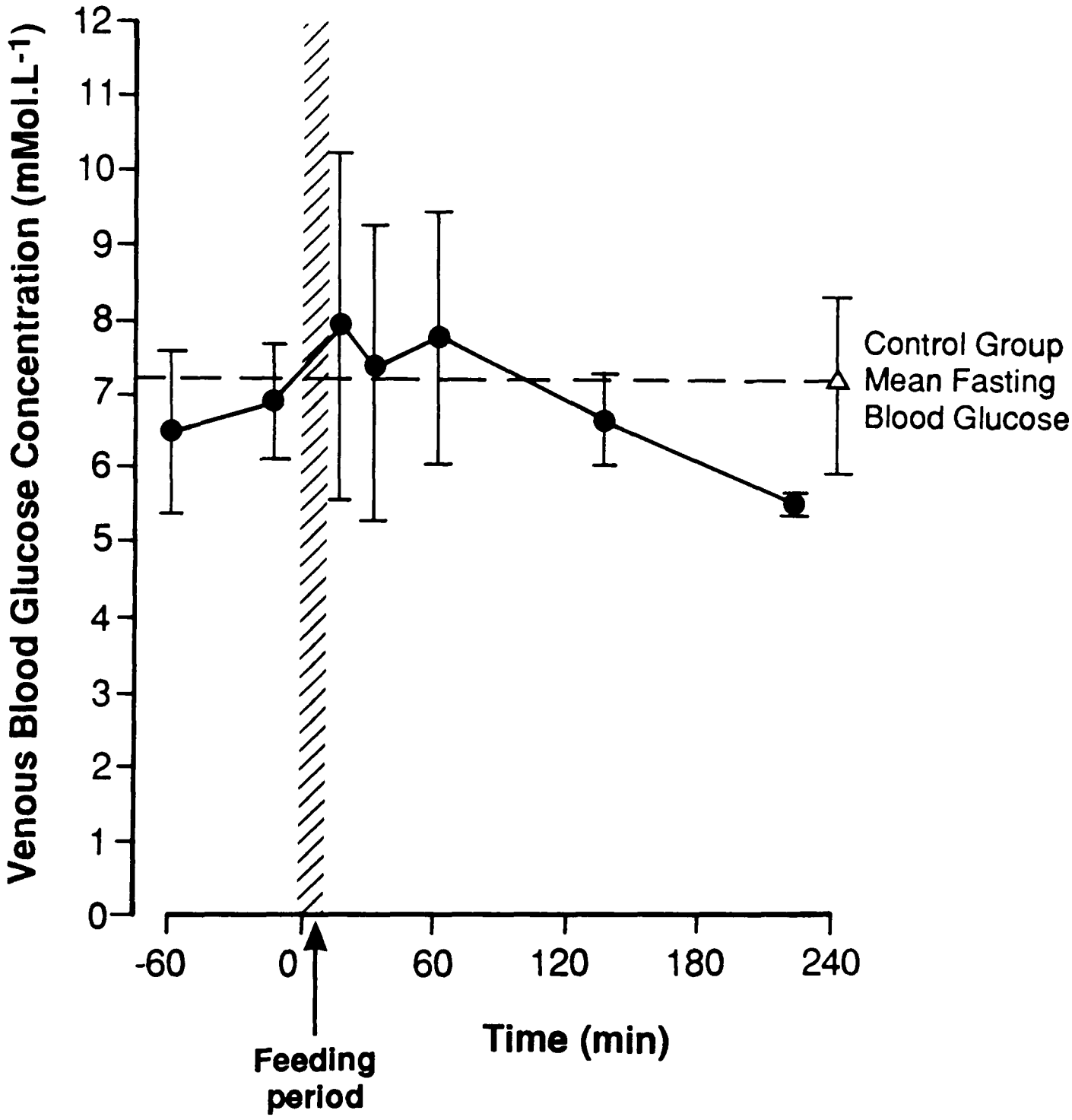


Figure 50 Blood Glucose Variation in the Ferret before and after Feeding

The effect of feeding milk on jugular venous blood glucose concentration in the ferret (n = 3 - 20 animals). The mean fasting blood glucose level in a group of ferrets is represented as a broken line extending from the open triangle for comparison

TABLE 12

A Comparison of End-Stage Blood Glucose Concentrations from Ferrets involved in a Variety of 2-DG Procedures

Experimental Procedure	Conscious State	Sacrifice Sample Point	Endstage Blood Glucose Concentration $mMol.l^{-1}$	Experimental "n"	% Change from Mean Fasting Blood Glucose
$[^3H]$-2-DG control	Anaes.	+45min	19.63 ± 1.60	3	+167
$[^3H]$-2-DG control	Anaes.	+45min	18.70 ± 2.28	4	+154
$[^3H]$-2-DG Vagal stimulation	Anaes.	+45min	22.77 ± 5.31	3	+210
$[^3H]$-2-DG Apomorphine stimulation	Anaes.	+45min	19.27 ± 5.64	3	+162
$[^3H]$-2-DG control	Consc.	+45min	6.87 ± 0.82	4	- 7
$[^3H]$-2-DG Apomorphine stimulation	Consc.	+45min	6.45 ± 0.84	4	- 12
$[^{14}C]$-2-DG control	Consc.	+60min	8.49 ± 1.23	6	+ 16
$[^{14}C]$-2-DG Cycloheximide stimulation	Consc.	+60min	14.90 ± 1.89	5	+103
$[^{14}C]$-2-DG Mustine stimulation	Consc.	+60min	7.90 ± 1.53	4	+ 8
$[^{14}C]$-2-DG X-ray stimulation	Consc.	+60min	13.85 ± 5.24	4	+ 88

effect of hyperglycaemia was in fact the results obtained in the anaesthetised ferret and it is seen clearly from the table that in both control and experimental groups, the animals were markedly hyperglycaemic (up to approximately 200% of fasting level) at the end of the experiment and therefore presumably to an extent throughout the procedure also. In contrast the conscious animals did not show this hyperglycaemia except apparently where the stress induced by particular emetics was very great as in the case of cycloheximide administration and X-irradiation. Neither apomorphine nor mustine administration led to the amount of emesis that would have been reasonably expected from these stimuli and the animals were relatively undisturbed; it was in these groups that the blood glucose remained within the normal fasting range, and, of course, in the control group animals from both experiments.

4.4 LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING APOMORPHINE ADMINISTRATION IN THE CONSCIOUS FERRET

Brief sporadic retching and vomiting only, was achieved in four ferrets challenged with apomorphine administered i.v.. All animals however displayed prodromata.

Based on the results obtained after administration of apomorphine under urethane anaesthesia it was expected that this experimental series would, in the absence of the obscuring factor of anaesthetic-induced hyperglycaemia, reveal more clearly the action of apomorphine on the brain stem nuclei. Relative metabolic activity however in the AP, NTS and DMVN of the apomorphine showed no significant change from the control group (see Table 13).

TABLE 13

Local Cerebral Glucose Utilization following Apomorphine Administration in the Conscious Ferret

	OPTICAL DENSITY RATIOS	
	Control Group (n=4)	Apomorphine Administration (n=4)
AP	3.75 ± 0.72	4.17 ± 0.34
NTS	3.29 ± 0.65	3.27 ± 0.11
DMVN	3.5 ± 0.96	3.58 ± 0.34
XII	3.05 ± 0.88	2.91 ± 0.18

4.5 LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING CYCLOHEXIMIDE AND MUSTINE ADMINISTRATION OR X-IRRADIATION IN THE CONSCIOUS FERRET

4.5.1 Cycloheximide

Intraperitoneal cycloheximide administration was followed as previous experiments had shown (see section 3.2.3.1.) by displays of emetic prodromata and then by retching and frank vomiting beginning at around 15 - 20 minutes after administration and continuing throughout the period of the 2-DG exposure in all animals.

An example of the autoradiograms produced from the ferrets thus exposed is illustrated in Fig. 51. Relative metabolic activity in the cycloheximide treated group for the AP, NTS and DMVN showed no significant change from the control group despite the profound emetic response observed (Table 14 and Fig. 52).

4.5.2 Mustine

Intravenous mustine administration in a group of 4 ferrets did not, during this 2-DG experiment cause the profound emetic response normally to be expected and previously demonstrated following this stimulus. All animals displayed emetic prodromata but only two showed actual retching and vomiting despite the use of a dose which had previously been 100% effective in producing vomiting in the ferret.

However, this stimulus was sufficient to promote a significant (50%) increase in neuronal metabolism in the area postrema (see Tables 14 and Fig. 52) and a typical autoradiogram from this series is illustrated in Fig. 53. No changes in metabolism were detected elsewhere in the brain stem.

TABLE 14

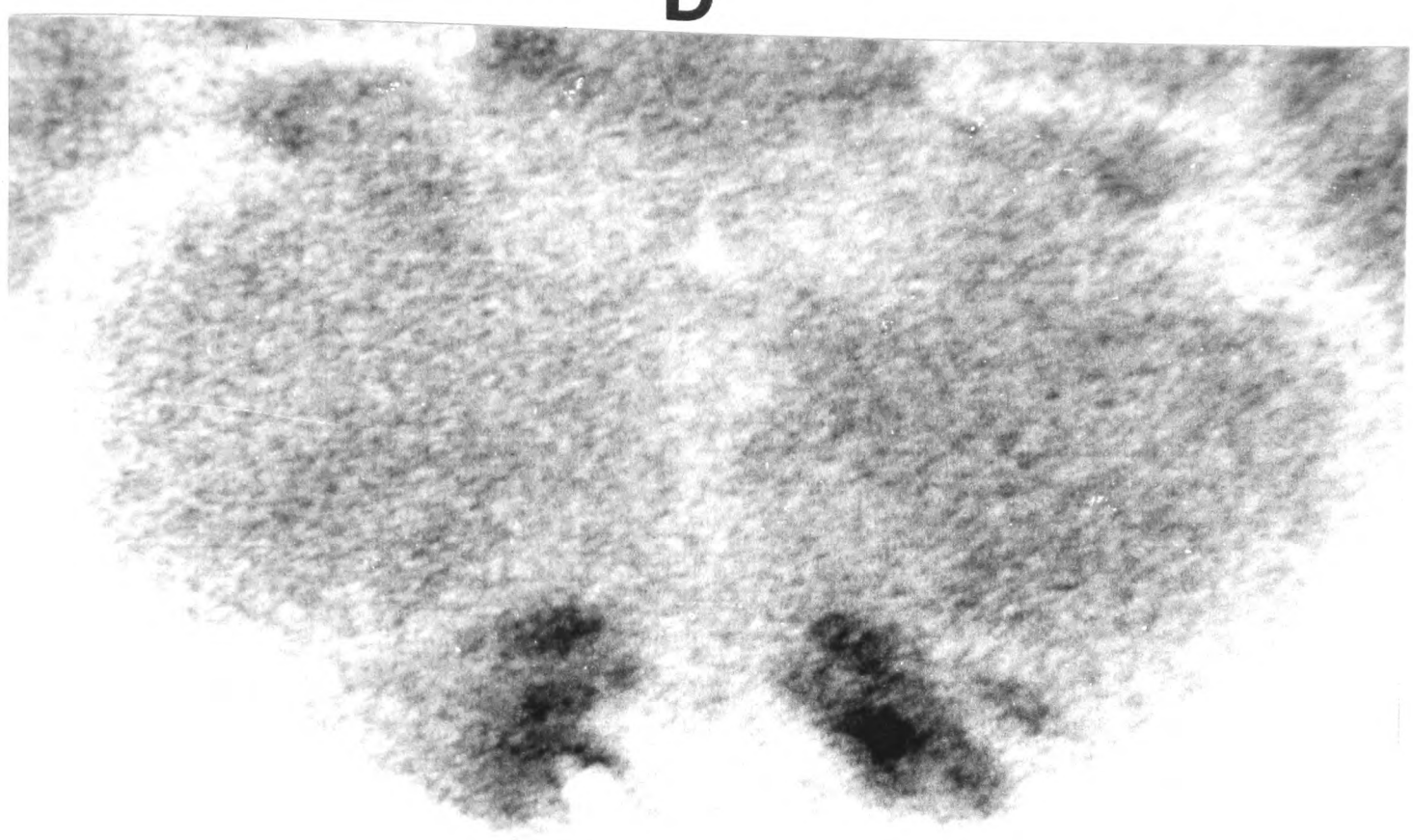
Local Cerebral Glucose Utilization following Cycloheximide, Mustine or X-ray Administration in the Conscious Ferret

	OPTICAL DENSITY RATIOS			
	Control Group (n=6)	Cycloheximide Administration (n=5)	Mustine Administration (n=4)	X-irradiation (n=4)
AP	1.55 ± 0.19	1.62 ± 0.28	2.20 ± 0.38**	2.18 ± 0.17***
NTS	1.67 ± 0.28	1.69 ± 0.12	1.72 ± 0.25	2.01 ± 0.20
DMVN	1.78 ± 0.35	1.75 ± 0.12	2.05 ± 0.30	2.34 ± 0.30*
XII	1.69 ± 0.36	1.36 ± 0.13	1.57 ± 0.18	1.86 ± 0.19

Figure 51 Autoradiogram following Cycloheximide Administration in the Conscious Ferret

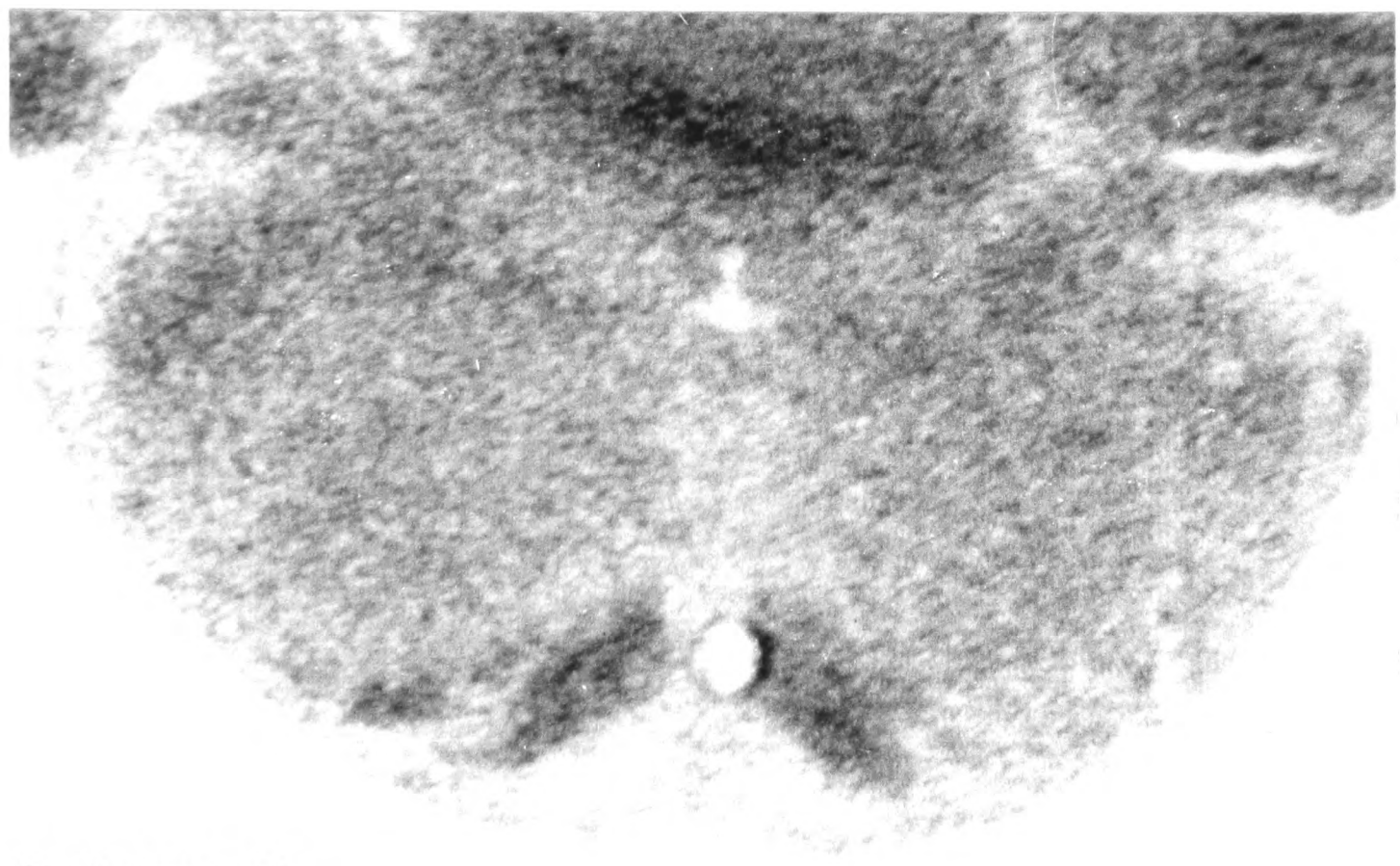
^{14}C-2-DG autoradiograms of ferret brain stem transverse sections at the level of the area postrema in two conscious unrestrained ferrets. The upper picture is from a control animal. The lower picture is from an animal treated with 20mgkg $^{-1}$ i.p. cycloheximide. Although a substantial retching and vomiting response with displays of prodromata were observed no difference in uptake of 2-DG was noted in the experimental animals in the targetted areas of the brain stem as calculated from autoradiograms such as those illustrated here

D



Control

V



Cycloheximide

Figure 52 Percentage Change in Glucose Utilization in the Brain Stem Nuclei of the Conscious Unrestrained Ferret in Response to Cyclohexamide Administration (20mgkg $^{-1}$ i.p.) Mustine Administration (1200μgkg^{-1} i.v.) and Xirradiation (800cGy)

The ^{14}C-2-DG isotope was used (n = 4 - 5)
Key: AP = Area Postrema, NTS= Nucleus Tractus Solitarii, DMVN= Dorsal Motor Nucleus of the Vagus, XII = Hypoglossal Nucleus

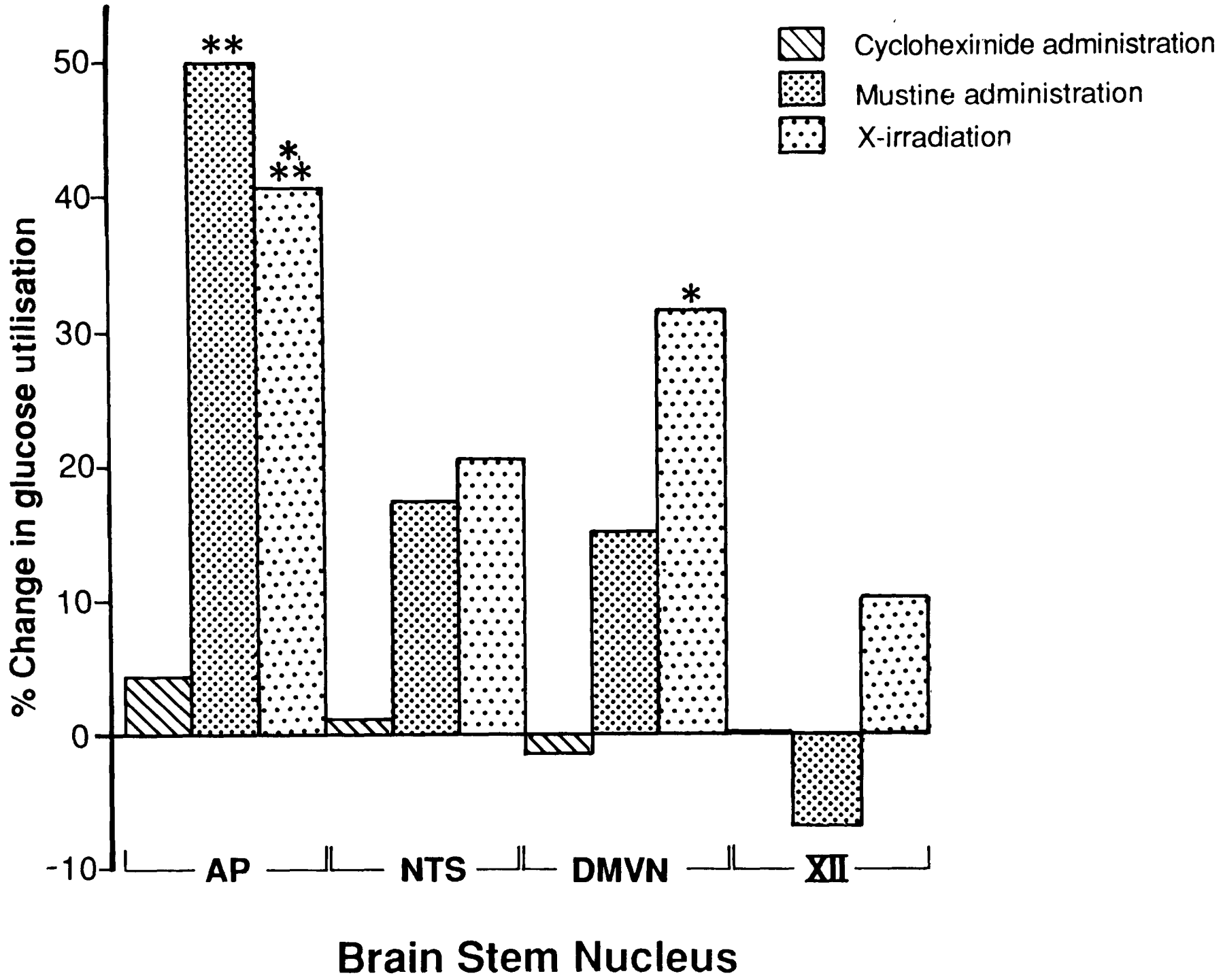
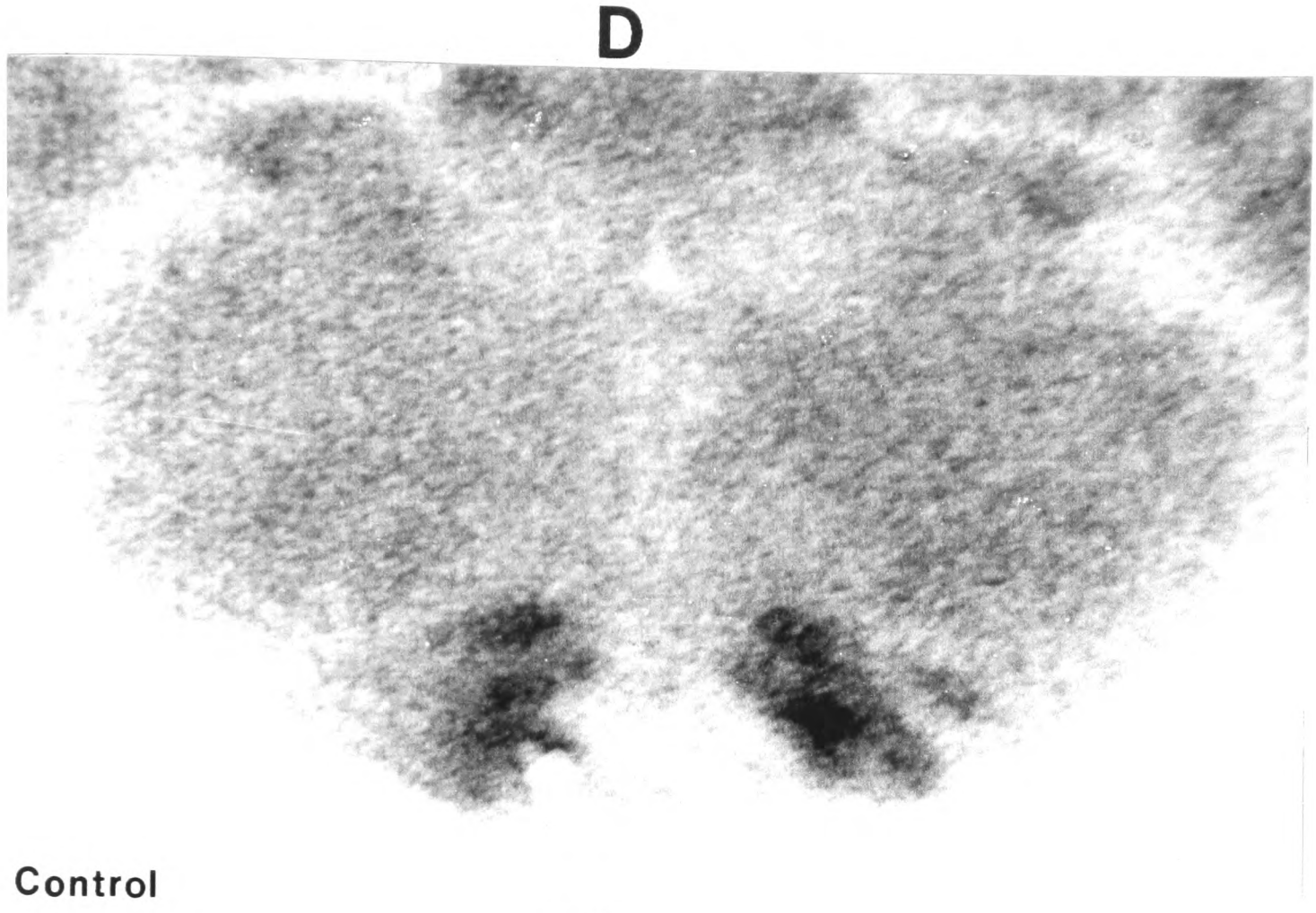

Cyclohexirnide administration
Mustine administration
X-irradiation
% Change in glucose utilisation
50
40
30
20
10
0
-10
**
**
*
*
AP
NTS
DMVN
XII
Brain Stem Nucleus

Figure 53 Autoradiogram following Mustine Administration in the Conscious Ferret

^{14}C-2-DG autoradiograms of ferret brain stem transverse sections at the level of the area postrema in two conscious unrestrained ferrets. The upper picture is from a control animal. The lower picture is from an animal treated with 1200µgkg $^{-1}$ i.v. mustine. Although, overall, the emetic response in this group of animals was relatively weak significant increases in relative metabolic activity were noted in the AP as illustrated in this autoradiogram.
Key: AP = Area Postrema



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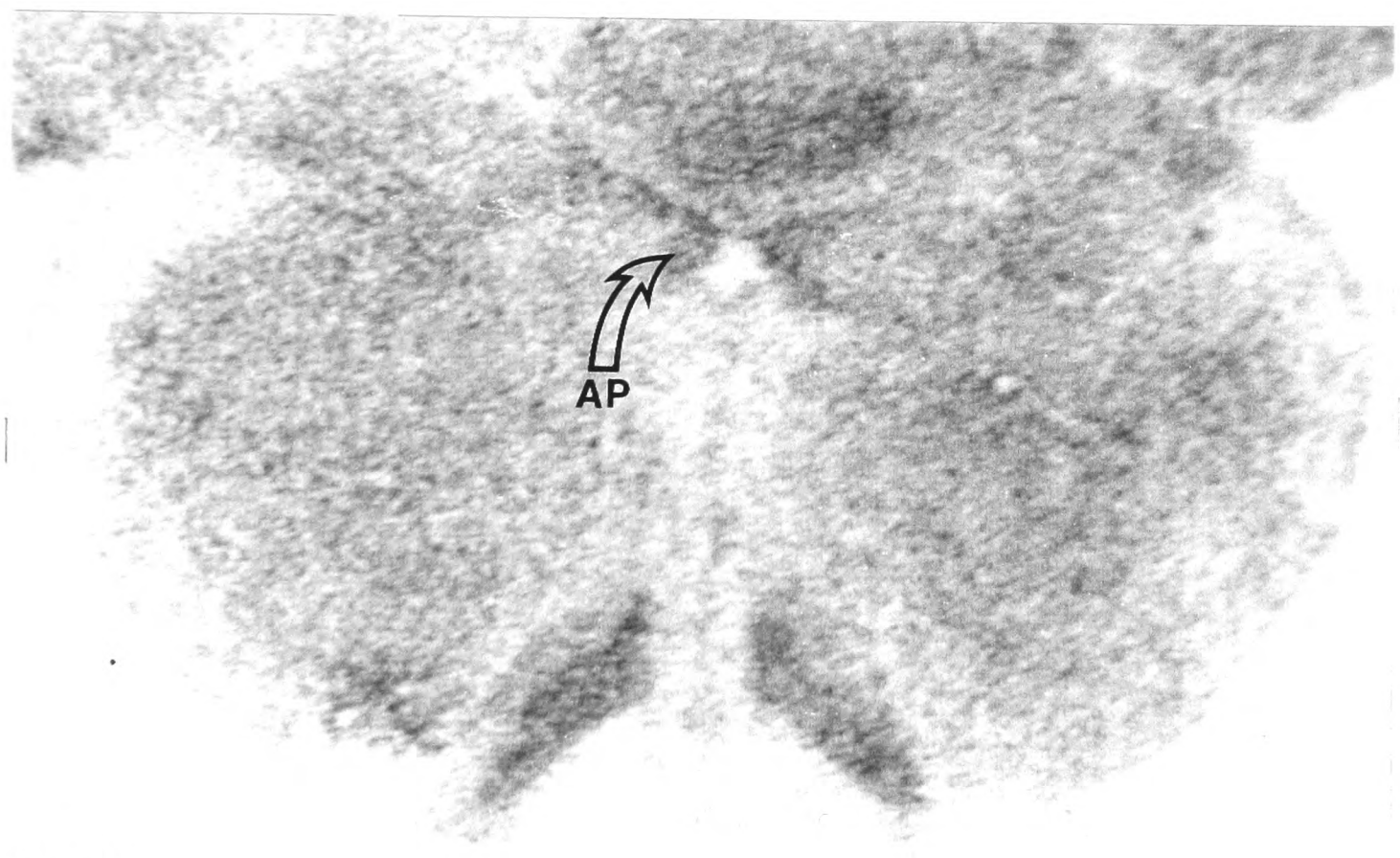


Mustine

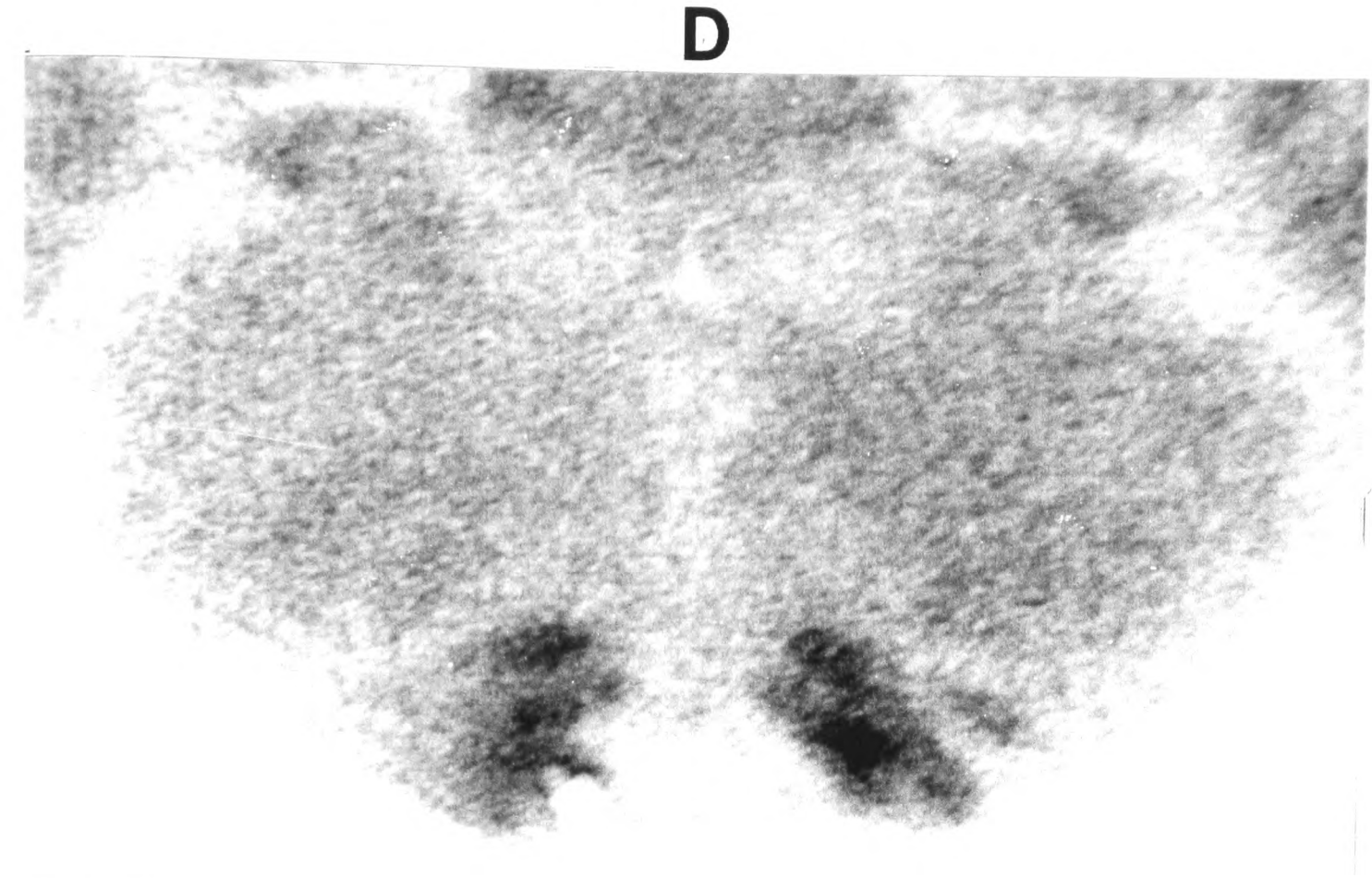
4.5.3 X-Rays

Irradiation with 800cGy of X-rays produced predictable retching and vomiting in four ferrets throughout the period of exposure to 2-DG and maintained the record of this form of stimulation as being the most reliable and reproducible for experimental purposes. Frank retching and vomiting began in all cases between 15 - 20 minutes after initiation of irradiation. An example of the autoradiograms produced from these groups of experimental animals are illustrated in Fig. 54.

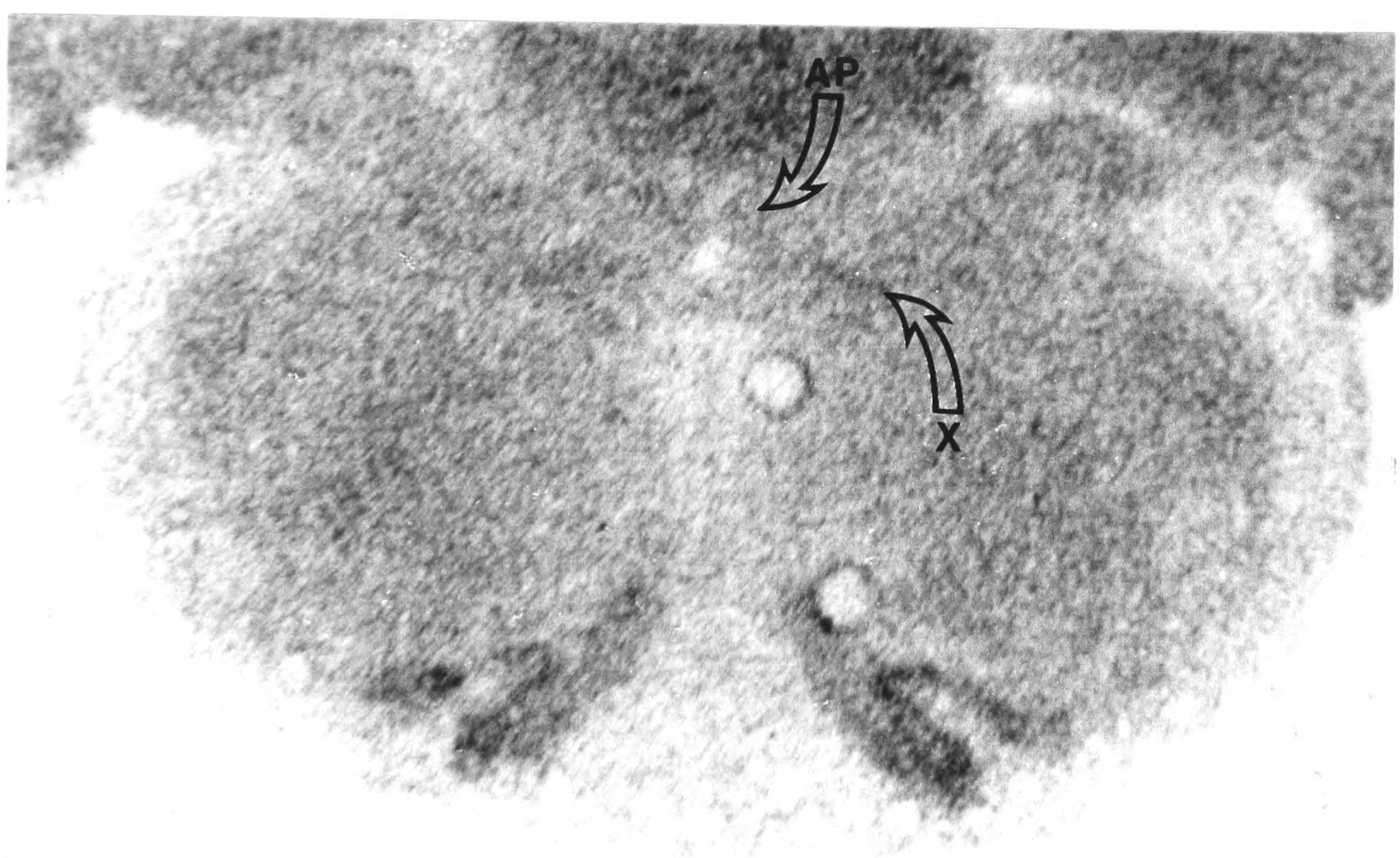
Relative metabolic activity in this experimental group showed significant changes from those in the control group in the A.P. and DMVN with rises in activity of these neuronal groups being calculated at 41% and 32% respectively. There was also a distinct trend towards an increase in activity in the NTS (20%) but this did not achieve a statistically significant level (see Table 14).

Figure 54 Autoradiogram following X-irradiation in the Conscious Ferret

^{14}C-2-DG autoradiograms of ferret brain stem transverse sections at the level of the area postrema in two conscious unrestrained ferrets. The upper picture is from a control animal. The lower picture is from an animal irradiated with 800cGy of hard X-rays (250Kv, 15mA, 0.5mm Cu + 1.0mm Al filtration, HVL-1.32mm Cu, mean dose rate 122 ± 13cGy,min^{-1}). Significant increases in relative metabolic activity can be seen in the AP and DMVN following a substantial emetic response in this group of animals. Key: AP = Area Postrema, DMVN = Dorsal Motor Nucleus of the Vagus-X



Control



X-Radiation

CHAPTER 5

DISCUSSION

"Get wisdom, get understanding : forget it not;
neither decline from the words of my mouth.
Forsake her not, and she shall preserve thee:
love her, and she shall keep thee.
Wisdom is the principal thing; therefore get wisdom:
and with all thy getting get understanding.
Exalt her, and she shall promote thee: she shall
bring thee honour, when thou dost embrace her."

Proverbs 4, vs 5 - 8

CHAPTER 5 DISCUSSION

5.1 INTRODUCTION

One of the major aims of this work was to examine the use of the ferret as a novel animal model for emesis research and to establish the range of emetic agents to which it would respond as a basis for detailed studies into the mechanism of action of radiation and cytotoxic drugs using a combination of nerve lesioning pharmacological challenges and 2-DG autoradiography.

This discussion is divided into several sections; first, the emetic responses of the ferret to the range of stimuli examined in this thesis are compared to available data from other experimental animals and man, thus allowing conclusions to be drawn about the suitability of this animal as a model forman. Secondly, the detailed mechanisms of action of emetics, primarily cytotoxics and radiation, are discussed in two sections, one dealing with the effect of nerve lesions and a second covering the effects of these stimuli on the relevant structures in the brain stem which have been reported to be involved in emesis. The latter component relies predominantly on the results of the application of the 2-DG technique. In addition, this section also assesses the applicability of 2-DG autoradiography as a new technique in the study of emetic pathways in the CNS. The discussion concludes with an overall assessment of the mechanism of radiation and cytotoxic drug-induced emesis in the ferret and other animals.

5.2 COMPARATIVE STUDIES OF EMETIC SENSITIVITY

5.2.1 Apomorphine

Apomorphine has been used for almost a 100 years as a convenient pharmacological tool to evoke emesis in man and experimental animals (Harnack, 1874). Apomorphine, whilst being structurally related to morphine (first synthesised in 1869) is a potent and relatively selective dopamine D_2 receptor agonist. Many studies have demonstrated that emesis evoked by apomorphine is produced by activation of the AP (e.g., Wang and Borison, 1952). The data on ferrets' reactions to apomorphine in comparison to other species will be reviewed, as many would regard a species that fails to vomit to apomorphine as an inappropriate model for man (Proctor et al., 1978). The majority of work identifying the range of species responding to apomorphine was undertaken by Wang, Borison and Hatcher and this aspect has been more recently reviewed by Wang in 1965 and Borison et al., in 1981.

Dogs, cats, pigeons and ferrets all respond to apomorphine. The insectivore Suncus murinus failed to respond to apomorphine over a dose range 0.1 - 100mgkg^{-1} s.c. (Ueno et al., 1987). The monkey is usually reported to be insensitive to apomorphine (Brizzee et al., 1955). In man, frank vomiting can be elicited and nausea is very common. As the species which failed to respond to apomorphine do respond to other emetic agents, these differences probably reflect

differences in the neuropharmacology of the emetic mechanism. The present experimental data show that the ferret clearly responds to apomorphine although this has been disputed in the literature (e.g. King, 1988; Tuor et al., 1988). The results of our studies are in line with those obtained in the other carnivores tested e.g. cat and dog.

It appears therefore that the response to apomorphine is relatively widespread throughout a variety of animal species and we might then speculate why some species, e.g. monkey and insectivores, fail to respond. As those animals respond to other emetic stimuli such as motion and chemicals acting via the AP, this implies a difference in the central neuropharmacology of emesis for different agents. Moreover we must be careful in this assessment to ensure that the dose range used in these studies was either sufficient to induce emesis or was low enough not to have induced the profoundly disturbed behaviour that can be induced by high doses of apomorphine and which of course could modify the animals' behaviour to such an extent that the emetic reflexes would have been interfered with (e.g. convulsions, stereotypy or sedation). This problem can best be addressed by examining the relative emetic sensitivities of a variety of species to apomorphine and, in addition, such an analysis will enable an assessment of precisely how sensitive the ferret is to apomorphine rather than merely noting its response or lack of it.

Examination of the dose responsiveness to apomorphine gives rise to the classification in Table 15. This shows that the cat, an animal often used for emesis research, is

relatively insensitive to apomorphine, requiring a dose of 24mgkg^{-1} s.c. to produce emesis (Borison, 1959). Interestingly, the cat also requires a dose of >4mgkg^{-1} given into the vertebral artery to induce emesis (Abrahamsson et al., 1973). The chicken also requires doses in the mg range to evoke emesis (Osuide and Adejoh, 1973 and Saxena et al., 1977). In the dog there are many studies in which it has been reported that "dogs are unusually sensitive to apomorphine-induced emesis" (Wang, 1980). Dose response data in the dog show that vomiting may be evoked by doses as low as 2.5μgkg^{-1} i.v. with the 100% response produced by doses within the range 15 - 40μgkg^{-1} depending upon which series is examined. The dose producing emesis when apomorphine is given s.c. is 30μgkg^{-1}. Comparing these data to the data in man we see that man appears to vomit to apomorphine in the range 20 - 50μgkg^{-1} i.v. and approximately 50 - 140μgkg^{-1} when given i.m. or s.c.. Whilst man clearly can vomit to apomorphine many authors comment that the response is variable, but all agree that profound nausea is in any case produced at very low doses (10μgkg^{-1} i.v.). It therefore appears that the dog and man have a similar sensitivity to apomorphine whereas the other species, ferret excepted, are either insensitive, or have a sensitivity approximately 100 times less.

TABLE 15

The Responsiveness and Emetic Sensitivity of a Variety of Species to Apomorphine Administered via Different Routes

	s.c.	i.v.	i.m.	p.o.
Man	50-150μg	20-30μg	70μg	-
Monkey	Unresponsive	Unresponsive	-	-
Dog	30μgkg^{-1}	2.5-15.0μgkg^{-1}	-	3-4mgkg^{-1}
Cat	25mgkg^{-1}	>4mgkg^{-1}	-	-
Ferret	25-50μgkg^{-1}	50-100μgkg^{-1}	-	-
Suncus m.	Unresponsive	-	-	-
Sheep	-	1mgkg^{-1}	-	Responds but data unreliable
Chicken	10mgkg^{-1}	>25μgkg^{-1}	-	-
Kite	-	-	3-4μgkg^{-1}	-

Footnote: Data drawn from multiple sources listed in reference section

The results reported here have demonstrated that the ferret responds to apomorphine administered by the i.v. and s.c. routes, the latter route being more reliable and sensitive. The s.c. dose range was 25 - 100μgkg^{-1} and 100μgkg^{-1} is the ED_{100}. Our investigations have shown a difference in the emetic response between the route of administration of the drug. For example a dose of 100μgkg^{-1} s.c. evoked retching and vomiting in 100% of animals tested whereas when given i.v. the same dose was less than 50% effective. This does not appear to be a simple matter of altered sensitivity by the i.v. route since the use of much higher doses (e.g. 500μgkg i.v.) was substantially less effective in producing emesis and in the single animal that responded to this dose the latency of 13.3min was greatly lengthened from the 4.8 $\pm$ 3.6min found in the 100μgkg^{-1} i.v. group. The reason for this discrepancy is not clear at present but as with other pharmacological agents this may depend upon functions of the rate of presentation of apomorphine to the A.P. (i.e. to its receptor site) and/or its relative agonist/antagonist activities at a given concentration (Goodman and Gilman, 1985). It is of interest that similar differences have been found for i.p. and s.c. doses in respect of apomorphine stereotypy in the rat (Melzacka et al., 1979).

It is also of interest that the only previous study of apomorphine in the ferret reported that emesis could not be evoked by doses less than 5000μgkg^{-1} s.c. and the authors of this report suggest there that there is a very steep dose-response curve in this species (Florczyk et al., 1982, see also Florczyk et al., 1981 and Gylys and Gidda, 1986).

This result is clearly at odds with the present study and also with a report published recently by Miner et al., (1987) demonstrating an emetic response in the ferret at 200μgkg^{-1} s.c.. At present we can offer no explanation for these discrepancies. However, it is worth noting that the aforementioned study by Florczyk and co-workers was undertaken on castrated male ferrets and three more recent studies also carried out on castrated males have similarly failed to show apomorphine induced vomiting in the same dose range in the ferret although retching was observed (Tuor et al., 1988, King, 1988, Gylys and Gidda, 1986). Whilst it is tempting to suggest cause and effect here, other variables may be involved, e.g. all these studies were carried out in N. America, and moreover, our own studies show that apomorphine-induced vomiting is present in females throughout their reproductive cycle. In Canada in particular the present day supply of experimental ferrets all derive from a small number imported some years ago and therefore genetic factors maybe playing a part in their lack of response.

Somewhat surprisingly, in view of its widespread use in vomiting research the monkey has been found to be relatively insensitive to apomorphine with no vomiting produced in the dose range 5 - 25μgkg^{-1}. This leaves the possibility either that dopamine receptors are absent from the A.P. or perhaps that the dose range used to challenge the monkey lies well outside its true sensitivity and that the known behavioural effects of apomorphine interfere at these dose levels. This problem of dopamine D_2 receptor induced emesis may possibly be resolved by using more specific and potent D_2 receptor agonists, e.g., tetralin. Indeed, there is one report of the

use of tetralin in the common marmoset showing that 2.5μgkg^{-1} s.c. was the minimum dose to produce emesis in 100% of animals tested (Costal et al., 1987). Whilst this may support the above contention it may be that there is a difference in the sensitivity to apomorphine for different primates and such a difference has been reported for motion sickness susceptibility (Money, 1970).

5.2.2 Copper Sulphate and Sodium Chloride

The present results demonstrate that the ferret responds to intragastric $CuSO_4$ in the range of 20 - 150mg% (30ml p.o.) and in that respect they fall into line with those from previous experimental animal models (e.g. Wang and Borison, 1952; Kayashima and Hayama, 1976. Since it is assumed that the substance is not being absorbed when it produces its effect, the test substance was given as a fixed volume of varying concentration based on the premise that the mucosal receptors would be activated by a critical concentration presented at the mucosal surface. In keeping with this, it has been shown that the afferent discharge responses of the mucosal receptors appear to be concentration related (Clarke and Davison, 1978 and Iggo, 1957). A fixed volume of 30ml was chosen for the ferret based on the quantity of fluid a ferret will drink without encouragement at one sitting.

Comparing the responses of the ferret to other species, we find it responds to $CuSO_4$ in a similar range to that seen in the dog (i.e. 20 - 50 cf 40 - 160mg%) with a similar latency (9 - 15min). The insectivore Suncus murinus however (Ueno et al., 1987) does not respond at all, and we have found no data in the monkey. It is difficult to arrive at an

estimate of relative sensitivities because of the wide variety of expressions of dose that have been used. This makes it very difficult to calculate the real concentration administered into the stomach. Humans are sensitive to $CuSO_4$ which has an approximate ED_{100} of 400mg% in man and a latency of 2 - 5min at a concentration of approximately 10mg% a figure broadly comparable to the latency value in the dog and the ferret (Meester, 1980). In contrast, it appears that the cat is less sensitive (requiring 1000 - 2000mg%) by comparison with man, dog and ferret (Brizzee and Marshall, 1960; Beleslin and Strbac, 1987. Although man responds in a similar dose range as the ferret such levels of stimuli have caused great concern over toxic side-effects (Miser and Robertson, 1978).

Whilst seawater and saline have been used as emetics on an empirical basis until recent times (Decker, 1971), there is very little experimental or clinical data with which to compare the results of the present study. Exceptionally however we do know that 1M NaCl placed on the gastric mucosa of the anaesthetised ferret does cause retching (Andrews and Wood, 1988) and that when induction of saline emesis fails in man considerable morbidity and occasional mortality has resulted through the effect of excessive $[Na^+]$ load on the brain (Meester, 1980), a situation that arises in the vagotomised ferret so treated.

Our data have extended the range of emetic test stimuli in ferret by also looking at mannitol, choline chloride, KCl and glucose, but again, comparative data are not available.

5.2.3 Ipecacuanha

Whilst ipecacuanha has been used as a therapeutic drug in Europe since the 17th Century (Vale et al., 1986) the experimental literature in animal models is incomplete (Manno and Manno, 1977).

Recent clinical trials (Meester, 1980, Manoguerra and Krenzelok, 1978 and Neuvonen et al., 1983) using ipecacuanha syrup in man have shown that given the correct dose, usually with a draught of water varying from 50ml to 400ml, vomiting can be produced in approximately 96% of individuals. The data are not absolutely precise or complete but representative figures indicate a vomiting latency of 5 - 40min after ingestion. The emetic response typically involves three productive bouts in the first hour and can continue spasmodically for up to 4 hours. Doses of ipecacuanha syrup range from 15 - 40ml in the adult the most common being 30ml.

von Podwyssotzki (1879) gave the first thorough description of the pharmacology and physical chemistry of emetine, one of the two principal ipecac' alkaloids, in 1875. Thereafter experimental work on ipecac' most often used emetine to represent its main pharmacological constituent although as late as 1930 Koppanyi was reporting on work in the dog with oral ipecac' syrup. More modern data on the effectof ipecac' syrup per se in a variety of animals are therefore sparse. although Louwin's review (1902-3) confirms its activity in a variety of animals including cat and dog. The only recent data on the dog showed ipacac' to cause vomiting in 99% of animals tested with 0.35mg kg^{-1} p.o. (Weaver and Griffith,1969).

We found the ferret to be similarly sensitive. Thus 30ml of ipecac' syrup was found to cause vomiting with a mean latency of 8.4 ± 0.2min, continuing for over 120min and being fatal in some animals. A body weight adjusted dose of ipacac' presented in 30mls of water caused vomiting in approximately 50% of animals tested with a latency of 24.2 ± 6.5min.

Louwin (1902-1903) reviewed the literature on ipecac' and its alkaloids and reported emetine itself to be emetic in the cat, dog and pigeon with Thumas (1891) inducing vomiting in the dog by application of small amounts to the medulla oblongata. Eggleston and Hatcher (1915) demonstrated emetine's emetic efficacy by the i.v. and oral routes in the dog and compared this with the efficacy of cephaeline (the other major ipecac' alkaloid) and that of a mixture of ipacac' alkaloids. The minimum effective emetic dose of emetine was found to be approximately the same for the i.v. and p.o. routes ($3mgkg^{-1}$) but with the i.v. route producing an effect in approximately 15min, as compared with about 45min for the oral route.

In the present studies on the ferret emetine caused vomiting by the oral route when presented as 30ml of a 150mg% ($50mg\ kg^{-1}$) solution made up in water or the syrup base. 150mg% was chosen to represent the concentration of emetine normally encountered in ipecac' syrup. It was also found that emetine administered i.p. ($20mgkg^{-1}$) caused vomiting in all animals. $20mgkg^{-1}$ was the single dose selected on the basis of the dose of cycloheximide used in these experiments; this being twice the dose that causes 100% inhibition of brain protein synthesis in rats (Grahame-Smith, 1972).

Interestingly, there appeared to be little difference in latency between the i.p. and oral routes (when presented in water) although of course the total dose administered was quite different in both cases. However, the latency of approximately 45min to 3mgkg^{-1} emetine (p.o.) in the dog quoted by Eggleston and Hatcher (1915) is remarkably similar to the latency of 40min obtained here in the ferret in response to a dose equivalent to 45mgkg^{-1} p.o..

5.2.4 Cisplatin

Pre-clinical toxicological evaluation of i.v. cisplatin in dogs and monkeys (Schaeppi et al., 1973) showed that dogs vomited to doses in the range 0.75mg - 10mg kg^{-1} i.v. with maximum effect at 3mgkg^{-1}, and monkeys at a dose of 1.25mgkg^{-1}. Subsequently it was shown that 3mgkg^{-1} i.v. cisplatin produced vomiting in 100% of dogs with a latency of 90 - 110min (Gylys et al., 1979) and this has been confirmed in more recent studies, e.g. Schurig et al., 1982 and Akwari et al., 1985.

Cisplatin is also active in the cat (London et al., 1979; McCarthy and Borison, 1980; McCarthy and Borison, 1981; McCarthy and Borison, 1984). These data show the minimal effective dose of cisplatin in the cat to be 5mgkg^{-1} i.v. with maximal effect at 7.5mgkg^{-1} i.v. and a paradoxical decline in emesis when the dose rises to 10mgkg^{-1} i.v.. At the ED_{100} the latency is approximately 70 minutes which accords well with the data in the dog, stretching out to about 4 hours at the lower doses and 2 hours at the 10mgkg^{-1} dose.

In man nausea and vomiting have been reported in the vast majority of patients receiving cisplatin (Kahn et al., 1978).

Even single doses as small as 4.0mg per person i.v. (0.08mgkg^{-1} or 1.5mg m^{-2}) can cause nausea (Higby et al., 1973) and vomiting has been consistently observed in patients receiving 50mg m^{-2} i.v. (approximately 1.3mgkg^{-1}) and above (i.e. up to 120mg m^{-2} or 3mgkg^{-1} i.v.) where vomiting occurs in 100% of patients (von Hoff et al., 1979).

Latency in man at the usual clinical doses is approximately 60 - 120min but delay in onset may occur for up to 6 hours (Borison and McCarthy, 1983) and indeed true delayed emesis (after 24hr) has been observed in 74% of patients on high dose cisplatin (Kris et al., 1985) with the incidence of vomiting being greatest in the 48 - 72hr period following therapy. By comparison of course the greatest proportion of vomiting occurs during the first 24hrs.

The emetic sensitivity of the ferret to cisplatin was first reported by Florczyk et al., (1981) and has subsequently been confirmed by a number of studies including the present one. Whilst all authors agree that the ferret is responsive to cisplatin given i.v. and that a dose higher than that for the monkey and dog is required there appears to be a discrepancy in the literature regarding the value of the ED_{100}. In their original studies Schurig et al., reported that all (4/4) animals responded with a dose of 6mgkg^{-1} i.v. and with increasing dose (8, 10mgkg^{-1} i.v.) the incidence remained the same but the latency decreased and the number of emetic episodes increased. Subsequent studies by Miner and Sanger (1986) and Miner, Sanger and Turner (1987) reported that doses of 7.1 and 10mgkg^{-1} i.v. were totally effective and commented that the discrepancy in the doses was due to a sensitivity

difference between two groups of ferrets. This study used a larger group of animals (10) for the 7.1mgkg^{-1} dose than that used by Schurig et al., 1982. Thus it appears unlikely that the results with these lower doses of cisplatin are fortuitous.

The present study was unable to evoke vomiting in animals at a dose below 10mgkg^{-1} and hence it appears that there may really be differences in the sensitivity of various groups of ferrets to cisplatin; this has also been observed by others (Andrews, personal communication). It is unclear whether this is due to differences in strain, sex, reproductive status or other factors but it is worth noting that the majority of studies have used male fitch ferrets in the 1 - 2kg range. In the present study we were unable to discriminate between the responses of males and females and fitch and albino animals. It is also noteworthy that different preparations of cisplatin have been used by the different groups (e.g. Neoplatin, Platinol, Sigma Chemical).

Whilst a discussion of the precise ED_{100} for cisplatin in the ferret may at first sight appear trivial it is important because the ferret is now widely used as a screen for novel anti-emetic agents particularly for ones effective against cisplatin. Therefore in order to avoid erroneous results it is crucial that each research group establishes the sensitivity of ferrets from its own source, to cisplatin; and that after a control group is established anti-emetic trials on subsequent groups of animals include some control animals (e.g. Miner and Sanger, 1986). This may be particularly important when conducting studies during the breeding season.

TABLE 16

The Emetic Sensitivity of Various Species to Cisplatin

	Emetic ED_{100} ($mgkg^{-1}$ i.v.)	Vomiting Latency (min)
Man	2.50	60-360
Monkey	1.25	-
Dog	3.00	90-120
Cat	7.50	70
Ferret	6.00-10.00	75

Whatever the final value of the ED_{100} is for the ferret it is clearly in the same range as that for the cat (the only point of similarity) but above that for the dog, monkey and man (see Table 16). The reason is unclear but may reflect differences in the neurohumoral pathways for detection of cisplatin used by cat and ferret on one hand and dog, monkey and man on the other.

5.2.5 Mustine

In man the current total therapeutic is usually $400\mu gkg^{-1}$ i.v. in single or divided doses and concomitant with this occur some of the highest recorded incidences of nausea and vomiting. Mustine causes vomiting in greater than 90% of individuals with a latency of between 30 and 120min (Borison and McCarthy, 1983). With a minimum latency to vomiting of 30min mustine stands out as probably the most rapidly acting emetic within the category of clinical cytotoxic agents.

Houck (1947) was the first to note the emetic sensitivity of the dog to mustine and Hunt and Phillips (1949) the sensitivity of the cat. It was not until 1958 that Borison et al. carried out the first formal study of emesis caused by mustine in the cat and the dog. The minimum effective dose used in this study was $500\mu g^{-1}$ in the dog also the ED_{100} producing a latency of approximately 122min (range 105 - 149min). At a dose of $5.0mgkg^{-1}$ the latency dropped to approximately 15min (8 - 21min). The cat was not so sensitive with a minimum effective dose of $1.5mgkg^{-1}$ i.v. (latency 90min). The ED_{100} was found to be $5.0mgkg^{-1}$ i.v. (mean latency 65min, range 15 - 150min). One other study on mustard in cats confirmed the 100% emetic efficacy of i.v.

mustard in the cat at 5.0mgkg^{-1} but the mean latency for this group was about 16 minutes. No data on mustine induced emesis in the monkey has been found and the only other data is on i.c.v injections in the dog (Papp et al., 1966) where 500μg (ED_{50} x 9) of dilute mustine delivered into the fourth ventricle caused vomiting in all animals with a latency ranging from 1 - 4 minutes.

The only other available data relevant to the emetic sensitivity of mustine in animal models come from work on phosphoramide mustard and cyclophosphamide. Phosphoramide mustard is a metabolite of cyclophosphamide. Nausea and vomiting occur very frequently (30 - 90%) after high dose (50mgkg^{-1} i.v.) cyclophosphamide (Harris and Cantwell, 1986). Latency to vomiting may be up to 2 - 12hours after administration (Fetting et al., 1982). I.v. cyclophosphamide caused unpredictable vomiting in the cat with a minimum effective dose being 150mgkg^{-1} and no ED_{100} was established. Over a dose range 150 - 480mgkg^{-1} the mean latency was 54 $\pm$ S.E. of 9min (Fetting et al., 1982). Phosphoramide mustard was effective at 100mgkg^{-1} and an ED_{100} was found to be 200mgkg^{-1} (latency 127 $\pm$ S.E. of 6mins). Neither agent produced predictable emesis by the i.c.v. route of administration but some vomiting was noted to 300mgkg^{-1} cyclophosphamide and 200mgkg^{-1} phosphoramide mustard.

In the ferret, only one other study reports on this class of cytotoxics (Hawthorn et al., 1988). Their study demonstrated that cyclophosphamide evokes a characteristic emetic response in the ferret which is dose related as has been

noted in man. The ED_{50} was evaluated at $100mg^{-1}kg$ i.p. and the ED_{100} at $200mgkg^{-1}$ i.p.. At the ED_{100} the latency was 21.6 ± S.E. of 8.0min. I.v. dosing was also carried out, with 200mgkg causing vomiting at a latency of 19.1 ± S.E. of 3.4min.

In the present study the starting point for dosing was $400\mu gkg^{-1}$ of mustine i.v., a dose representing the usual total human therapeutic dose. This produced behaviour suggestive of nausea which we have termed prodromata of vomiting but no frank emesis. At $1.2mgkg^{-1}$ i.v. vomiting occurred in all animals tested with a latency of 28.1 ± 7.2 minutes.

Generally, the sensitivity of the ferret to mustard is in line with the dog and man, all three being much more sensitive than cat. Interestingly, latencies for all the animal groups are similar when an ED_{100} is administered. The sensitivities of a variety of animals and man are compared in Table 17.

5.2.6 Emetine

For comparison, studies in the literature of the emetic potential of emetine are discussed above under Ipecacuanha and related to the data in this study which showed vomiting in all ferrets challenged with $20mgkg^{-1}$ i.p. of the drug (latency 35.9 ± 7.2min). There is little data from modern times in vomiting animal models. In 1961 Bhargava et al., showed vomiting to emetine in the dog by the i.c.v. ($0.2mgkg^{-1}$) and i.v. routes ($3mgkg^{-1}$). The average latency to the i.v. route was 18 minutes. This appears to be the last work studying emetine in animal models until the present study took place. Generally speaking we can say that man, dog, cat and

TABLE 17

The Emetic Sensitivity of Various Species to Mustine

	Emetic ED_{100} ($mgkg^{-1}$ i.v.)	Vomiting Latency (min)
Man	0.4	30-120
Monkey	-	-
Dog	0.5	8-21 (Av 15)
Cat	5.0	15-150
Ferret	1.2	28.1 $\pm$ 7.3

pigeon vomit to emetine by the i.v., i.p. or p.o. routes but there are too few data for comparative susceptibility to be estimated as has been done for instance with apomorphine.

5.2.7 Diacetoxyscirpinol

Vomiting has been found in a number of responsive animal models but exact details of susceptibility are lacking. Thus emesis has been noted in response to DAS administration in pigs (Vesonder et al., 1973), pigeons (Szathmary, 1983), dogs and monkeys (quoted in Goodwin et al., 1978) and ducks (Ueno, 1983). In the duck the minimum effective emetic dose was found to be $0.2mgkg^{-1}$ s.c. and in the dog $0.62mgm^{-2}$ (equivalent to approximately $0.04mgkg^{-1}$). In man the threshold for vomiting appears to be $2.4mgm^{-2}$ or approximately $0.06mgkg^{-1}$ and the ED_{100} approximately $3mgm^{-2}$ or $0.08mgkg^{-1}$ (Goodwin et al., 1978; Murphy et al., 1978).

In the ferret $1.5mgkg^{-1}$ of DAS i.p. produced vomiting in all animals tested with a latency of about 22 minutes. Interestingly at this dose there were neurological manifestations of toxicity with marked ataxia; also we were able to document the occurrence of hot erythematous skin following dosing, a sign displayed by human cancer patients treated with anguidine. The dose used to challenge the ferret in these experiments was based in the first instance on work done on the dog on a related trichothecene called Fusarenon-X (also related to T_2) (Matsuoka et al., in 1979). This is the only paper looking at the mechanism of action of trichothecenes as emetics and used $0.3mgkg^{-1}$ Fusarenon-X injected i.v. in

dogs. Vomiting was induced in 5 - 15 minutes after injection. Taking into account the comparative emetic properties of DAS and Fusarenon-X, the difference in route and the often greater sensitivity of the dog to such toxins a five fold increase in dose was used since no attempt was being made to construct a dose response curve.

5.2.8 Cycloheximide

Cycloheximide caused vomiting in man 5 - 60min following i.v. or p.o. administration at a dose of approximately 2mgkg^{-1} in about 33% of people challenged. Daily doses of 6 - 8mgkg^{-1} resulted in continuous nausea. By comparison the antipyretic effect was delayed for up to 90 - 180 minutes but it could not be concluded beyond all doubt that this effect was due to inhibition of protein synthesis (Young and Dowling, 1975). Cycloheximide has also been shown to have some anti-tumour activity (Lucas et al., 1953 and Reilly et al., 1953 cited in Grollman, 1966). No data were found for dog, cat or monkey for comparison.

For the ferret a dose of 20mgkg^{-1} i.p. was chosen, based on the dose that causes 100% inhibition of brain protein synthesis in the rat (Grahame-Smith, 1972). As with human use, cycloheximide caused profound nausea (as judged by presence of prodromata) in the ferret and was also marked by persistent diarrhoea. Cycloheximide 20mgkg^{-1} i.p. produced vomiting in all animals with a latency of about 17 minutes and a duration of at least 2 hours.

5.2.9 X-radiation

Man, monkey and dog display broadly similar response patterns to X-radiation but the cat, although a responder does so at levels of irradiation an order of magnitude greater than the others.

The ferret is sensitive to X-radiation with a vomiting ED_{100} of approximately 125cGy. Thus it is somewhat more sensitive than man (≏800cGy) or dog (≏600cGy) or monkey (≏800cGy). Nevertheless it is demonstrably closer to these models than the cat (≏5500cGy). It should be pointed out that an ED_{100} for vomiting in man and monkey has not been so far demonstrated. The calculated ED_{50} for the ferret is approximately 95cGy. Recent data indicate that man's vomiting ED_{50} may well be as low as 180cGy (Young, personal communication) which would suggest the ferret to be a very useful animal model for further studies.

More recent data in the ferret have confirmed our results. Most of these studies have used gamma photons from ^{137}Cs or ^{60}Co radiation sources. Thus Gylys and Gidda (1986) reported emetic responses in the range 100 - 600cGy with a latency of approximately 30min. The response peaked at around 60min post-irradiation. The 'plateau' effect of the response also occurred just as we originally found i.e. there was a level of radiation at around 200 - 400cGy above which no increment of radiation causes a decrease in latency or indeed much increase in emetic response. King's work in the ferret has also confirmed our figures for ED_{100} and ED_{50} (King, 1988). However no study has yet been done to match the spectrum of

doses or the number of animals used in our study and we await other confirmatory evidence from larger studies using X-ray sources. Even very recent work by Tuor and colleagues (Tuor et al., 1988) involved only 3 animals at 200 cGy and 2 at 400cGy using a ^{137}Cs source. Vomiting was noted in all animals. Latency at both radiation doses compared favourably with our results i.e. around 12min at 400cGy and 20min at 200cGy.

Interestingly, at the vomiting ED_{50} the latency to first emesis for the ferret of approximately 30min is remarkably close to the comparable figure for man (≏30min), monkey (≏40min), dog (≏30min) and cat (≏30min). It is important to note here that inter-species comparisons such as these, are fraught with difficulties because of the different methodologies employed, especially in timing of responses. Also the amount of detail included in these papers is often quite sparse. These difficulties are compounded by the wide variation in individual responses to radiation exposure evident in higher animals.

A summary of the basic radiation-induced emesis data in man, dog, monkey, cat and ferret is given in Table 18. The ferret is clearly more sensitive to radiation than the other animal models or man. The ferret's response is, however generally in line with the dog and man, the two standards against which we wished to compare our results. The results are not comparable with the cat which is relatively radioresistant. The ferret ED_{50} in particular compares well

TABLE 18

Tne Emetic Sensitivity of Various Species to X-radiation

	Vomiting ED_{50} (cGy)	Vomiting ED_{100} (cGy)
Man	139-230	800 [ED_{80}]
Monkey	450	800 [ED_{80}]
Dog	230	600
Cat	1500	4500
Ferret	95	125

with man and the dog. This is probably a more useful figure to quote for comparison purposes because of the difficulties in establishing a true ED_{100} for man. Although the ED_{100} for the ferret appears much lower than that for the man and the dog it does mean that at relatively low doses e.g. 200cGy we can be sure of a 100% response in control groups for experiments where the same animal cannot be used as its own control because of the lethal effects of radiation. It also means that we can study the radiation-induced vomiting response without the danger of using doses that affect the CNS and which may produce the equivalent of transient functional incapacitation.

The ferrets in our study displayed clear changes in behaviour as a response to X-irradiation. This behaviour pattern which we have termed the prodromata of vomiting comprises a number of individual components which also occur in response to the whole variety of emetic stimuli that have been summarised in this study. Even at the lowest dose of radiation used (50cGy), where no frank vomiting was observed, there was still a recordable incidence of prodromata. If such behaviour is analagous to nausea in man then even at such very low doses of radiation the ferret is affected to some extent, and vomiting does not supervene without prior awareness of "illness". Importantly, even at this dose mild diarrhoea was present in most animals in this group. Diarrhoea was in fact universally present throughout the dose range tested. With increasing dose the diarrhoea became mucoid, watery and eventually bloody with intensity being greatest at about 60min

after irradiation. Often animals replicated the behaviour of defaecation at the higher doses without actually passing any faeces.

In man Westbrook et al., (1987) and Danjoux et al., (1979) noted the occurrence of diarrhoea at this early post-irradiation stage as well as the more commonly recorded incidence of late onset diarrhoea i.e. up to 72hr post-irradiation. Both these groups note that the early diarrhoea appears self-limiting and rarely requires treatment and we found in the ferret that even the profound diarrhoea elicited at 800cGy stopped after 5hr and the animals showed a remarkable degree of recovery. Late onset diarrhoea has been recorded in the dog (Gralla et al., 1979) and monkey (Eldred and Trowbridge, 1954 and Henschke and Morton, 1957) but the literature reveals very little of the early reactions in animal models other than the basic information on vomiting. Henschke and Morton mention that early post-irradiation diarrhoea occurs also in the monkey, but no data for comparison with the ferret are available for the cat or dog.

In general the literature does not record well the behavioural reactions of the various standard animal models of vomiting to radiation and therefore it is difficult to make comparisons with the ferret. However, our data should form the basis for studies to determine the nature of nausea, rather than vomiting, and the effectiveness of various anti-emetic regimes against nausea. Costall et al., (1987) have published a possible quantitative methodology with reference to another animal model of nausea, the monkey.

Better documented in the animal literature are the behavioural changes and effects of high radiation doses. We were able to observe evidence of this transient functional incapacitation (TFI) in the ferret at doses above 800cGy. Profound mucoid diarrhoea was accompanied, at doses up to the maximum of 1600cGy by lethargy, sedation and ataxia. These high doses coincide with a plateau of vomiting latency and a decline in the emetic response. Two hours after irradiation peripheral vasconstriction was evident, the animals were difficult to rouse and had showed little improvement even after 6hr. This pattern is similar to that recorded in the monkey (Middleton and Young, 1975) although radiation doses used in our experiments did not reach a level causing such CNS depression that emesis was actually prevented. This implies that the reaction we elicited was at the milder end of the spectrum of transient functional incapacitation in the ferret. Mattsson and Yochmowitz (1980) pointed out that at 300cGy whole body ^{60}Co radiation, the human reaction is very similar to the dog in that vomiting alternates with sleep, and nausea is always present. Such subdued behaviour would last for 4 - 6hr before improvement was noted and normal activity was resumed. This general pattern is very much like that observed in the ferret at this dose and was distinguishable from the previously described symptoms of minor TFI.

5.2.10 Conclusions

We have assessed the sensitivity of the ferret to various emetic stimuli and compared the response to other animal models

TABLE 19

The Rank Order Emetic Sensitivity of Various Species to Different Emetic Stimuli

	Radiation	Cisplatin	Mustine	Copper Sulphate	Apomorphine
Most Responsive	Ferret	Monkey	Man	Man	Man
	Man	Man	Dog	Ferret	Dog
	Dog	Dog	Ferret	Dog	Ferret
	Monkey	Cat	Cat	Cat	Cat
Least Responsive	Cat	Ferret	-	-	Monkey (unresponsive)

of vomiting and to man. For the four most commonly used animals and man the emetic potency to the five most studied stimuli is summarised in rank order in Table 19. This table illustrates three points. First, the emetic sensitivity to one stimulus does not predict the sensivitity of the same species to another stimulus; for example the monkey is most sensitive to cisplatin but is unresponsive to apomorphine. Second, the cat is, in general, the least sensitive to each of the stimuli studied. Third, the ferret, with the exception of its response to cisplatin, occupies a position closer to that of man and dog than any other animal model. Presumably these differences reflect the subtly different underlying mechanisms by which each agent causes emesis.

5.3 MECHANISMS OF ACTION OF APOMORPHINE AND INTRAGASTRIC EMETICS IN THE FERRET

5.3.1 Apomorphine

The present study has demonstrated that abdominal visceral nerve lesions do not influence the emetic response to apomorphine, showing that it is unlikely that the abdomen is a major site of action for apomorphine-induced emesis. By the same token the gastric relaxation produced by apomorphine and mediated by the vagal input to the NANC neurones (Abrahamsson, 1973) is not the prime cause of the emesis produced by this drug. These observations confirm those reported in the dog (Borison and Wang, 1953). The present study has not identified whether apomorphine acts at the AP in the ferret but other workers (Andrews and Hawthorn, personal

communication) have demonstrated that lesioning of the AP in the ferret renders animals refractory to apomorphine as has been shown in other species e.g. cat and dog.

Two outstanding questions remain. First, is the entire AP required for apomorphine-induced emesis or is the apomorphine sensitive area confined to a small discrete portion of the AP (Borison and Brizzee, 1951 and Brizzee and Borison, 1952). This question requires resolution because a refractory response to apomorphine has been universally employed, as the sole criterion for a functionally completely lesion of the AP. This test has been most often employed in studies in which the site of action of a novel emetic agent (e.g. cisplatin) has been under investigation. If it transpires that the apomorphine sensitive area of the CTZ is distinct from the general chemosensory area of the CTZ then erroneous conclusions may have been made about the central site of action of a number of agents. The second question is how apomorphine influences the AP. Apomorphine is a dopamine receptor agonist and our own study with domperidone and that of Miner and Sanger (1987) using metoclopramide have shown that both of these agents can abolish the apomorphine response thus suggesting an involvement of dopamine receptors. Interestingly $5HT_3$ receptor antagonists were without effect on the response to apomorphine.

One of the current concepts of the function of the AP is that it has chemodetector cells which are presumed to respond to 'toxins' and release a transmitter to fire the neuronal

outputs of the AP to elicit vomiting. Whilst this mechanism may be involved in the response to apomorphine it is more likely that apomorphine is acting directly on neurones within the AP as dopaminergic receptors (Domperidone binding sites) have been shown to be present in the AP (Schwartz et al., 1986) and both dopamine and apomorphine activate AP neurones (Carpenter, 1983). However dopamine -hydroxylase has also been found in the AP which could suggest a role for noradrenaline rather than dopamine (Leslie, 1985).

Further support for an action of apomorphine on the AP comes from the present study which demonstrated an increase in 2-DG uptake by the AP following emetic doses of apomorphine in the ferret.

It would appear then, that dopamine could be neurotransmitter in the AP but the observation that relatively potent dopamine antagonists e.g. domperidone, do not block vomiting by non-dopaminergic emetic agents which are however thought to act at the AP implies that it does not have a role to play in this area, as a final common transmitter for vomiting. More likely dopamine receptors are one class of many present on the small neurones of the AP.

The involvement (if any) of such dopaminergic receptors in radiation and cytotoxic induced emesis is unclear. However it should be borne in mind that dopamine is found in the CSF and platelets, from which it can be released, and thus agents which alter dopamine levels in these areas could possibly evoke emesis in a manner analagous to apomorphine.

One anomaly remains with apomorphine-induced emesis in that the shortest reported latency (Miner and Sanger, 1987) to vomiting in the ferret is about 3min (via the s.c. route). This may imply that either the concentration of apomorphine needs to build up in the AP before a critical threshold is reached or that a certain period of activation of the AP neurons is required before an emetic response is generated (This could possibly serve as a mechanism to prevent "accidental" activation of the emetic reflex in response to brief surges in plasma or CSF dopamine levels or indeed of other transmitters). Interestingly a similar situation in respect of the appearance of apomorphine sterotypy elicited in the rat after i.p. v.s. s.c. dosing have been found (Melzacka et al., 1979).

One of the major problems in assessing the mechanisms of action of anti-emetics is the lack of mechanistic studies in man. A unique example of such a study is that by Lindstrom and Brizzee who ablated the AP in man to relieve intractable vomiting secondary to space-occupying lesions of the CNS. Subsequent to surgery these patients were demonstrated to be refractory to apomorphine given s.c. (Lindstrom and Brizzee, 1962). This remains the only demonstration in man of the site of action of a centrally acting emetic.

5.3.2 Intragastric Emetics

Emesis can be induced in the ferret by intragastric administration of sodium chloride, glucoseand mannitol. The most likely explanation for this response is that it is mediated via an osmotic action on the gut; this is supported

by the observation that equi-osmotic levels of each agent are required to induce vomiting. In addition to the above stimuli, the much-used experimental intragastric stimulus, copper sulphate also evokes vomiting in the ferret.

5.3.2.1 Pathways by which intragastric stimuli evoke emesis

In the present context we are dealing with the emetic response to intragastric stimuli evoked with a relatively short latency (<15min) and the mechanisms addressed relate to this phase but do not exclude aditional ones which may be involved if these agents enter the circulation and act on the CTZ.

The results from the present study and the experiments of Wang and Borison previously alluded to (e.g. Wang and Borison, 1951 and 1952) using copper sulphate clearly implicate the abdominal vagus, with the greater splanchnic nerves having a minor influence, unless sectioned in addition to the vagus. The role of the vagus is dramatically illustrated by the observation that animals with a previous abdominal vagotomy die of sodium poisoning following sodium chloride ingestion because of their failure to vomit. The involvement of a peripheral site in vomiting produced by intragastrically administered agents is supported by Wang and Borison's observations the ablation of the AP fails to abolish the emetic response to intragastric copper sulphate in the dog; a result that was duplicated recently in the cat by Beleslin and Strbac (1987). This observation is particularly interesting because it implies that intragastric stimuli evoke emesis by vagal afferent activation of the VC rather than by prior activation of the AP.

However this does raise the question of the exact function of the abdominal vagal afferents, activation of which we already have demonstrated increases ^{3}H-2-DG uptake in the ferret AP.

Examination of the temporal course of the effects of visceral nerve lesions on the emetic response to intragastric stimuli revealed that the latency was not static but varied with the delay in testing after the lesion. Before discussing these changes further it is necessary to examine what is known of the detail of the mechanisms by which intragastric stimuli actually cause vomiting.

5.3.2.2 Activation of Emesis via Abdominal Visceral Afferents

The abdominal viscera are supplied with afferent fibres from both the vagus and splanchnic nerves, with the splanchnic nerve primarily supplying the upper portion of the gut. Because the major influence on the emetic response to intragastric stimuli was produced by vagotomy in our studies (and others) this review focuses on the role of the vagus although the splanchnic nerves will be discussed in passing. In fact the abdominal vagus is essentially an afferent nerve with about 80 - 90% of the axons being afferents (Andrews 1986) in most species including the ferret (Asala, 1984; Asala and Bower, 1984). These afferent fibres are almost exclusively unmyelinated. Whilst it is known that retching and vomiting in the ferret can be induced by activation of abdominal vagal afferents using electrical stimulation (Andrews et al., 1985) what we seek is evidence that the emetic stimuli used can activate these vagal afferents.

Two main types of information are signalled in vagal afferents from the gut to the CNS, mechanical and chemical, and these will be considered separately.

The gut mechanoreceptors signal information regarding the degree of distension of various gut regions and the level of contractile activity. Whilst overdistension, particularly in the small intestine is a potent emetic stimulus it is unlikely to be involved in the present study. In fact even rapid injection (<5sec) of 50ml of 154mM sodium chloride in the ferret failed to induce emesis. The main reason for this is that the stomach has a considerable ability to relax to accommodate increasing volumes. Rapid delivery of gastric contents into the duodenum could evoke emesis but this is unlikely in the present study as, if the orogastric injection had actually distended the intestine, it is likely that emesis would have occurred during the injection rather than several minutes later. Emesis is associated with abnormal motor patterns in the gut (Akwari, 1983 and Akwari et al., 1985) and this type of effect cannot be excluded as a cause of emesis in the present study but it is still necessary to explain how these motor changes are evoked. There is some evidence that activation of vagal afferent chemoreceptors (see below) can evoke changes in gut motility by a vago-vagal reflex (Andrews and Wood, 1988) and that this motility change could then be detected by the mechanoreceptive afferents in order to evoke emesis.

Whilst an involvement of mechanoreceptors cannot be excluded the weight of evidence is against this. The most

likely explanation is an involvement of the gut mucosal chemoreceptors. Thus vagal afferents have receptors which are located in the mucosal layer of the gut and signal information about the chemical nature of the luminal contents. Of particular relevance to the present study are the observations that many act as osmoreceptors (Andrews, 1986) and thus are ideally suited for detecting the hypertonic stimuli used here such as sodium chloride, sucrose, glucose and mannitol. The case for an involvement of osmoreceptors is strengthened by the observation that the emetic potential of one agent could be predicted from its osmotic pressure. The gut also possesses carbohydrate receptors and these too may possibly be involved. In the distribution of the hepatic portal vein vagal receptors sensitive to sodium ions have been described and again involvement of these cannot be excluded (Andrews and Hawthorn. 1988).

The above receptor types could account for emesis induced by a number of the emetic stimuli used in the present study but it is difficult to envisage how they could be involved in the response to copper sulphate. Many of the chemoreceptors in the gut are described as being 'polymodal', responding to a number of luminal stimuli so copper sulphate could act on these but this has not been tested directly. In the rat (a 'non-emetic' animal) gastric mucosal afferents responding to copper sulphate have been reported and this apears to be the only report directly showing afferent activity evoked by this widely used emetic stimulus (Clarke and Davison, 1978). Thus vagal afferents with the capability of discharging in response

the lower jejunum. Taken together these studies show that the upper gut (stomach, duodenum, proximal jejunum) are all sites from which the agents used could evoke vomiting. On teleological grounds alone it is possible therefore to argue that the more proximally located receptors should be the most sensitive as they are most likely to be exposed to potential toxins first and thus initiate appropriate action to rapidly eject a toxin before significant absorption had occurred (Davis et al., 1986).

5.3.2.3 The Problem of Temporal Changes in Emetic Response

It was decided to investigate the role of the vagus and greater splanchnic nerves in the emetic response to intragastric stimuli using the same approach as previous workers i.e. sectioning the appropriate nerves and examining the response to emetics at a fixed interval after lesioning. In some animals the response to either sodium chloride or copper sulphate was tested on several occasions after lesioning and it became apparent that the response was not constant particularly in terms of latency, after either vagotomy alone or in combination with section of the greater splanchnic nerves. Because of the small numbers involved the responses to sodium chloride and copper sulphate are considered as if they were equivalent. Three days following vagotomy no emetic response was observed (NaCl) but at 7 - 10 days a response is present but delayed compared with controls ($CuSO_4$). By 3 weeks the responses could be resolved into two components viz. one, an ultrashort latency response occurring within one minute of

administration, a reaction rarely if ever seen in non-lesioned animals and a second component usually being most apparent at a time point after which vomiting had stopped in the control group animals. Preliminary studies using the combined lesion in a few animals at this time showed that both components of the response were absent in some animals. Of the animals that did respond the majority showed an early response similar to that in animals with vagotomy alone, but failed to show the delayed phase. Viewing these results together highlights the difficulty in attempts to derive a mechanism for the action of an emetic based on examination of responses at a single time point after nerve lesioning. This may have implications for the study of other agents and this issue is addressed elsewhere.

In addition it may be that whilst in the intact animal the vagus is the major pathway for emesis induced by intragastric stimuli, after its removal another mechanism is recruited. This appears to be supported by the studies with sodium chloride in which no animals responded 3 days post-lesion whereas by 7 - 10 days a response had returned in the vast majority of animals. In animals with a combined lesion no response to sodium chloride was observed implying that by 7 - 10 days post-vagotomy the splanchnic nerves had taken over the emetic function of the vagus. At 3 weeks an ultra-fast response developed which is independent of the vagus and the greater splanchnic nerves. The question then presents itself as to what the mechanism of these changes might be.

At present we can only speculate until more data becomes

available but among the possibilities could be the following:-

a. At some point in the time course it appears that the splanchnic nerves may "take-over" the function of the vagus and support at least part of the emetic response. This is all the more significant because it has already been shown that section of the splanchnic nerves alone has no substantive effect on the emetic response not only to intragastric stimuli but to all the stimuli used in this thesis.

b. Since the nerves which control upper GIT motility are cut in the experiment it is conceivable that some of the changes in latency observed are due to changes in the rate of emptying of the intragastric compounds from the stomach and their delivery to more distal parts of the gut upon which they may then exert their emetic effect. The effects of vagotomy on emptying pattern may also alter with time, initially slowing the delivery of emetic agent to the detector thereby increasing latency and later speeding it resulting in a 'dumping-like' syndrome and enhancing it.

c. The gut mucosa contains a number of agents which when given i.v. induce emesis in the dog by an action on the AP (e.g. NPY, CCK). (It is known that vagotomy induces a number of structural changes in the gut). Gut denervation may influence the release mechanisms and sensitize the cells to release these agents in quantity when later exposed to hypertonic solutions or copper sulphate. These agents once released could evoke emesis by an action on the AP having reached the general circulation.

d. Vagotomy by depriving the NTS of significant afferent input may induce changes in the neuronal organisation of the NTS or even the AP to which it also projects. This might give rise to an increase in the sensitivity of the emetic mechanism to other inputs to which it was previously relatively insensitive (e.g. AP or greater splanchnic nerve).

Whatever the precise mechanism involved the implications of these results may be significant for the understanding of the mechanism of radiation and cytotoxic induced vomiting where there are already clear indications of the involvement of at least two pathways, one nervous and one humoral. It may very well be the case that the apparent conflict in the results from different species reflects the time at which testing takes place and in some cases at least the humoral mechanism may have been induced in animals which would otherwise have been dependent on the intact vagus.

5.3.2.4 Neuropharmacology of Intragastric Emetics

It is interesting that there is parallelism between vagotomy and $5HT_3$ in their effect on radioemesis and cytotoxic vomiting but with intragastric emetics vagotomy has profound effects whereas metoclopramide has none. This suggests that while gastrointestinal mechanisms are important in both groups of stimuli the pharmacological mechanisms operating within the GIT that mediate their response to the two groups may be different e.g. the release of 5-HT from enterochromaffin cells activating vagal afferents may only occur in response to the

profoundly traumatic stimuli of irradiation or administration of cytotoxic drugs whereas such stimuli as sodium chloride or copper sulphate may not, in the short term at least, cause sufficient damage to evoke this type of response. An alternative explanation could be that rather than the two groups of stimuli acting in the same region of the gut e.g. the small intestine, where enterochromaffin cells are densely populated, they may act at different regions with radiation and cytotoxics involving mainly the small intestine and the intragastric hyperosmotic stimuli (and $CuSO_4$) acting on the stomach and proximal duodenum. Some evidence in support of this idea has come from the observations of Blackshaw et al. (1987) and Andrews and Wood (1988) showing that hypertonic stimuli placed in the stomach or duodenum of the ferret can evoke retching in the anaesthetised animal via a vagal pathway.

An additional possibility is that the "emetic receptors" sensitive to intraluminal stimuli in the upper gut (e.g. stomach) do not use 5-HT whereas those insensitive to the same stimulus but located in the lower regions of the gut (e.g. ileum) do use 5-HT. Thus when an emetic stimulus enters the stomach it would be detected and rapidly ejected before it reached the lower set of receptors. Hence if we were to by-pass the former group of receptors by injecting the emetic directly into the small intestine we might be able to demonstrate a role for $5\text{-}HT_3$ receptors in the emetic response to intraluminal stimuli.

From our observations we would predict that a $5HT_3$ receptor antagonist would have little effect on sodium chloride and copper sulphate induced emesis and indeed this appears to be the case (Andrews, personal communication). However, it is important to stress that we have only investigated the short-term responses to the intragastric emetics discussed and it is clear from the data of Wang and Borison cited extensively in the present study that in the dog with a vagotomy copper sulphate can have an emetic effect by a direct action on the CNS via the AP; the latency of such effects is greater than 2 hours (cf. controls of 9 - 15min) and the threshold dose is eight times greater than that in intact animals.

5.3.2.5 Mechanisms of Action of Ipecacuanha

For agents given into the stomach where the drug is given in a vehicle (comparing human and animal data where the stimuli are thought to act at the level of the GI tract) all the electrophysiological data suggests that the afferent discharge rate is related to the concentration of the agent applied to the mucosa. It is on this basis that investigations of ipecac' syrup were carried out. However, as the drug is given in syrup, itself a hypertonic stimulus, due consideration was given to the effect of the vehicle on the response to the drug. Because Ipecac' mayhave two sites of action, i.e. on the gastrointestinal mucosa and on the CNS the situation is further complicated as, in the case of the former site the concentration at the mucosa may be the critical one whereas in the latter case the measure of effective dose is more likely to be on a drug weight per kg body weight basis. These problems

are highlighted when we come to examine the data from the present study where we attempted to look at the effects of ipecac alone in two vehicles, and of its most abundant emetic ingredient, emetine given in syrup or water by mouth at a concentration close to that found in ipecac.

Ipecac in syrup and emetine in syrup and syrup alone all elicited vomiting with a similar latency i.e. 9 - 14min. On first inspection this would suggest that the emesis is primarily an effect of syrup. As the syrup (66% w/v) is effectively approximately 0.7M and is therefore hypertonic it is likely that the emesis is driven through the mechanisms previously outlined. Further consideration was given to this problem.

When ipecac is given on a per kg basis in syrup and the same amount of ipecac is given in water a striking difference is observed (see Table 8). The latency to ipecac in syrup is about half that for ipecac in water whereas the mean numbers of vomits are similar (although retches are much greater in the ipecac in syrup group). Similarly when 150mg% emetine is given in syrup the latency is about 14min compared with a latency of 40min when given in water. In syrup, the number of retches and vomits for emetine were substantially increased. Taking those two sets of experiments together illustrates that whether ipecac or its major emetic ingredient is given the latency can be substantially influenced by the nature of the vehicle. In both cases the latency of the vomiting when the drug is made up in syrup is the same as if only syrup is given (see Table 8). These data would suggest that the true latency to emetine and

ipecac itself lies somewhere between 25 - 40min.

Interestingly this range of latencies is comparable with that observed when emetine is given by the i.p. or i.v. routes (Bhargava et al., 1961) when one of the other active ingredients, namely cephaeline, is given directly into a Pavlov pouch. This illustrates one of the problems of giving emetic agents by the oral route in that firstly the vehicle itself may be emetic and secondly the vehicle may influence the rate at which the agent itself either reaches the chemoreceptor in the gut or alternatively is absorbed (i.e. it may affect gastrointestinal motility).

If one therefore compares two different doses of ipecac' both given in syrup the latency is not significantly different but the emetic potential is, indicating that while the latency maybe determined by the syrup vehicle, the amount of vomiting is determined by the non-osmotic stimulus. It is clear from these data that the picture is a complicated one and the interpretation of the pathways involved is made more difficult by the nature of the stimulus itself which has both peripheral toxic and osmotic effects as well as possible central effects. Nerve lesions were therefore carried out in an attempt to clarify the situation. Giving ipecac syrup to a vagotomised animal fails to evoke vomiting and results in convulsions and death. The simplest explanation for this observation would be that the peripheral or vagal detector system has failed to respond to a toxin; hence the toxin penetrates the CNS and kills the animal. From our previous experiments we knew that hypertonic stimuli could evoke vomiting by a vagal mechanism

(e.g. NaCl) and it was possible therefore that this result reflects the fact that we are abolishing the emetic response to the syrup component of the ipecac' syrup which would normally prevent emetic alkaloids of ipecac being absorbed. After vagotomy these alkaloids are absorbed and result in toxic effects on the CNS culminating in convulsions and death.

As emetine is a component of ipecac' and the above studies have shown that a dose of emetine equivalent to a dose of ipecac when given in water is emetic we decided to investigate the effect of vagotomy on this response, in order to avoid any complications produced by the effect of the syrup per se.

Surprisingly, vagotomy was without effect on the vomiting response to emetine. This suggests that as described elsewhere emetine is acting at a central site. However it is apparent that this conclusion is at odds with the conclusion from the ipecac' and syrup experiments as, if emetine was acting at a central site after absorption we would have expected the vagotomised animals challenged with ipecac syrup to have vomited, albeit with a delayed response.

At present our experiments do not allow us to resolve this discrepancy and the best that can be done without undue speculation is to highlight such inconsistencies and their implications.

The experiment showing that vagotomy completely abolishes the emetic response to ipecac' syrup suggests that this entire response is mediated peripherally, by the vagus. However, it was surprising that the emetic response to emetine was not influenced by vagotomy. How can these two discordant

observations be resolved? Firstly, it should be remembered that syrup itself is emetic and like many other hypertonic stimuli can act via the vagus. If we suppose that the emesis seen with ipecac syrup is primarily due to the syrup rather than the 'emetic' components then whilst we would expect vagotomy to abolish the early component, the data from the emetine vagotomy study would lead us to expect a delayed burst of vomiting due to absorption and hence a central action of emetine probably on AP, as previously reported. However we do not observe this but instead convulsions occur at the time we would expect to see vomiting.

It should be borne in mind that ipecac syrup contains more than emetine as the active principle (e.g. orthomethylpsychotrine, cephaeline). It may be that with ipecac syrup, vomiting is primarily driven by the syrup and is then reinforced by the absorbed emetic alkaloids acting centrally. Ipecac also contains other alkaloids which have convulsant activity and thus it is possible that when vomiting is not initiated soon after ipecac' administration the animal absorbs a larger quantity of these agents and they produce convulsions and ultimately gross CNS depression. This supervenes before the absorbed emetic agents such as emetine and cephaeline achieve a concentration sufficient to evoke emesis. In this case the CNS is overwhelmed by a number of toxins in ipecac' only some of which have emetic activity.

This somewhat unsatisfactory discussion of this set of results serves to illustrate the considerable problems of identifying the site and mechanism of action of an emetic agent

containing a large number of chemicals each with different effects on the body which can interact. The level of confusion with ipecac is the more worrying because of its widespread clinical use and the fact that it has been under study for well over 100 years.

5.4 MECHANISMS OF ACTION OF CYTOTOXIC AGENTS AND X-RADIATION IN THE FERRET

5.4.1 Cytotoxic Agents

The results of the present study are discussed in terms of the possible site(s) of action of each agent and compared to data from other species. This section concludes with an overall assessment of the mechanism of cytotoxic drug-induced vomiting and in particular attempts are made to explain why agents with the common feature of cytotoxicity but underlying diverse structures are able to evoke emesis. The implications of these studies for anti-emetic therapy are discussed.

A number of general problems arise from the consideration of data arising from variety of sources. From the point of view of mechanism account has to be taken of:-

a. Similarities and differences in latencies; what factors contribute to the latency of an individual cytotoxic agent?

b. Pattern of emetic response; what determines the characteristic 'fingerprint' of retching and vomiting for each agent.

c. Duration of emetic effect; what characteristics of a cytotoxic agent determine its duration action.

An attempt will be made to answer some of these questions in the discussion that follows hereafter.

5.4.1.1 Patterns of Cytotoxic-induced Vomiting

It is striking that four of the agents studied in this investigation, mustine, cycloheximide, emetine and DAS have tightly grouped latencies of between 17 - 36min, and studies from other workers have shown latencies in a similar range e.g. cyclophosphamide 19.8 ± 3.6min (Andrews et al., 1987; Hawthorn et al., 1988). It is noteworthy that the radioemetic agent in this group gives a latency similar to that found for radiation in the ferret. However these latencies are clearly distinguishable from all reported data on cisplatin in the conscious animal which has latencies reported in the range from 68 ± 6min to 99 ± 4min (Florczyk et al., 1982). The only other agent which has been found to have a similarly long latency is adriamycin (84 ± 6min) (Schurig et al., 1984). The second feature of note is that each agent has a characteristic pattern of retching and vomiting associated with it (see Sections 3.2.3.1 to 3.2.3.5). Some produce more intense emesis early on in the response with a gradual decline over the observation period whilst others produce emesis almost continuously throughout the observation period e.g. cycloheximide. Also it can be seen that the interval between bursts of retching and vomiting may range from a few minutes to nearly an hour. It is clear even from this limited amount of data that there are differences between the precise nature of the animal's response for each agent and whilst these differences, particularly with respect to the pattern of emesis, have occasionally been alluded in the past (e.g. Borison and McCarthy, 1983) the work of this thesis represents the first attempt to record and quantify these differences in a single species.

Concerning the observations that we have made above, two major questions raise themselves. Firstly, how can we account for the latencies of each agent and secondly, what factors contribute to the genesis of the pattern of emesis for each agent.

a. Latency

There are several factors that might influence the latency to any given emetic.

i. The cytotoxic agent may require conversion from its injected form to its active form which also happens to be emetic. As far as the literature reveals this is not the case for any of the agents tested here but it does apply to cyclophosphamide which requires conversion to phosphoramide mustard. The latency of emetic response to cyclophosphamide is in the same range as that for all the non-cisplatin agents and interestingly it is similar to that for mustine an agent closely related to phosphoramide mustard. Therefore it appears that conversion to an active principle is unlikely to make a substantial contribution to a 'delayed' latency.

ii. Cytotoxic agents may be given by different routes e.g. in our study emetine and cycloheximide were given i.p. whilst DAS, mustard and cisplatin were given i.v.. Two pieces of evidence suggest that the route of administration is unlikely to be of significance provided the ED_{100} has been achieved. This is exemplified by the following

observations. The latency of the response to cisplatin is almost the same whether the compound is given i.v. or i.p.. The same is also true for cyclophosphamide where the i.v. latency is 21.6 ± 8.0min and the i.p. latency at the same dose is 19.8 ± 3.6min (Hawthorn et al., 1988).

iii. The differences between cisplatin and the other cytotoxics may reflect a difference in the exact mechanism by which they give rise to emesis. There are two possibilities here; it may be that rather than having a direct effect on the CTZ they cause the release of an emetic agent in a distant part of the body (most likely the gut) and thus the latency period represents the time each agent takes to mobilise sufficient quantities of this substance to trigger emesis. An alternative possibility promoted by Harris in 1982 is that the latency is accounted for by interference with the intracellular mechanism for the production of "rapidly turning over enzyme systems that are responsible for the breakdown of a neurotransmitter" (Harris and Cantwell, 1986), the assumption being made that this mechanism operates within the AP, although it is by no means certain that all of the cytotoxic agents act at this site. The hypothesis put forward by Harris was derived largely from an examination of the emetic latencies of a range of cytotoxic agents in man and their emetic potency, based on incidence of side-effects of therapy. Comparing such data with experimental data

derived from the present studies in the ferret presents several difficulties. Firstly, whilst we have precise emetic latency values for the ferret to a range of cytotoxic agents comparable data are not available in man and latencies are normally quoted as a range sometimes spanning minutes to hours for the same compound (Borison and McCarthy, 1983). Secondly all the data in the ferret for latency, is based on data obtained at the ED_{100} for each compound, whereas in man not all compounds achieve an ED_{100} for emesis. One agent which produces close to an ED_{100} in man is cisplatin and therefore it is worth considering this agent in more detail. Referring back to Table 16 we can see that the latency to cisplatin is broadly comparable in all species so far studied and this might lead one to think of a common underlying biochemical mechanism. Harris's hypothesis proposes that a prime site of action of cisplatin is initially on the level of a 'central' enzyme blockade rather than by inhibition of protein synthesis or interference with DNA. Thus one might expect that agents which worked at a site 'proximal' to the critical enzyme e.g. by an action on DNA, would have a longer latency than cisplatin. This appears not to be so as the potent protein synthesis inhibitor cycloheximide has a shorter latency than cisplatin in both man and ferret. A similar argument can be made for the response to mustine, emetine and DAS in the ferret.

Additionally Harris and Cantwell (1986) commented that vomiting due to the alkylating agents (e.g. cyclophosphamide) and adriamycin is delayed, possibly due to the time it takes for enzyme levels to fall after inhibition of mRNA. Whilst the latency for cyclophosphamide and adriamycin vomiting is similar in man (Table 20) it is quite different in the ferret. Thus whilst some components of the hypothesis may translate across species others do not. What then can be said in summary about this hypothesis? Most importantly, it represents one of the first attempts to explain the mechanism rather than merely the site of action of cytotoxic drugs. However whilst some data supports the theory it has never been tested directly by examination of the biochemistry of the AP. Secondly difficulty lies in translating the mechanism across species. It is possible that the latency differences observed are a consequence of different turnover rates of the "pivotal" or critical enzyme if it is the same in each species or indeed the use of different key enzymes in different species. A further problem is that the hypothesis assumes that all cytotoxic agents in a given species act at the AP and this is clearly not the case; it appears that in the ferret at least many agents act primarily at a site in the gut. Even if the agents are shown to act primarily at the gut level in some species it merely serves to shift the site of action and we still have to explain how the agents activate the visceral afferents. Perhaps the mechanisms suggested by the hypothesis for the AP may be adapted to explain the emetic action of these cytotoxic agents at the level of the gut.

TABLE 20

Man and Ferret Compared with respect to Vomiting Latency to Various Cytotoxic Agents

	Vomiting Latency (min)	
	Man	Ferret
Cisplatin	60 - 360	69 ± 6 - 99 ± 4
Mustine	30 - 120	28 ± 7
Emetine	-	36 ± 7
Cycloheximide	5 - 60	17 ± 5
DAS (Anguidine)	-	22 ± 9
Cyclophosphamide	120 - 720	22 ± 8
Adriamycin	120 - 720	84 ± 6

Footnote: Data drawn from multiple sources listed in reference section

b. Temporal Pattern

One of the most puzzling features of cytotoxic-induced vomiting is how very short courses of therapy can then evoke vomiting over periods of a few hours or days (depending upon the agent used). Due to a number of practical constraints we have only been able to study vomiting over relatively short periods in the ferret and it must be explained that the mechanisms proposed only apply to these time regimes and different mechanisms may apply to the later phases of emesis. Whatever the site at which the emetic agents work we have to explain how the agents and particularly cisplatin can cause activation of the emetic mechanism over such extended periods. If the scheme proposed by Harris is correct, then the inhibition of the 'pivotal' enzyme must continue for some time. Over these longer time courses it may be necessary to consider an involvement of other mechanisms such as damage to the gut (e.g. cisplatin, see Allan and Smyth, 1986) or cerebral oedema due to changes in the permeability of the blood-brain-barrier. Further studies are required to identify the mechanisms involved in these delayed phases of emesis.

A second area for consideration is what determines the pattern of emesis. If either an enzyme is inhibited or a secondary agent is released from the gut by the cytotoxic drug then one might expect emesis to be continuous, but in all cases it occurs in bursts, although the interval between the bursts is variable. It is possible that the intervals represent the time taken for the emetic agent to accumulate and trigger

emesis. It is also possible that it represents the time for the endogenous emetic factor to be synthesised in sufficient quantity to trigger emesis. Until we know the precise mechanism by which cytotoxic agents and indeed other emetic agents finally evoke emesis it is not possible to make further comments on the mechanisms of the production of the vomiting pattern.

Nevertheless an appreciation of the factors contributing to the genesis of temporal pattern is of importance in understanding the optimum times at which to administer anti-emetic therapy. It is of particular interest that $5HT_3$ receptor antagonists are not only able to block cisplatin emesis in animals and man when given prior to therapy but can also rapidly stop vomiting after it has started (Sanger personal communication). This latter observation would suggest that cytotoxic agents do indeed cause the continuous release of an ememtic factor whose action can be antagonised by a $5HT_3$ receptor antagonist.

5.4.1.2 The Role of the Area Postrema in Cytotoxic-induced Vomiting

Research into the site of action of cytotoxic drugs and the mechanism of their associated emesis has tended to concentrate on the AP because during the nearly 40 years since the delineation of the CTZ, systemic toxins such as drugs and other chemicals have been assumed to act there.

However, as Borison reiterated in a recent paper on cisplatin-induced vomiting in the cat (McCarthy and Borison, 1984), not all chemically induced vomiting is mediated

by the AP mechanism, citing the action of verartrum alkaloid on the nodose ganglion and the action of pilocarpine on the orbito-frontal cortex as examples. Moreover involvement of the AP in vomiting due to a systemic chemical stimulus does not necessarily exclude the possibility of the involvement of other potential chemoreceptor sites, including the visceral afferents of the gut.

Nevertheless the initial approach has been, for many systemic chemical toxin challenges such as cytotoxic drugs, to look at the effect of ablation of the AP on the vomiting so induced by these compounds.

Such data only suggest the involvement of the AP, and not necessarily that it is a primary site of action of blood-borne or CSF-borne chemicals. Indeed, exogenous emetic agents could activate the AP in two ways. Firstly, 'chemosensory' elements (possibly ependymal cells) of the AP may detect the emetic and cause release of a neuroactive agent which then fires AP neurons (similar to the carotid chemoreceptors). Secondly, the emetic may provoke the release of an endogenous emetic agent or 'mediator' from a target site distant from the AP (Andrews and Hawthorn, 1988). Such a system has been proposed for radioemesis in which peptide YY is the putative mediator released from the gut to act on the CTZ of the AP causing vomiting. It is possible for both mechanisms to be in operation at separate times for a single agent, simultaneously for a single agent, or separately for different agents.

There is also the tehnical problem of the extent of the lesion produced by AP ablation. A variety of ablation

techniques have been used including surgical removal, electrocautery, and cryosurgery but the controversy over the extent to which these lesions can be described as discrete continues (Borison et al., 1987; Harding et al., 1985). Because of the very small size of the AP even in larger animals like the dog, absolute precision in ablation is difficult to achieve and some damage or contusion of underlying structures is inevitable. The compact nature of the nuclei of the caudal brain stem adds to this problem. All of the critical nuclei involved in the control of emesis appear to lie in close apposition or juxtaposition in this area. Indeed Leslie (1986 and 1988) describes the dorsal motor nucleus of the Vagus, the sensory nucleus of the Tractus Solitarius and the AP as the dorsal vagal complex or DVC. To make matters worse close consideration of the neuroanatomy of the AP and the subjacent area subpostrema or subnucleus gelatinosus of the NTS show that they are remarkably similar in structure. Moreover the subnucleus gelatinosus has been shown to correspond to the dorsomedial region or division of the NTS in the cat (Taber, 1961) and this has led Leslie to propose that the AP is simply an extension of this part of the NTS into the lumen of the fourth ventricle which has lost its blood-brain barrier and become intensely vascularised (Leslie, 1988).

The situation is further complicated by the connectivity of the AP whose principal efferent links are to the parabrachial nuclei, NTS and cerebellum; and whose afferent ones are from the NTS, gastric vagal afferents, and DMVN.

Thus it is clear that AP ablation will involve underlying

nuclei and underlying connecting neuronal links even if it is carried out with meticulous care; in any mechanical AP ablation not only will the CTZ have been removed but also the afferent neural connections of the AP from higher centres, vagal afferents from the gut, NTS and DMVN. Lastly, it has also to be considered that AP ablation does not necessarily equate to CTZ ablation per se if the zone proves to be only part of whole AP structure. A number of other functions have been attributed to the AP aside from being a CTZ for toxins and so a pure CTZ lesion is probably impossible.

This leads to mention of another area of difficulty in interpreting AP lesions. Not only is there still argument as to the extent of the tasks that the AP carries out both in breadth (e.g. CTZ for emesis, control of blood pressure, appetite control) and depth (e.g. chemoreception, transduction, integration) (see for instance Leslie, 1986 and Borison, 1984) but the precise way in which these functions are carried out is still not known. A review of the data covering this aspect of the AP which attempts to reconcile known and putative functions with ultrastructure and neurochemistry was published by Leslie in 1986. Briefly Leslie summarizes current understanding of the AP by referring to the following special features; position in the brain, lack of a blood-brain-barrier specialised ultrastructure and circulation, receipt of neuronal information directly from thoracic and abdominal viscera, dispatch of neuronal information directly to forebrain structures involved in autonomic control, and a large complement of binding (receptor) sites for neuroactive

compounds, to which its neurones react by discharge. Finally its loss is detectable only in subtle physiological anomalies. It is concluded from this evidence that the AP is indeed a 'multisensor device' in combination with a 'central processing unit'; in essence a 'biocomputer' that monitors many vegetative functions and applies small corrective controls via other brain-stem nuclei. As Borison (1986) has pointed out and Leslie's work has highlighted many of the prodromal or acute symptoms associated with emesis are also shared in part with the other physiological processes that the AP seems to mediate.

Data on the effect of AP ablation on cytotoxic drug-induced emesis are limited to that in the dog and cat, and largely to cisplatin and mustard with some additional material from cyclophosphamide. As yet there are no published data in the ferret from the result of AP ablations and as has been stated in our aims the present study did not encompass such lesions because of our wish to concentrate on the vagus. Cisplatin-induced vomiting was abolished both in the cat (McCarthy and Borison, 1980 and 1984) and the dog (Akwari et al., 1985) by ablation of the AP which seems to be clear cut evidence that at the very least the CTZ is an important site of action for cisplatin. However McCarthy and Borison are at pains to point out that this may not necessarily be so, citing the long latency (at least 60min) for cisplatin (and many other clinically cytotoxic drugs) as one reason why. Having assigned the role of toxin-sensor to the AP and found it to act efficiently in terms of speed of response to substances such as apomorphine, it is difficult to invoke its primary

action in detecting and reacting to cisplatin, a comparatively slow response. Further to this they suggest that the apparent irreducibility of the latency to vomiting in response to cisplatin in the cat implies that some intermediate mechanism may be involved in its action. As has been pointed out, not only does the figure for latency in the cat agree remarkably well with that of the present study in the ferret, but considering the data in Table 16 it can be seen that the minimum latencies for man, dog, cat and ferret are closely aligned. This would seem to add weight to the argument that there is an intermediate step common to all species tested that is required before emesis can be invoked. However, it is worthy of note that at least from our experience in the ferret, prodromata were recorded usually about 30min after dosing so that the effect of any intermediate step can certainly be observed well before vomiting takes place indicating perhaps that such a step might involve the gradual build up or breakdown of a substance. This latent period would be the time when enzyme inhibition is taking place according to the hypothesis put forward by Harris (1982). However, this is perhaps too slow for such an effect because data on the emetic effect of protein synthesis inhibitors in the ferret show that they vomit by and large between about 10min and 30min after dosing. Since Harris's proposal would put the protein synthesis inhibitors as second in speed of action to an enzyme blocker it is not unreasonable to expect that cisplatin if acting as such should have a latency less then the protein synthesis inhibiting cytotoxics. Cisplatin's least but

swiftest effect is on protein synthesis so that one might speculate that its latency would fall close to the other compounds whose primary action is on protein synthesis. It has, in fact, a more potent action on DNA, the effect of which one would predict to be slowest of all, which indeed it seems to be, at least as far as those compounds that have been tested here.

However, it is possible that the relatively long latency for cisplatin is accounted for neither by production of an intermediary emetic metabolite nor by upsetting the delicate balance of turnover of a critical protein, for there is evidence that it has effects at sites remote from the AP. Florczyk et al., (1980) showed that cisplatin blocked gastric emptying in the mouse via an effect on the pyloric region. Then in 1982 Akwari and Lucas studied the effect of i.v. cisplatin in the dog and showed that after a latent period of 90 - 120min hyperkinesis of the jejunum with accompanying lengthy bursts of action potentials appeared (cited in Akwari, 1983). Vomiting was preceded by oral migration of these action potential burst patterns from the jejunum up to the stomach. These episodic bursts of action potentials which disrupted gastrointestinal pace-setter potentials continued in isolated groups from time to time over the next 2 hours resulting in vomiting, a situation that seems to parallel closely the clinical one in which delayed emetic episodes have been reported in cancer patients up to 8 hours after therapy (and in some cases much longer).

Subsequently ablation of the AP was carried out in dogs

treated with cisplatin to see whether or not there was any association between the AP and the establishment of this altered gastro-duodenal myoelectric activity (Akwari et al., 1985). After AP ablation gastric and duodenal migrating myoelectric complexes were unaltered and the disruptive effect of cisplatin on these patterns was unaltered. However, AP ablation in the dog abolished the cisplatin induced orally migrating action potentials at the same time as apparently conferring complete protection against vomiting. These experiments indicate that there is a dual action of cisplatin in influencing gastrointestinal motility associated with emesis; disruption of gastrointestinal pacesetter potentials by cisplatin is independent of its action at the AP, and establishment of the orally migrating action potentials which precede vomiting in the dog is wholly dependent on the integrity of the AP. The close temporal association of the onset of these migrating action potentials with the start of frank vomiting and the clear abolition of both by AP removal seems, at least in the dog, to indicate that the AP is the prime target for the action of cisplatin in the induction of emesis or the action of some secondary mediator or metabolite of cisplatin. The bearing of lesions of the abdominal vagal afferents on this story will be dealt with in the next section on the effects of peripheral vagal lesions. The release of such a secondary mediator from the gut by the action of cisplatin on the mucosa is a possibility for a candidate to effect both the AP dependent changes and AP independent changes. Unfortunately the evidence for intestinal mucosal

toxicity of cisplatin (Allan and Smyth, 1986) indicates that the damage is not apparent for a number of hours and is maximal between 12 and 24hr post injection, a time scale which does not match with the onset of nausea and vomiting and the authors concluded that prolonged gastrointestinal symptoms did not correlate well with the intestinal mucosal toxicity of cisplatin.

Whereas the data on the effect of AP ablation on cisplatin induced vomiting in the dog and cat agrees well, those for mustine in the same two species does not. Removal of the AP was found to abolish mustine-induced vomiting in the dog but was found to be ineffective in the cat (Borison et al., 1958) and as yet we have no data in the ferret for comparison. As was pointed out in the introduction these authors then suggested the CTZ might be playing its role either by being directly stimulated, or by being stimulated indirectly from a mediator (released for instance from the gut) or by being part of the afferent neural pathway from a peripheral receptor site. Papp et al., (1966) took up the problem by injecting mustard into the cerebral ventricles of dogs with and without the AP present. Animals without an intact AP did not vomit to a dose of mustard nine times the emetic ED_{50} in intact animals. The experiments also showed the effectiveness of mustard instilled into the fourth ventricle; 100μg placed into the fourth ventricle of a dog caused vomiting within 10 minutes with the latency dropping to 5 minutes or less at a dose of 500μg. By the i.v. route, for comparison, Borison et al., (1958) had given figures of $500\mu gkg^{-1}$ i.v. giving a mean latency of

approximately 120min and 10mgkg^{-1} giving a mean latency of approximately 16 minutes. From a consideration of these data Papp and co-workers concluded that mustard does indeed provoke early emesis in the dog by its action at the CTZ in the AP. However, there is a technical criticism which they allude to which can be made of all such manoeuvres. Papp et al. concede that the acidity of the vehicle solution at the higher doses of mustard instilled into the fourth ventricle could cause vomiting in the absence of mustard. This may account for the sensitivity of the AP to locally applied substances of very many types (e.g. Carpenter et al., 1983). Moreover in the specific case of mustard we are dealing with a substance that is a vesicant and consideration must therefore be given to this non-specific action on the AP when viewing the results of i.c.v. injection.

The only other evidence for the site of action of mustard based cytotoxic drugs is that from cyclophosphamide and phosphoramide mustard (Fetting et al., 1982). Unfortunately, the investigations of these compounds in the cat produced equivocal results both from direct challenge experiments in normal animals and AP ablated animals. The evidence suggested some partial role for the AP in this type of emesis. The situation was a complicated one in which phosphoramide mustard, the active metabolite of cyclophosphamide took approximately 2hr to produce vomiting in the normal cat compared to about 1hr for the parent or original compound. Clearly it is difficult to draw any conclusions from such data. Perhaps these equivocal results in the cat have some relationship to the fact

that mustard induced vomiting is not prevented by AP ablation in the cat.

Overall, mustine induced vomiting is, in general, quicker in onset than cisplatin and displays a dose response relationship whereby with increasing dose, latency falls from 2hr to about 15min. Thus, the pattern of response to mustard is clearly different from that to cisplatin. Reviewing mustard as an alkylating agent in the light of Harris' hypothesis (1982) one would expect a latency similar perhaps to that found with cisplatin. With latencies recorded as short as 15 minutes in the dog and 20 minutes in the ferret (this thesis) it would seem difficult to invoke the primary cytotoxic action of mustine as an alkylator as also being responsible for the triggering of emesis; indeed Borison suggested as much when he concluded that such a short latency for mustine might mean that it "possesses an emetic stimulant property independent of its cytotoxic action". Further, the conflicting data on the effect of AP ablation on mustine vomiting in the cat and the dog implies that the importance of different target sites varies from species to species (Borison and McCarthy, 1983) and from agent to agent; hence the contrasting concordance of data on the effect of AP ablation on cisplatin-induced vomiting in the cat and the dog.

5.4.1.3 The Role of the Gastrointestinal Tract in Cytotoxic Induced Vomiting

We have shown that section of the abdominal vagus in the ferret has a profound effect upon emesis induced by a wide variety of agents, either totally abolishing or markedly

reducing the response to cisplatin, mustine, cycloheximide, emetine and DAS (see also Hawthorn et al., 1988). Of these, cisplatin and mustine have been claimed to act in the dog primarily via the AP. Unfortunately the vagus does not seem to have been seriously considered as a site at which cytotoxic agents can act. It is worthy of note that there is only example, that of mustine in the cat, in which both visceral nerve lesions and AP ablation have been studied. Here AP ablation had no effect but abdominal visceral nerve lesions markedly reduced or abolished the response, a phenomenom which is also true for radiation-induced emesis in the cat (Borison et al., 1958; Borison et al., 1987).

Although the sensitivities of the cat and the ferret differ to a variety of agents (including mustard, where the ferret is more sensitive) in respect of mustard emesis both species seem to depend on the integrity of the vagus. Unfortunately vagal lesion data are not available in the cat for cisplatin vomiting whereas in the ferret the vagus is clearly essential. Conversely, in the dog, data are not available on the effect of vagal lesions on mustard induced vomiting where again, in the ferret, we have demonstrated the importance of the vagal pathway. Our ability to build up a clear picture of the relative importance of peripheral pathways vs central ones (via the AP) is severely curtailed by the paucity of data from experiments looking at the effects of AP ablation, abdominal vagotomy and drugs in the main experimental animal model species. What is clear though, is that in the ferret at least, with some supporting evidence

from the cat, the role of the abdominal visceral afferents of the vagus in systemic toxin-induced emesis is far greater than one might have assumed. Such a conclusion has been extended more recently to the action of cyclophosphamide in the ferret (Andrews et al., 1986). It is perhaps worth a reminder here that vagotomy does not affect the mechanical capability to retch or vomit, a fact that we have demonstrated as part of our control procedures, when ferrets with a previous abdominal vagotomy were challenged with s.c. apomorphine and vomited reliably. This was also reported by Andrews et al., 1986.

This re-emphasis of the gut as a site of detection is perhaps not so strange if viewed in the light of its primacy in biological terms as the area where most toxins, in man at least, are detected and reacted to. It is quite acceptable for the gut and vagal afferents to be invoked in the case of challenge by oral emetics, like copper sulphate; it may not be unreasonable to suggest a similar mechanism for circulating toxins which might act either on epithelial mucosa cells or at the interface with the vagal afferents. It is possible that an action at this point could release a secondary mediator which then stimulates vagal afferents and/or the AP. Then again because of vagal afferent connections with the AP, the AP itself may become involved as part of the nervous pathway to the 'VC'. Alternatively one might postulate a direct action of an emetic on the AP which then, via the DMVN, alters gastric motility patterns which are subsequently detected by the vagal afferents as being equivalent to a direct toxic action on the gut via its lumen (e.g. copper sulphate or erythromicin).

This would then be signalled via the NTS as the trigger for vomiting to commence. Such a scheme would involve both the AP and the vagal afferents and might explain the longer latencies obtained where we would expect to find very short ones (i.e. when an emetic acts on the AP and then through the NTS to activate the motor components of vomiting).

Where AP integrity is not required for vomiting, as in mustard induced vomiting in the cat which is the only data available from this situation, clearly a route to activation of vomiting independent of the AP must be available. Whilst this could involve a third site (e.g. the forebrain in the cat for mustard (Borison et al., 1958) and cisplatin (London et al., 1979) it seems reasonable to suggest that this is a case where the toxic agent has an effect that directly influences the afferent discharge in the abdominal vagi.

In the ferret, we do not know if this situation applies, as we have no data on the effect of AP ablation on cytotoxic drug-induced vomiting to compare with that from the vagal lesions. However, we do have some data on the involvement of central structures, from our 2-DG experiments. In the case of cycloheximide 2-DG experiments, for reasons which we attempt to explain later it is not possible to use the data obtained therefrom. However, for mustine-induced vomiting (disregarding negative results which are difficult to interpret in 2-DG terms) the 2-DG autoradiography showed a marked increase in activity of the AP. So, at the very least we are able to say that although the vagus is essential for mustine-induced vomiting in the ferret, the AP is still somehow

involved in the process either by direct stimulation by mustard or through neural interconnections or via a humoral mediator released by the gut.

How we can reconcile the importance of the peripheral pathway for the site of action of cytotoxic drugs with the biochemical theory of their mechanism as emetics (Harris, 1982) is not clear. At first glance such evidence would seem to negate such a mechanism since the hypothesis is based upon the AP as a site of action. However, it may be that the biochemical actions of cytotoxic agents as enzyme inhibitors, protein synthesis inhibitors and alkylators or a mixture of these is exerted at the level of the gut mucosal cells instead of the CTZ, as we have already suggested.

We have already referred above to the effect of cisplatin on gut mucosal cells (Allan and Smyth, 1986) which seems to be outside the acute timeframe with which we are concerned. Of more interest perhaps is the work of Lieberman et al., (1970). This study looked at the effect of protein synthesis on intestinal epithelial cell damage induced by nitrogen mustard, X-radiation, and cytosine arabinoside. In particular it was found that mustard caused inhibition of DNA synthesis within 10 minutes of administration and it was concluded that this agent and X-radiation would have a primary biochemical effect on DNA (e.g. alkylation) within a few minutes of exposure. The results of this work showed that, paradoxically, cycloheximide could actually prevent this damage and that protein synthesis was somehow a key process in the response of certain cell types to lethal stimuli, specifically mustard or X-radiation.

Interestingly, in thinking about whether this effect was due to protein synthesis inhibition in particular or a result of another property of cycloheximide the authors state that emetine, a closely related compound, was unsuitable for testing because of its own damaging action on the gastrointestinal mucosa. This was also true of another widely available protein synthesis inhibitor, puromycin.

All these apparently unrelated facts including the data revealing the enzyme-inhibiting effects of emetine (Grollman and Jarkovsky, 1974), the profound effect of cycloheximide-induced inhibition of brain protein synthesis (e.g. Serra et al., 1982) on pharmacologically based stereotypy and the direct pharmacological blocking effect of emetine which we have previously discussed (e.g. Achari et al., 1972) at least identify a variety of mechanisms available to different cytotoxic agents that could account for their observed emetic activity and the timescale in which it appears. The possibilities may be summarized as follows:-

a. Damage to gastrointestinal mucosal cells with subsequent

   i) direct effects on vagal afferents

   or ii) indirect effects on vagal afferents via a local media

   or iii) indirect effects on the AP via a humoral mediator

b. Direct pharmacological action on

   i) vagal afferents from the gastroinestinal tract

   ii) gut motility

   iii) the area postrema

c. Primary biochemical action affecting the rapidly turning-over enzyme systems envisaged by Harris (1982 and 1986) as

i) enzyme inhibitors - fast acting

ii) protein synthesis inhibitors - fast acting

iii) DNA alkylators and cross-linkers - slow acting

iv) DNA synthesis enzyme inhibitors - slowest acting

What is clear from the preceding arguments is the paucity of experimental data upon which our ideas have rested even as far back as the studies of the 1950's. Currently because of the interspecies variability in response to cytotoxic challenges, our ability to draw firm conclusions about sites of action and mechanisms of action and apply them to man is limited. Data to substantiate or refute Harris' hypothesis are still unavailable although attempts have been made in this thesis to discuss the validity and applicability of the hypothesis in the light of our own work and the information available on the mechanisms of action of the individual compounds.

5.4.2 X-radiation

In contrast to data on cytotoxic drug-induced emesis in experimental animals those for ionizing radiation are more extensive. Moreover, apart from the variation in source generation in the case of radiation, a single if somewhat enigmatic stimulus is involved, which contrasts markedly with the cytotoxic agents which comprise a highly heterogeneous group of chemicals, with a variety of different actions at the subcellular, cellular and tissue level. Neither, it may be possible to argue, do we have the problems of route of

administration, differential rates of absorption, the effect of active secondary metabolites, direct pharmacological effects, or persistence of the stimulus in the system.

5.4.2.1 The Role of the Area Postrema and the Abdominal Vagal Afferents in Radiation-Induced Vomiting

The controversy over the function of the AP in mediating radiation-induced vomiting has continued for thirty or more years. Between 1954 and 1986 there were 10 studies in the cat, dog and monkey looking at the relative importance of the AP and the abdominal vagi. The studies reported here provided the first evidence in the ferret but more has come forward recently.

A summary of these data is made in Table 21.

Borison et al., (1987) reviewed the data from 7 studies and compared them to their own in the cat. These authors concentrated on the AP and were highly critical of methodology in the other studies, including the latest in that series (Harding et al., 1985). Moreover Borison and colleagues conclude unequivocally that (a) a complete emetic pathway for radioemesis bypasses the area prostrema and (b) radioemesis does not depend upon the generation of a chemical factor that selectively activates the A.P.

Notwithstanding criticisms of ablation technique, recording methodology and pre- and post-testing by Borison et al., (1987) it will be seen from Table 21 that there is a body of evidence from a number of different researchers

which indicates apparently that:-

a. The A.P. is essential for radioemesis in the dog, and solely responsible for the mediation of such vomiting

b. The A.P. is essential for radioemesis in the dog but is not solely responsible for this.

c. The A.P. is not essential for radioemesis in the cat

Full complementary data are not yet available in the ferret but initial studies indicate an equivocal situation akin to that in the monkey.

Most of the debate about the validity of these data from various species revolves around the discreteness of the AP lesions. If such arguments are put to one side for a moment we are left to consider, much as we did in detail for the cytotoxic drugs, just what the results imply. If excitation of AP neurons is required for radioemesis in some species then there are several possibilities concerning the mechanism of this excitation. Direct excitation is a possibility since the head has often been found to be the second most emetic area of the body with respect to the target of irradiation (e.g. Gerstner, 1960). However this mechanism is unlikely since shielding of the head does not prevent vomiting in animals irradiated elsewhere other than the abdomen (Chinn and Wang, 1954 and Wang et al., 1958). A popular idea is that radiation could cause the release of some substance into the circulation which acts directly to excite the neurons of the AP.

TABLE 21

The Effect of Area Postrema Ablation or Section of the Abdominal Vagus on Radiation-Induced Vomiting in a variety of Species

	Area Postrema required for vomiting	Abdominal Vagus Required for vomiting
Monkey	Yes	Yes
Dog	Yes	No
Cat	No	Yes/No
Ferret	Possibly	Yes

Footnote: Data drawn from multiple sources listed in reference section

Alternatively irradiation could result in the release of a mediator from a site distant from the AP, which then stimulates afferent nerve activity in the abdominal vagus and, via the connections with the AP (both direct and via the NTS) cause excitation of that organ. Then again such connecting fibres may just be routing through the AP which performs some integrative function upon the reflex as a whole. The remote site for the release of a humoral radioemetic factor has always been thought to be the gastrointestinal tract, particularly that part which lies in the area of abdomen known as the epigastrium.

Bearing in mind these possibilities and taking the dog as the animal in which the AP is most clearly implicated (5 studies) as being important for radioemesis we must look at other complementary data. The studies of Wang et al., (1958) and Carpenter et al., (1986) showed that the vagus was not necessary for vomiting in the dog. It has already been demonstrated that motor vagal fibres are not essential for vomiting, (see for instance Dresbach, 1947 (quoted in Brizzee, 1956) and Hatcher and Weiss, 1923), although some lengthening of latencies has been noted. It was also demonstrated that the removal of the abdominal sympathetic chains did not have significant effect on vomiting either. Interestingly in the Wang study of 1958 Wang et al. found that vomiting prior to death 3 - 4 days post irradiation was still present in dogs with the AP ablated but absent in dogs with vagi and sympathetics lesioned. So even in the species which most clearly displays its dependence of acute vomiting on the integrity of the AP, the abdominal vagus can still act apparently independently to cause vomiting after removal of the AP.

Since many of these observations strongly suggest that one of the pathways to radiation-induced emesis at least in the dog is via a humoral mediator, effort has been directed towards the isolation of such a factor.

One interesting candidate for an intestinally derived emetic factor has been reported recently (Harding et al., 1984 and 1985). Originally these investigations found that vomiting could be induced in dogs by the i.v. injection of a side-function of porcine intestinal extract obtained during the purification of Secretin. This potent emetic agent in the dog turned out to be peptide YY (PYY) and is found primarily in endocrine cells of the gastrointestinal mucosa. It is actively emetic in the dog via the i.c.v. or i.v. routes and this vomiting can be prevented by ablation of the AP but not by pre-treatment with drugs like Domperidone, Spiroperidol or Naloxone. Also receptors for PYY in the area postrema have been demeonstrated autoradiographically (Robertson et al., 1986). Peptide YY has alsobeen shown to increase blood flow in the area postrema by comparison to other circumrenticular organs (Tuor et al., 1988).

Interest in PYY as a potential candidate for the factor released from the gut by radiation which would act on the AP to cause vomiting lead us to be the first to test the compound for its effect in the ferret. At doses ranging from 1 - 10μgkg^{-1} i.v. we detected prodromata and found retching. Vomiting was infrequent and sporadic but when it did occur the latency was very short indeed (2.7min at 5μgkg^{-1}). This work has subsequently been repeated in the ferret and PYY was found to be about 36% effective (ie caused emesis in 4 out of 11 animals tested) in causing emesis at a dose of 3μg.kg-1, a dose similar to that in the dog, but under experimental conditions that the authors themselves control could have adversely affected an accurate result. So far the ferret and the dog are the only two species in which PYY

has been found to be emetic; interestingly, evidence for the mechanism of radioemesis in these species is at present conflicting, with the AP highlighted in the dog and the vagal afferents in the ferret.

In man evidence of the activity of PYY is scarce but Allen et al., (1984) have shown that the peptide (at very low doses, $2pmolkg^{-1}min^{-1}$) causes a significant delay in gastric emptying in man. No evidence of nausea or vomiting was noted at this dose in man.

However PYY is not the only candidate for a humoral mediator since neurons in the AP are excited by a wide variety of substances including excitatory amino acids, biogenic amines and several other neuropeptides (Carpenter et al., 1983). Two of these possibilities were pursued by Carpenter et al., in 1986. The evidence that prostaglandins, for instance, are good candidates is circumstantial, and preliminary, on the authors own admission, despite them having good credentials for such a function e.g. synthesised in response to injury, tissue concentrations rise after irradiation, time course of increase similar to latency of irradiation and excitatory at AP. In contrast, they also found support for a molecule that would block dopamine D_2 receptor activity in the way that they had demonstrated for Domperidone and which totally blocked radioemesis in the dog, as Dubois et al., (1984) had also found. Carpenter et al., 1986 also demonstrated that Domperidone prevented excitation of AP neutrons after irradiation. The simplest idea that the mediator molecule is in fact dopamine was discounted because of lack of an obvious source that does not

also store serotonin or histamine. Moreover, if Domperidone were blocking the action of a D_2 agonist at the AP in this situation one might have expected other D_2 antagonists to have been equally effective, which they are not, either in the dog or man. A peripheral site of action for Domperidone has to be considered perhaps as an agent that prevents the release of a mediator from the gut or simply as a regulator of gastrointestinal motility. Willems and Lefebvre (1986) maintain that such actions on the gut are via the D_2 receptors in the AP anyway. Whatever the role for Domperidone in the dog it is clearly not the same in the monkey (Dorval et al., 1985) and in the present studies we have shown that Domperidone is ineffective against radiation-induced vomiting in the ferret (as well as against cycloheximide, DAS and emetine). Perhaps there is some association between the fact that the AP appears to be so important to radioemesis in the dog and the fact that Domperidone so effectively blocks such vomiting in the dog too. Electrophysiological studies in the AP of the dog have also been carried out. Carpenter's group have reported results which give support to the idea that the AP performs a pivotal role in the genesis of radioemesis in this species (Carpenter et al., 1986). They demonstrated that AP neurons become spontaneously electrically active after irradiation during the 1 - 4hr post-exposure period. This activation correlated well with the onset of prodromata and frank vomiting in the anaesthetized dog.

In all the literature there is one reference alone to the situation in man. In Lindstrom and Brizzee's 1962 paper on the

removal of the AP from the brains of a small series of patients with intractable vomiting secondary to brain tumours, one patient underwent radiotherapy after the ablation surgery. Unfortunately the dose used was 4000cGy to the head alone which weakens any conclusions that might have been drawn but it is interesting to note that this patient did not vomit.

Since the cat appears to be dependent predominantly on the abdominal vagus for radioemesis, the monkey showing some dependence and our studies in the ferret revealing its reliance on peripheral mechanisms, we shall now examine the precise effect of the various peripheral autonomic nerve lesions and the related effects of putative anti-emetic drugs.

5.5 NEUROPHARMACOLOGICAL STUDIES OF RADIATION AND CYTOTOXIC DRUG INDUCED VOMITING

5.5.1 X-Radiation

Looking at the pattern of emesis in response to radiation (at 200 and 800cGy) in the ferret we found that most vomits occurred during the first 30min declining sharply thereafter (see Fig. 39). Lesions of the greater splanchnic nerves showed no effect on the incidence of vomiting at 800cGy. This is consistent with the result of electrical stimulation of the splanchnic nerves, which, unlike vagal stimulation, failed to show retching (Andrews et al., 1985). Following chronic abdominal vagotomy emesis to 200cGy was abolished and this leasion appeared to produce a permanent (tested after 6 months) protection against emesis evoked by this level of radiation (Fig. 40). In contrast, vagotomy resolved the emetic response to 800cGy into two parts viz. in the first 30min following radiation vomiting was markedly reduced or abolished, whereas

in the subsequent hour vomiting was comparable to control levels (Figs. 40 and 41). This resolution into vagally-dependent and vagally-independent phases has a number of implications for the mechanism of emesis. The results suggest that vomiting at a low dose of radiation (i.e. 200cGy) and the initial phase of vomiting to high dose radiation (i.e. 800cGy) are exclusively dependent on activation of vagal afferents. The 'vagally-independent' component of high dose radiation could then possibly be mediated by an action at an extra-abdominal site.

Two extra-abdominal sites for the initiation of radiation-induced emesis are worthy of consideration. Because of the implied involvement of vagal unmyelinated afferents with $5HT_3$ receptors, any area supplied by afferents with these characteristics could be involved in emesis if either 5-HT was released locally or could reach the receptor site via the circulation. Pre-release from the platelets or the gut producing an elevated plasma 5-HT concentration is a possible source. The heart has afferents which can be activated by $5HT_3$ receptor agonists and which can be blocked by the $5HT_3$ receptor antagonists which have been used in the present study (Andrews and Hawthorn, 1987). When activated these afferents evoke the Bezold-Jarisch reflex and this reflex has been used as a screen for $5HT_3$ receptor antagonists. The cell bodies of the relevant vagal afferents are located in the nodose ganglion. A large proportion of the cell bodies with unmyelinated axons respond to iontophoretically applied 5-HT and have $5HT_3$ receptors. 5-HT in the circulation could act here if it penetrated the blood-neurone barrier known to exist

around sensory ganglia. Could effects at these sites be responsible for the extra-abdominal site of radiation-induced emesis? The weight of evidence at present suggests not; the main reason being the data from experiments with 800cGy. Abdominal vagotomy merely reduced vomiting when tested at 7 - 10 days after operation. The residual response was not abolished by $5HT_3$ antagonists (Andrews and Hawthorn, personal communication) suggesting that even if radiation acts at the nodose ganglion or the heart it is not via $5HT_3$ receptors. Also if 5-HT is released systemically from the gut then not only should gut afferents be activated but extra-abdominal sites should be activated with a very similar time course and certainly not 30min later. Thus an involvement of the vagal $5HT_3$ system in this appears unlikely but a role for other agents for which the nodose ganglion has receptors (e.g. bradykin) cannot be excluded at present.

It is appropriate now to reconsider the general proposal that an endogenous humoral emetic mediator of radioemesis might logically act via the AP. As has already been alluded to, such an hypothesis has been proposed by Harding et al., (1984 and 1985) who suggested that the emetic agent could be Peptide YY. However, as stated, our own studies in the ferret failed to show this agent to be unequivocally emetic in this species and once again it is necessary to look for an alternative emetic agent to fulfill this function in the ferret. Some indications as to the nature of such a substance are provided by the pharmacological studies reported here. High dose Metoclopramide treatment ($2mgkg^{-1}$, 3 - 5 doses i.v.) had already been shown to be one of the more effective anti-emetic

treatments for cytotoxic-induced vomiting in man (Gralla et al., 1981 and Gralla, 1983). Our own studies then showed that this agent virtually abolished the emetic response to 200cGy and reduced that to 800cGy in the ferret (Fig. 44 and 45). The effect against 800cGy was confined to the initial phase of emesis leaving the second phase unaffected (Fig. 41). Thus in its actions against both 200 and 800cGy, high dose Metoclopramide mimicked the effect of abdominal vagotomy. It is worth noting that Domperidone was without effect against 200cGy (Fig. 44). Metoclopramide is well known as a gastrokinetic agent and in conventional doses (20mg.p.o. x 2) appears to act via the release of acetylcholine (McClelland and Sanger, 1983). At higher doses it acts as a Dopaminergic antagonist and also as an antagonist at $5HT_3$ receptors (Bradley et al., 1986, McRitchie et al., 1984 and Buchheit et al., 1985). As Metoclopramide is only anti-emetic to a significant extent at doses above those which are gastrokinetic, and Domperidone was ineffective under these circumstances, it appears likely that the high-dose anti-emetic effect of Metoclopramide is due to an interaction with $5HT_3$ receptors. This conclusion is further supported by the observation that the more potent prokinetic, the substituted benzamide BRL 24924 was shown to be a more effective anti-emetic than Metoclopramide as was the yet more potent and selective $5HT_3$ receptor antagonist BRL 43694 which is apparently devoid of prokinetic effects (Boyle et al., 1987; Miner etal., 1987).

Taking these observations together they firstly suggest that 5-HT is intrinsically part of and intimately involved in

the emetic response to radiation. As we have demonstrated that radiation-induced emesis at 800cGy can be divided into an initial vagally-dependent phase and a second vagally-independent phase (which may or may not be mediated via the CTZ of the AP) the results reported here suggest that radiation provokes a release of 5-HT which causes activation of abdominal vagal afferents during the initial phase. Moreover it is known that during X-irradiation the 5-HT content of enterochromaffin cells of the intestine falls significantly (Matsuoka et al.,1962) and more recently an inhibitor of 5-HT synthesis, para-chlorophenylalanine (PCPA), has been used to antagonize cisplatin-induced emesis in the ferret (Barnes et al., 1987).

The 2-DG studies reported have shown that the AP in the ferret is also activated by radiation-induced vomiting. These studies do not tell us the mechanism of the activation but nevertheless provide important supportive evidence for the involvement of the AP in at least part of the reflex response to this stimulus, in the ferret.

Confirmation of the validity of the data from the present study on the effect of the prokinetic and $5HT_3$ receptor antagonist BRL 24924 against 200cGy radiation in the ferret has come from Andrews et al., (1986) and Miner et al., (1987) and confirmatory work on the effect of BRL 43694, the more highly specific $5HT_3$ receptor antagonist, has been done by Boyle et al., (1987).

Our results with Domperidone against 200cGy of X-rays in the ferret are in contrast to those of Dubois et al., (1984) in

the dog and Gylys and Gidda (1986) in the ferret, but in agreement with those in the monkey (Dorval et al., 1985). If anything we found that Domperidone made retching worse after irradiation. The failure of dopamine antagonists like Domperidone to effectively prevent cisplatin-induced emesis in man (Tonato et al., 1985 and Saller et al., 1985) has been borne out in these experiments and also confirmed previous suspicions that Metoclopramide was not exerting its activity through an effect on D_2 receptors in the AP, for instance.

As we have already said it has been suggested (Alphin et al., 1986) that Metoclopramide acts as an anti-emetic through its gastrokinetic effects. However it is clear that at doses at which Metoclopramide can be shown to be clearly gastrokinetic it has no concomitant anti-emetic activity (Brogden et al., 1982; Harrington et al., 1983). That is not to say that use of a drug that is gastrokinetic in order to stop disordered gastrointestinal motility is not advantageous in situations where such dysrhythmias are the concomitants of nausea and vomiting (Geldoff et al., 1986).

Conclusions

The results reported here on the effect of BRL 43694 and BRL 24964, clearly demonstrate that at higher doses of radiation there is a portion of the radioemetic response that is blockable by BRL 43694, a $5HT_3$ receptor antagonist which only has documented activity against $5HT_3$ receptors. This component has been referred to as the vagally-independent component of vomiting, the implication being that such vomiting depends on a humoral mediator (as yet unidentified), released into the circulation and probably acting via the AP (see also

Andrews et al., 1986 and Andrews and Hawthorn, 1988). At the low dose of radiation vomiting is dependent entirely upon vagal integrity and completely blockable by $5HT_3$ antagonists and we are led to the conclusion that radiation somehow provokes the release either directly or indirectly of 5-HT from the gut which subsequently evokes an abnormal discharge in the abdominal vagal afferent nerves.

5.5.2 Cytotoxic Agents

We also had the opportunity to investigate the effect of Domperidone, Metoclopramide, BRL 24924 and BRL 43694 on vomiting produced by Cycloheximide, DAS, Emetine and Mustine.

As with radiation, Domperidone was found to have no beneficial effect on vomiting in response to this group of cytotoxic agents, which bears out the findings of others in animal models (Dorval et al., 1985; Miner et al., 1987). Indeed there was a paradoxical shortening of latency to vomiting with DAS, similar to the worsening of retching noted at 200cGy treated with Domperidone. Domperidone however, always remained effective against apomorphine-induced vomiting with or without the presence of the abdominal vagus. Dopamine D_2 receptors are clearly implicated in this response in the ferret but they do not appear to play a significant role in cytotoxic or radiation-induced vomiting in this animal model and it implies once again that the anti-emetic action of Metoclopramide, from wheresoever it may be derived, is not dependent on antagonism of such receptors either peripherally or at the AP.

For the range of cytotoxic agents that was studied in the work reported herein Metoclopramide in high doses showed no

clear effect on cycloheximide, DAS or emetine-induced vomiting. However, in the case of cisplatin and mustine, as with radiation, there was a significant effect with virtual abolition of emesis in both cases. As vagotomy successfully reduced emesis to all stimuli in these categories it was somewhat unexpected that Metoclopramide did not in the case of cycloheximide, DAS and emetine. There is no evidence as far as we are aware in the literature of the use of anti-emetics against vomiting due to this group of cytotoxics, with which to compare our results.

Miner et al. (1986) reported the effectiveness of Metoclopramide against cisplatin-induced vomiting in the ferret, corroborating our results and prompting the investigation of the anti-emetic potential of other candidate $5HT_3$ receptor antagonists. The opportunities of the present study were limited to the investigation of the effect of BRL 24924 (the compound with gastrokinetic as well as $5HT_3$ antagonist properties). It was shown that although vomiting could not be prevented with the use of BRL 24924, there was a large and significant decrease in emetic potential and a corresponding increase in latency, a pattern much closer to that produced by vagotomy than possible with Metoclopramide. The effectiveness of $5HT_3$ receptor antagonists was demonstrated subsequently in the ferret against cisplatin (Miner and Sanger, 1986; Miner et al., 1986; Miner et al., 1987; Costall et al., 1986; Andrews et al., 1987) and cyclophosphamide (Hawthorn et al., 1988) and mustine, dacarbazine, doxorubicin and actinomycin-D (Smith et al., 1986) in the dog. The more selective and potent $5HT_3$ receptor antagonist BRL 43694 which we showed had

impressive anti-emetic effectiveness against low and high-dose radiation, has also been tested against cytotoxic drugs and found to be effective for cisplatin, cyclophosphamide and doxorubicin vomiting (Boyle et al., 1987).

The evidence is building up now from many sources other than those reported here to demonstrate the effectiveness of the $5HT_3$ receptor antagonists as potent antiemetics against a wide range of cytotoxic drugs. Moreover it is important to note for the possible implications for clinical application that such drugs are able, when given intravenously to intervene rapidly during the course of a vomiting bout to prevent further vomiting (Miner and Sanger personal communication; Andrews personal communication) in a manner that has never been reported with any other anti-emetic compound.

Although the majority of the data have been obtained in the ferret model where we have shown the vagus to be an important requirement for cytotoxic and radiation-induced vomiting, that from the study of Smith et al., (1986) was obtained using the dog, but as we have illustrated the dog shows a great dependence on the AP for the integrity of its vomiting response to cytotoxics and radiation. So, despite the interspecies differences which we have discussed with respect to the relative importance of the AP or the gastric afferents as sites of action for cytotoxic drugs and X-radiation, antagonism of $5HT_3$ receptors appears to be effective in both systems, indicating perhaps that wherever they might be located, such receptors perform a key role in the emetic pathway.

However, referring back to our result for high-dose radiation in the ferret, it will be recalled that BRL 43694 was apparently able to abolish the so-called 'vagally-independent' phase of vomiting such that we are able to say that even in the ferret there must be a system for the induction of radioemesis that does not rely on the integrity of the vagus but is still blockable by $5HT_3$ receptor antagonists. Our work in the ferret at low doses of radiation (200cGy) demonstrates that such a mechanism does not appear to come into operation until the emetic stimulus is great enough to induce it. These experiments suggest that both low and high doses of radiation evoke the release of a neuroactive agent which can act locally to activate vagal afferents but which can also be released in greater quantity so that it enters the circulation perhaps to activate the AP directly. The hypothesis of further extra-abdominal and possibly central sites of action for $5HT_3$ receptor antagonists which might account for these findings has been discussed recently (Andrews and Hawthorn 1987).

Work reported earlier in this thesis on the effect of vagotomy on sodium chloride and copper sulphate induced vomiting in the ferret, lends support to the idea that some kind of humoral emetic mechanism may be inducible; for we found that having abolished such vomiting in the short term by vagotomy, a short latency emetic response appeared when the animals were re-tested sometime later. It could be that we were observing in these experiments the establishment of the kind of humorally-based emetic mechanism that we suppose to be the norm that has been observed in the dog, where the vagus has been shown to be relatively unimportant, specifically in

radiation-induced vomiting. That the dog also posesses an established vagally-dependent emetic mechanism for purely intragastrointestinal emetic stimuli is in no doubt (Wang and Borison 1951). Moreover $5HT_3$ receptor antagonists appear to be ineffective against such stimuli (Sanger, personal communication) implying that the intraluminally-based system for toxin detection is "hard-wired" and that this pathway does not rely on $5HT_3$ receptors. However, this evidence does not necessarily undermine the hypothesis that $5HT_3$ receptors perform an important function in the emetic mechanism since such intragastric emetics can cause vomiting from an action on the stomach alone where there is little 5-HT present (Kayashima et al., 1978; Andrews and Wood 1988). Even after pyloric ligation ferrets will still vomit to intragastric sodium sulphate (Andrews, personal communication).

Crucially although the evidence from the work presented here on the effects of vagotomy and $5HT_3$ receptor antagonists on radiation-induced vomiting in the ferret appears to point to a twin central and peripheral site of action for the $5HT_3$ receptor antagonists, very recent evidence (Andrews, personal communication) enables us to view our results on radiation and cytotoxic-induced vomiting in a different light. Thus, it has now been demonstrated that administration of BRL 43694 ten days after abdominal vagotomy and just prior to irradiation produces no change to the pattern of vomiting at 800cGy. If BRL 43694 were to have been acting at a remote site to block $5HT_3$ receptors then we would have expected this vagally-independent phase of vomiting to have consequently been abolished, which it was evidently not. The most plausible conclusion is that

vagotomy promotes the formation of compound whose action is not blockable by $5HT_3$ receptor antagonists. The release of this substance is, moreover, dependent on the degree of emetic stimulus to which the animal is subjected, since for instance vomiting induced by 200cGy is abolished permanently by vagotomy, an effect exactly paralleled by administration of the $5HT_3$ blocker BRL 43694, but similar testing of vagotomised ferrets with 800cGy after three weeks showed that the residual vomiting was still present (Andrews, personal communication).

Thus we see that for a relatively low intensity radiation insult, vomiting in the ferret is dependent on a mechanism that is reliant upon the integrity of the abdominal vagus and blockable by $5HT_3$ receptor antagonists, a situation that is paralleled by that for cytotoxic drugs. At high doses of radiation in the ferret two mechanisms appear to be operating i.e. the vagally-dependent $5HT_3$ blockable mechanism equivalent to that observed at low doses of radiation, and in addition, a vagally-independent mechanism not blockable by $5HT_3$ antagonists relying on a novel humoral mediator released only after a sufficiently powerful emetic stimulus is applied.

As far as the site(s) for the target of $5HT_3$ receptor antagonists is(are) concerned some progress has been made recently. Neuropharmacological studies have demonstrated the presence of $5HT_3$ receptors on vagal afferent and enteric neurons (Fozard 1984; Richardson et al., 1985; Round and Wallis, 1988; Ireland and Tyers, 1987). The existence of such receptors was first suggested by Gaddum and Hameed in 1954 and described in more detail by Gaddum and Picarelli in 1957 when the receptor subtype 5HT-M, later to be re-classified $5HT_3$

was given its designation (Bradley et al., 1986). Additionally as has already been pointed out it had earlier been noted that 5-HT could evoke discharge of vagal afferent C-fibres (Paintal, 1964) and of particular interest to our studies is the evidence that 5-HT levels fall significantly in the enterochromaffin cells of the gastrointestinal tract during X-irradiation (Matsuoka et al., 1962). Plenty of evidence exists for the presence of 5-HT in the dorsal vagal complex and its presence specifically within the AP has been confirmed (Leslie, 1987). Indeed there is even some suggestion that 5-HT may be directly associated with vagal nerve fibres. However, until very recently $5HT_3$ receptors in particular had not been identified in the central nervous system. At the end of 1987 Kilpatrick et al., described the first experimental evidence for the existence of the $5HT_3$ binding sites (receptors) in rat brain tissue, and there are indications that the AP has the highest level of $5HT_3$ binding sites. In any case in view of the ideas that we have put forward above it may not even be necessary to postulate an extra-abdominal site of action for $5HT_3$ receptor antagonists. As these drugs also have behavioural effects it may well be that such $5HT_3$ receptors if they are confirmed to exist in the CNS subserve an entirely different function to emesis. The functional significance of medullary $5HT_3$ receptor sites and their relationship to emesis still remains to be elucidated.

What we do not have at present is a satisfactory way of linking our new-found knowledge of the role of $5HT_3$ receptor antagonism, vagal function and peptide YY action in cytotoxic and radiation-induced vomiting to an hypothesis such as that

proposed by Harris (1982). This seeks to explain the spectrum of emetic potency of a variety of emetic agents on the basis of differential effects on rapidly turning over enzyme systems that are responsible for the breakdown of a key neurotransmitter which stimulates receptors in the CTZ of the AP when its concentration rises in response to emetic challenge. Perhaps as we have suggested this system is to be found in the gut at the interface with the vagal afferent endings, and that here the transmitter is 5-HT. Perhaps, as Miner et al (1987) have suggested, the balance between the extent to which this enzyme system is inhibited by any particular cytotoxic drug regime and the varying rates of 5-HT release or synthesis explains the different latency periods and potency obtained with different cytotoxic drugs. Some support for the concept of a link between the role of 5-HT in emesis and a possible effect of cytotoxic agents on 'critical enzymes' can be gleaned from a paper by Goldstein and Goldstein (1961) who suggested that narcotics for instance act by inhibiting a rate-limiting enzyme, the end-product of which is necessary for normal brain function. Way et al., (1968) further suggested from the evidence that inhibitors of protein and RNA synthesis diminish the development of tolerance and physical dependence to morphine, that the proteins affected may be enzymes associated with 5-HT (Ronnback and Hansson, 1986).

Despite our lack of understanding, preliminary clinical studies with several $5HT_3$ receptor antagonists (ICS 205-930,

GR 38032F and BRL 43694) have shown their potential for controlling cytotoxic drug-induced emesis (Leibundgut and Lancranjan, 1987 and Cunningham et al., 1987). In the first of these studies vomiting was prevented in 31 out of 47 courses of high-dose cisplatin (in combination with other chemotherapeutic cytotoxic drugs); the authors comment that these results compare favourably with those for high-dose metoclopramide and add that treatment seemed to be free from side effects. In the second study patients who had displayed vomiting refractory to first line antiemetics were chosen. Thirty one courses of cytotoxic chemotherapy were given involving the use of combinations of drugs including among others cyclophosphamide. Of these only one patient experienced nausea and vomiting during the 24 hours following the treatment whereas they had previously found domperidone, metoclopramide and dexamethasone largely inaffective. The authors point out that such successful antiemesic activity has not previously been achieved in this situation by the use of a single drug and indeed combination anti-emesis has met with only limited success. Side effects were virtually absent in these pilot studies, the results of which have now been substantiated with data from a number of very recent trials in man against cytotoxic chemotherapy-induced emesis (e.g. Carmichael et al., 1988; Gralla et al., 1988; Joss et al., 1988) and against radiotherapy induced emesis (Priestman et al., 1988).

Complete control of vomiting to the various chemotherapy treatment regimes employed in eleven of these recent trials was as high as 85% on the first day of treatment. Mild headache and some diarrhoea/constipation were reported but these side effects have not been reported in human volunteers receiving BRL 43694 alone for instance. The only study on the effect of $5HT_3$ antagonists against radiation-induced emesis in man (Priestman et al., 1988) treated 23 patients with 800 -1000cGy to the upper abdomen. Complete abolition of vomiting was achieved over the first three days post-irradiation in 70% of patients with 96% having less than 3 episodes of emesis over that time period. This result compares very favourably with the 50% control achieved with metoclopramide or nabilone. No significant side-effects were noted in this series of radiotherapy patients. Most interestingly, noted in a study of the pharmacokinetics of BRL 43694, is the fact that the duration of action of this drug exceeds its life in the plasma (Sanger, personal communication).

These initial data bear out those from animal studies in respect of efficacy in particular. Moreover, the ferret has showed its ability to be a good predictor of results in man proving its usefulness both in the study of mechanism of cytotoxic drug-induced and radiation-induced emesis.

5.6 2-DEOXYGLUCOSE INVESTIGATIONS OF CENTRAL EMETIC CONTROL

5.6.1 Application of 2-DG Methodologies in the Ferret Model

The justification for the modified approaches used in these studies rests on the fundamental assumptions that (a) upon sacrifice of the animals all isotope in the brain tissue is in the form of phosphorylated 2-DG with little or no free 2-DG and (b) that a simple correlation exists between brain tissue glucose use, total isotope concentrations at sacrifice and the optical densities of resultant images on photographic emulsion (McCulloch, 1982).

With i.v. administration using a dwell time of 45 minutes in normoglycaemic animals, residual 2-DG is very low in brain regions of high glucose use. However, this assumption may be less valid in certain other situations. First, in an area of low metabolic activity e.g. white matter (where ^{3}H-2-DG is quenched causing artificially low OD readings), the ratio may be distorted because densitometry measures the effect of free radioactive 2-DG in the cell as well as that of the phosphorylated form which alone reflects the true metabolic activity of the cell. Secondly, the fraction of total radioactivity which is present in the form of unphosphorylated 2-DG is increased by moderate hyperglycaemia (Kelly and McCulloch, 1983). This may affect the result in two ways: 1) high levels of non-radioactive glucose in the plasma compete with the radioactive tracer for entry into the brain cells throughout the experimental period, thus "blunting" the effect of the stimulus and resulting in high levels of residual

2-DG in brain cells at the point of death, 2) this in turn leads to an influx of 2-DG into brain cells (resulting from death) and this unphosphorylated tracer is measured by densitometry along with the phosphorylated 2-DG with the effect described above. Thirdly, the use of the intraperitoneal route of delivery of the isotopic tracer rather than the intravenous route may give rise to higher levels of unphosphorylated 2-DG at the end of the experimental period, i.e., the point of sacrifice of the animal (Kelly and McCulloch, 1981). This may lead to false conclusions from the comparisons of optical density (O.D.) ratios, as the numerator and denominator are affected to varying degrees by the inclusion within the optical density readings of an element due to the residual unphosphorylated 2-DG.

Finally, consideration must be given to the semi-quantitative technique itself. The most widely used semi-quantitative index is the O.D. ratio of the region of interest relative to some notionally unaltered region. However, there is evidence that a fixed ratio of isotope concentration in the CNS will not yield consistant O.D. values in separate experiments, because these are dependent upon the exposure time of the autoradiographs and the absolute amounts of isotope used (Kelly and McCulloch, 1983).

In any case, the relationship between tissue isotope concentration and O.D. is sigmoidal rather than linear (over the range 40 - 130 nCi/g for ^{14}C). Consequently, O.D. ratios will not necessarily reflect simply relative tissue isotope

concentrations, but being a function of absolute tissue isotope levels, will be altered by the position of the points of inflection of the curve. Furthermore, the shape of the O.D. vs tissue isotope concentration curve may be radically altered by varying the time for which the X-ray film is exposed. As a result the O.D. of a brain area may be significantly affected by altering the exposure time of the autoradiographs.

White matter has an intrinsically low and stable metabolic activity. This factor is often exploited in the construction of reliable O.D. ratios. However, this low metabolic rate gives rise to the possibility that residual un-phosphorylated 2-DG is higher in white matter. This potential source of problems (McCulloch, 1982) is unavoidable in the practical sense, but is presumably constant in any series of animals in which 2-DG autoradiography is performed with the same conditions and stimuli. In some cases, however, it is possible to avoid such problems by using left-right comparisons within sections as controls rather than between animal comparisons using reference to white matter.

Intraperitoneal injections have been criticised on the grounds that they leave a large percentage of unphosphorylated 2-DG at the end of the 45 minutes experimental time period (Kelly and McCulloch, 1981 and 1983). However, other investigations disagree with this conclusion (Meibach et al., 1980) and Kelly states that, if the route of administration alone is altered whilst the full quantitative protocol is followed, then the final rate of glucose

utilisation as calculated from the operational equation is relatively unaltered particularly for areas of high glucose use (Kelly, 1982).

Hyperglycaemia "blunts" the effect of the experimental stimulus by competing for uptake into the brain throughout the experiment and this results in high residual levels of unphosphorylated 2-DG at sacrifice. This distorts the results of densitometry, as it is measured along with phosphorylated 2-DG. If levels of hyperglycaemia vary markedly throughout the experimental time period and between animals and groups, then the situation is further complicated. Every attempt must therefore be made to maintain stable and comparable normoglycaemia in individual animals and between individuals and groups. This involves using a suitable period of pre-experimental withdrawal of food which is long enough to allow plasma glucose to stabilise at a low level but short enough to prevent brain cell metabolism switching to other sources of energy. Stress from operative procedures, animal handling, and the laboratory environment, should also be avoided as far as possible.

Semi-quantitative technique. O.D. ratios based on the areas of interest compared to white matter have been criticised on the grounds that the resulting index will vary as a function of the duration of exposure of the X-ray film and as a function of the absolute tissue isotope concentrations. However, more recent work by Mitchell and Crossman (1984) and Sharp et al., (1983) has shown that these criticisms are

invalid. Mitchell and Crossman (1983) assert that the variation in O.D. values reported by Kelly and McCulloch (1981) were due to a failure to subtract the background O.D. of the X-ray film from the O.D. values of the areas in question. Sharp et al., (1983) did so in the calculation of their data and ratios of the O.D's. of predetermined fixed ratios of ^{14}C concentrations derived from their data are indeed constant, though not across the whole range. It thus appears that the O.D. ratios can be used as a relative index of isotope concentration which is independent of variations in both exposure time and the absolute levels of isotope provided the X-ray film itself is not approaching saturation.

Furthermore, Sharp et al., (1983), showed that O.D. ratios provide a good index of local cerebral glucose utilisation and allow comparison between animals or groups of animals provided they are treated throughout the experiment in exactly the same fashion and maintained in the same physiological state. This can only be objectively confirmed if blood glucose is measured concurrently. Comparisons of the functional state of neural structures can be made using O.D. ratios between groups of animals in different physiological states but it is important to note that, under these circumstances, O.D. ratios do not provide an accurate index of local cerebral glucose utilisation. Moreover, the above criticisms do not take account of the fact that many investigators, as with the present study, want an index of functional activity, not a measure of absolute glucose use. Where the 2-DG technique is

being used to detect changes in the behaviourally relevant activity of discrete neural systems, appropriately chosen normalised indices as described can be preferable to estimates of rates of glucose use.

In studies concerned with the behavioural correlates of neural activity the reason for transforming optical density is to remove extraneous sources of variance in the data used for between-animal comparisons.

The variance introduced by these extraneous factors that affect the overall darkness of the radiographic image degrades the power of statistical comparisons between control and experimental animals (Gallistel, et al., 1982). Normalizing transformation using optical density ratios removes errors due to variations in dose of isotope tracer, thickness of cryostat section, X-ray film development anomalies, and plasma glucose levels.

In removing the variance due to these extraneous factors a normalizing transformation may be preferable to transformation into rate of glucose utilisation for the following reasons:-

1) The experimental procedure is simplified and does not necessarily require arterial cannulae, venous cannulae, frequent blood sampling, measurement of plasma glucose, measurement of plasma radioactivity, fitting of a curve to the values for isotope concentration as a function of time and the computation of the integral of this curve, measuring of the

optical density of the standards and derivation of a factor relating this to concentration of isotope. 2) It involves fewer measurements and hence less measurement error which, in any case, can be experimentally estimated in each animal. The only measurement errors entering into a normalized index are those involved in measuring O.D. (via the grey levels) which may be estimated by making repeated measurements. 3) It can remove the influence of any factor affecting the measured darkness of the image as a whole (e.g. variations in section thickness), whereas estimating rate of glucose use only removes the influence of some of these factors.

Use of normalized indices cannot be made without thought being given to possible sources of artifact. For example; O.D. ratios may be undesirable where there are substantial differences in the overall darkness of the images from different brains. Secondly, the index suffers from "ceiling effects" and the relationship breaks down when comparisons are attempted between structures already in the darkest part of the section. This is the region where the OD vs isotope curve is not linear. Finally it is always just possible that the experimental effect is upon the stable area or 'norm' that is being used as the invariable factor in the calculation of the ratio!

A summary of the details of the modified 2-DG techniques as used in the present study is given in Table 22.

TABLE 22

Experimental Measures and Analytical Approaches Employed in the Present Study

i. Maintenance of normoglycaemia - to minimize residual 2-DG and the competition of non-radioactive glucose.

ii. Extension of experimental time for i.p. experiments - to ensure minimal levels of residual 2-DG. Use of i.p. route to match blood profile of 2-DG to nature of stimulus and response.

iii. End stage blood sampling - to check on levels of residual 2-DG and plasma glucose.

iv. Intermittent blood sampling in the anaesthetized preparation - to check on levels of plasma 2-DG and plasma glucose.

v. Use of radioactive standards in the autoradiographs - to check response curve of 2-DG to optical density.

vi. Independent studies on blood glucose profile in the ferret - to have a standard with which to compare the results of plasma glucose measurements made under experimental conditions.

vii. Simplification of experimental procedures - to minimize stress and consequent hyperglycaemia e.g. i.p. administration in the free-running animal.

viii. Standardization of experimental conditions - to minimize the effect of inter-group differences.

ix. Normalization of data by construction of O.D. ratios - to remove variance due to factors discussed above.

x. Standardization of all autoradiographic procedures - to eliminate substantive difference in overall darkness of the images from different brains, allowing greater confidence in the application of normalization.

xii. Choice of isotope dose, exposure time and film type - to ensure "ceiling effects" do not degrade the normalization procedure.

In this study, despite efforts to do so, a free running system in the conscious ferret in which continuous blood sampling could take place could not be set up and the option of working on the anaesthetised animal was pursued initially. Very recently work by Harding and colleagues has shown how such a restraint system, in this case of the hindquarters, (as commonly used in the rat studies with ^{14}C-2-DG) profoundly affects the emetic response of the ferret (Harding, personal communication, 1987; Tuor, et al., 1987). However, it has proved possible according to this group to carry out multiple blood sampling in the conscious ferret using a sling-runner mechanism to which each ferret is acclimatised over a period of time.

Initial experiments in this study used ^{3}H-2-DG and produced visually good autoradiographic images. These however, suffered from the disadvantages discussed above and resulted in obvious false negatives. Despite such disadvantages and the long photographic development time we showed that we were able to detect significant changes in the brain stem nuclei of interest.

Experiments designed to investigate ferret blood glucose levels under several circumstances were done in the present study. A normal value was determined for the population of ferrets used and then this was compared with the effect of feeding, anaesthesia and eventually all the other emetic stimuli employed. This confirmed that the variations found under anaesthesia were sufficient to 'blunt' the effect of an emetic stimulus.

It was then decided to modify the technique to take account of the problems discussed above, and so whilst remaining with a semi-quantitative approach we adopted the use of the ^{14}C-2-DG isotope intraperitoneally admininstered with a dwell-time of 60min. This was intended to reduce white matter quenching and match the stimulus to the blood 2-DG profile, to reduce residual plasma radioactive 2-DG and to decrease the turnaround time for the experiments. Our principal interest was to study cytotoxic drugs and X-radiation and it was also hoped therefore that these profound emetic stimuli would lend themselves more readily to detection though changes in activity of brain stem nuclei than briefer and less severe stimuli.

We took three stimuli in this category each with different characteristics. Cycloheximide represents a class of substances which are cytotoxic, strongly inhibitory of protein synthesis, and analagous to other compounds like DAS and Emetine, also cytotoxic and highly emetic. Mustine represents a class of substances which are cytotoxic, thought to act as alkylating agents, highly emetic and possibly analagous to ionising radiation in their effects on living organisms. X-radiation is highly emetic in the ferret, is cytotoxic, affects DNA directly (Boon et al., 1984) but also a constellation of other systems and is unique in character in exposing all somatic systems instantaneously and comprehensively.

Cycloheximide produces a substantial amount of vomiting in those animals also given ^{14}C-2-DG during the autoradiographic experiments. Changes in activity in the AP, NTS, DMVN were

all, surprisingly, less than 5% and were not statistically significant. Changes in these nuclei after administration of other emetics were easily detectable so that this negative result is probably not due to experimental error.

The reason for this result is not clear but several possibilities warrant consideration. Firstly, partial activation of a brain region may yield an undetectably small alteration in 2-DG uptake (Lindroos et al., 1986). Further, a change in the electrical activity of neurons is not always reflected as a detectable change in 2-DG uptake (Prohansky et al., 1980).

Yet again, it maybe that recent suggestions by Carpenter and Co-workers (Auker et al., 1983) made after studying the visual and infra-red detection systemsof the rattlesnake will prove to apply for the ferret AP and its associated nuclei. They have attempted to explain an apparent discrepancy between single-unit activity and ^{14}C-2-DG labelling in the optic tectum by postulating that in this case in addition to action potential, excitatory synaptic potentials contribute significantly to neuronal Na^+ load and that either, not all synaptic excitation is mediated by increases in Na^+ conductance or that excitatory synaptic activity is not necessarily reflected in the number of action potentials generated. Carpenter has further suggested that the ionic mechanism underlying AP neuron excitation is a K^+ conductance decrease (known to be involved in the generation of some responses to serotonim (Gerschenfeld et al., 1974) and -aminobutyric acid), a conclusion made partly from knowledge of the effect of cyclic

AMP on AP function here he has shown it to be a good candidate for common emetic second messenger (Carpenter, personal communication, Carpenter et al., 1988, Deterre et al.,1982).

Lastly there is the possibility that cycloheximide, through an inhibitory effect on brain protein synthesis, may interfere with the process of uptake and phosphorylation of 2-DG by neurons. Evidence discussed by Nudo and Masterton (1986) which suggests that 2-DG labels active terminals more than somata would locate the site of the effect of cycloheximide at the synapses where processes of transmitter re-uptake, and re-synthesis occur as well as activation of any second messenger systems. Glucose utilisation in the neuron occurs in many processes: For example the Na^+/K^+ ATPase pump, protein synthetic reactions and a host of other metabolic reactions. Under stable conditions one might postulate that compared with 'pump' requirements the others would be reasonably constant and therefore increased requirement for neuronal firing would result in increased glucose uptake (Mata et al., 1980), a phenomenon that we see reflected in increased 2-DG uptake under experimental conditions. Further, if however, protein synthesis were markedly reduced (by cycloheximide for instance) then little net change in 2-DG uptake may be detected because the large decrease in energy requirements for protein synthesis might balance out the increase required for neuronal firing.

Cycloheximide in the rat alters the behaviourally excitant effects of serotonin (5-HT) (Grahame-Smith 1972) and has been shown to inhibit dopamine release in brain tissue

(Grahame-Smith and O'Shaughnessy 1985). Analagous protein synthesis inhibitors like emetine also block transmission in sympathetic nerve endings (Ng, 1968) and reduce adrenergic neuronal transmission (Achari et al., 1972). Thus involvement of brain protein synthesis in neurotransmitter function (Grahame-Smith, 1986; Green et al., 1976) may also explain why the effects on the brain of cycloheximide and other powerful inhibitors of protein synthesis are not detectable using 2-DG autoradiography. These ideas are also discussed in Setion 5.6.2..

The poor emetic response after mustine administration was surprising in view of its previously reliable record in producing vomiting, although all the animals did, in fact, display prodromata. A significant change in metabolic activity was detected in the AP (50%) but not in the NTS or DMVN, a situation similar to that observed after apomorphine stimulation under anaesthesia. During the mustine experiment however, the detected change was much larger and moreover the blood glucose remained within normal limits. Time constraints precluded attempts to carry the topographical analysis further to subdivide the NTS for instance, which would be a profitable exercise, in view of the known 'delegation' of tasks within the nucleus to specific sub-loci. Future studies would include further implementation on a routine basis of the computerised histology/autoradiography superimposition techniques in order to refine analysis of the autoradiograms. Use of newer techniques becoming available with increased computing power, to create 3-D images based on parameters such

as O.D., may allow the easier detection and visualization of changes in activity along the longitudinal as well as the horizontal and vertical axes. This may well reveal changes that are currently being inadvertantly hidden by the 'averaging' technique such as that which we employed in order to reduce sampling errors.

As expected 800cGy of X-radiation produced vomiting in all animals in the group exposed to the 2-DG probe, in much the same way as cycloheximide did in the experiment discussed above. Similarly end-stage blood glucose was raised and yet in this case we were able to detect significant increases in relative metabolic activity in several of the targetted nuclei i.e. AP and DMVN. Experiments in the rat (Ito et al., 1986) have shown that at 4 days post-exposure to radiation the brain as a whole takes up 15% less ^{14}C-2-DG than control brains and this depression in glucose utilisation is spread in an apparently random fashion throughout a wide variety of brain structures. Bearing in mind that the 2-DG determinations in that study were carried out a long while after irradiation by comparison to our experiment there is no real evidence from our results that any general depression of glucose uptake has affected the validity of our approach i.e. radiation does not appear to depress brain 2-DG uptake in the time-frame of our experiment. Moreover, the moderate background hyperglycaemia, provoked probably by the stress of the severe vomiting, did not limit the ability of the technique to detect significant changes in relative metabolic changes in the brain stem nuclei under observation.

In summary, the adoption of the ^{14}C-2-DG, 60min, i.p. semi-quantitative technique is well adapted to investigation of emetic-associated brain activity. Use of the ^{14}C isotope also lends itself to full quantification should the requirement arise. The i.p. route does favour a blood 2-DG profile that better fits the type of stimulus employed in these experiments (Meibach et al., 1980) but it might be possible, using a free running gantry-mounted indwelling catheter system, to co-ordinate the giving of the 2-DG bolus with the onset of the vomiting response and thereby establishes i.v. methodology in the ferret.

Although ^{3}H-2-DG autoradiograms appear to give better contrast and better resolution, the numerical results derived from them are prone to error for the reasons we have previously outlined, and for such reasons ^{14}C-2-DG is preferable for quantitative autoradiography.

5.6.2 2-DG Studies of the CNS Response to Emetic Stimuli in the Ferret

It had previously been established that electrical stimulation of the central cut end of the ventral abdominal vagal trunk of the ferret elicited retching, a reaction that was not produced by stimulation of the left greater splanchnic nerve (Andrews et al., 1985). There is mounting neuroanatomical evidence concerning the central connections of the vagus in a variety of species (for a brief review see Leslie and Gwyn, 1984). This has shown that the afferent fibres of the vagus terminate in the caudal portion of the NTS and in the AP. A heavy concentration of these afferents is located in the most dorsomedial region of the NTS called the parvocellular subdivision, subnucleus gelatinosu, or area subpostrema (Gwyn and Leslie, 1979). The larger proportion of such afferents projecting to this region of the NTS is derived from the stomach wall (e.g. Gwyn et al., 1985) and gastric afferents have been described as projecting directly to the AP (Kalia and Mesulam, 1980). It was an aim of this study to see what functional links might complement these anatomical ones using 2-DG as an index of neuronal metabolic activity.

Electrical stimulation of the abdominal vagus of the ferret produced significant increases in activity in the AP, NTS and DMVN providing for the first time evidence of functional connectivity in this system (Andrews et al., 1986). It is impossible from this study alone to distinguish between primary activation of the NTS or AP neurons by vagal afferents and a secondary activation via one or the other. Nevertheless

this remains apparently, the only piece of evidence showing that the AP is involved in emetic behaviour provoked through stimulation of vagal afferents. It does provide support for the idea that vomiting evoked via the vagus is mediated via the AP.

AP ablation sometimes protects against radiation-induced vomiting (Brizzee, 1956; Brizzee et al., 1958; Chinn and Wang, 1954; Harding et al., 1985) or motion sickness (Brizzee et al., 1980 and Wang and Chinn, 1954) and it is therefore possible that the 2-DG data showing the involvement described above mean that the AP is involved in the pathways causing vomiting under such circumstances irrespective of its function as a chemoreceptor trigger zone.

Gonzalez et al., (1986) examined the effect of gastric distension on 2-DG uptake in the medullary nuclei of the rat. They used 20ml of water to distend the stomach of a 200g rat, an extreme level of expansion of the stomach wall which would almost certainly have non-specifically stimulated gastric sensory receptors not involved in the detection of distension. By analogy with the ferret for instance, such a degree of distension would almost certainly have provoked vomiting. Nevertheless this study did show increased metabolic activity in the dorso-medial region of the NTS (underlying the AP) and the commissural subnucleus of the NTS, areas previously shown to receive gastric afferent sensory information. Interestingly, there are indications that the DMVN was activated in the stimulated animals; no information was given on the AP which, although illustrated in autoradiograms, did not appear to have been measured.

The work of Kostreva (1982) in the dog and the cat and Ciriello et al., (1983) and Savaki et al., (1982) in the rat has shown that electrical stimulation of the cervical vagi, which produced a pressor response, resulted in increased 2-DG uptake in the medial dorsal and dorsolateral subnuclei of the NTS near the obex. There were also increases in the AP. The depressor reflex that was elicited produced changes in the dorso-medial subnucleus of the NTS, and stimulation of the carotid sinus nerve primarily increased activity in the dorsal and dorsolateral subnuclei of the NTS. These studies were the first to demonstrate the use of 2-DG to map the central pathways of cardiovascular reflexes, and the results largely substantiated the data obtained from other technical approaches. These results are of interest for two reasons. The first is a technical one; it highlights the importance of screening out interfering stimuli as far as possible during 2-DG experiments. The multifunctional nature of many nuclei may lead to false conclusions if a potential target area is activated in a non-specific way with respect to the precise stimulus employed in the experiment. Secondly, the fact that the AP was markedly activated under these conditions lends weight to the evidence that the AP is involved in mediating functions other than emesis, e.g., fine control of blood pressure (Ferrario et al., 1972; Barnes et al., 1984).

After our experiments with direct stimulation of nervous pathways, similar experiments were performed, but this time using apomorphine administered i.v.. Apomorphine thus administered did not elicit frank vomiting in the ferret,

although weak retching was noted. A significant increase in relative metabolic activity in the AP of the treated group was noted, but changes in the NTS and DMVN were minimal. All the available evidence suggests that apomorphine acts by stimulating the AP to cause vomiting in those species which exhibit the response, (see for instance Wang and Borison, 1952; Borrison 1959 and Lindstrom and Brizzee, 1962). No AP ablations to test apomorphine responses were carried out in the ferret. The ferret is, nevertheless, responsive to apomorphine, although, as shown, most effectively via the subcutaneous route. It, therefore, was used to test the integrity of the vomiting reflex when other routes conveying emetic stimuli were interrupted. Thus the present 2-DG results are consistent with the assumption that apomorphine acts via the CTZ. Physiological recordings during these experiments revealed a small and transient fall in blood pressure which quickly recovered. It is unlikely that the results indicated were due to changes in B.P. as the AP, although implicated in the fine control of BP, Kostreva (1982), appears to be activated only by consistent rises in B.P.. Recordings of intraluminal gastric pressure during our experiments detected the reflex relaxation of the proximal stomach that is a concomitant of the prodromata of vomiting (Andrews, 1986). Whether this relaxation is a consequence of or a contributory factor to nausea is a matter of debate (see Willems and Lefebvre, 1986) but, in any case, it is most likely due to vagal activation of NANC fibres. No changes in 2-DG uptake were found in the DMVN, and it is not yet known whence fibres controlling the inhibition of gastric motility arise.

Even the weak retching elicited by apomorphine was insufficient to cause detectable changes in the DMVN in these experiments; neither was any significant change detected in the NTS. It may be that the emetic stimulus was too weak to cause changes in the NTS and DMVN detectable by our technique. A more interesting possibility is that the pattern of change in activity found here, confined as it is to the AP, represents the lowest level of activation of the emetic reflex i.e. 'turning on' of the CTZ alone without sequential activation of the NTS and the effector nuclei. This pattern may indeed represent minimal activation of the system in the same way that electrical stimulation gives a pattern that reflects maximal stimulation (see also discussion of 2-DG pattern from mustine-induced vomiting). In contrast to the experiments using apomorphine in the anaesthetised ferret, experiments in the conscious animal did not give clear cut results. The limited response to apomorphine, with only sporadic and brief retching and vomiting following the prodromata of emesis, appears to have been insufficient to produce significant changes in the target nuclei. A rise of 11% in relative metabolic activity was noted in the AP, however, with no change in the NTS and DMVN. Thus, there is some support from the experiments in the conscious animal for the tentative conclusions made above from those in the anaesthetised ferrets i.e. that at low levels of humoral emetic stimulation, the AP but not the NTS or DMVN is active.

Apomorphine has been investigated using 2-DG

autoradiography with respect to its effect on a wide variety of brain structures (see Sokoloff, 1984 for a review). However, the animal model used in these investigations was usually the rat, which does not vomit. Also, the dosages of apomorphine used in these studies (e.g. McCulloch et al., 1982) were between 50 - 5000% higher than those used in the present studies and, indeed, at the lower doses used in the McCulloch study, no significant changes were detected in any brain area.

In studies of cytotoxic agents, mustine provoked less of an emetic response than expected; it did however. give rise to prodromata in all animals, and retching and vomiting in half. There was a marked and significant rise in relative metabolic activity in the AP (50%) but, although rises were also detected in the NTS (17.5%) and DMVN (15.5%) these were not statistically significant. It might reasonably have been expected that mustine would have caused detectable changes in all three nuclei but perhaps the poor emetic response accords with the type of results we obtained with apomorphine for instance, i.e., primary activation of the AP without detectably significant changes in the NTS or DMVN. Alternatively it may be that these changes are occurring under the circumstances of mustine and apomorphine stimulation but the technique as used may not be capable of detecting them.

The available evidence on the site of action of mustine is conflicting because it points to the CTZ as being the site of action in the dog but the gastrointestinal tract in the cat. The present evidence in the ferret with mustine and with

cyclophosphamide (Hawthorn et al., 1988) has indicated the importance of the upper gastrointestinal tract as the site of action of these drugs. Initially this would seem not to agree with our 2-DG result in the ferret in response to mustine. However, our evidence from the electrical stimulation experiments indicates that the AP can be involved in this process either through direct anatomical links to the AP via the vagus, or through primary activation of the NTS. This is not necessarily inconsistent with the evidence that vagotomy prevents mustine-induced vomiting in the ferret, since such vital vagal emetic fibres may pass via the AP. This would account for our ability to detect a large change in the AP when only a small change was detectable in the NTS and DMVN. On the other hand it could just be that two independent mechanisms are used by mustine with activation of AP either by mustine itself or a molecule released from its action on the gut mucosa or by direct activation of the dorsal vagal complex (and hence AP) by the vagal afferents.

Again, considering our 2-DG results for radiation-induced emesis, we have to contend with the conflicting evidence from a number of animal models on the mode of action of radiation as shown by other experimental approaches. X-irradiation produced large and statistically significant increases in relative metabolic activity in the AP and DMVN. The change in the NTS was about 21% but this was not statistically significant. The actual emetic response to X-irradiation in these experiments was as predicted, and involved the full range

of prodromata, with extensive retching and vomiting, throughout the period of exposure to 2-DG. Activation of the AP does not necessarily imply that radiation-induced emesis is mediated via a humoral agent acting on the AP alone, because of the possibility of vagal afferents passing to the AP as well. Work on X-ray induced vomiting in the dog, cat, monkey and ferret has firmly implicated the pathway of activation of emesis as via the CTZ in the dog and monkey and via vagal afferents in cat, monkey and ferret. Borison maintains that these differences are only apparent because of the discrete nature of AP ablation in his hands which avoids damage to underlying structures. It could be, of course, that the effect of AP ablation is merely equivalent to that of a high level vagotomy. Unfortunately postrectomy and abdominal vagotomy have not yet been carried out in all animal models. The evidence from lesions of the AP and abdominal vagus in the monkey appears to show that both pathways are important. It is perhaps the relative importance of each of these pathways and the balance attained in any one species that accounts for the apparent inter-species differences. Further experiments are necessary to see the effect in the ferret of AP ablation on a variety of emetics including X-radiation. The 2-DG technique has shown that, whatever the precise mechanism of activation is, the AP is in fact involved. That the DMVN was activated accords with evidence that parasympathetic efferents to the viscera are involved in the vomiting process. These probably control the preparative changes in muscle tone and motility of the stomach

and upper small intestine, that precede emesis and accompany vomiting when it occurs.

The apparent lack of activity of the NTS suggests that the NTS is not directly involved by stimulation of abdominal vagal afferents. This might imply that the peripheral vagal route is simply not involved in radiation-induced vomiting in the ferret. The vagal lesion experiments described above, however, show that it is. Moreover, a 20% increase in activity was detected, so perhaps as suggested above, this activation is secondary to a primary activation of the AP by vagal afferents.

One final observation is worthy of mention and this concerns the autoradiographic pattern obtained from the ferrets exposed to X-radiation. In a small number of autoradiographs, 2-DG uptake was high in the lateral region of the NTS. It has been suggested that, in the cat, the function of the lateral NTS is as a sensory nucleus for afferent information from the lungs (Kalia and Mesulam, 1982). This region is also close to that area of the dorso-lateral reticular formation of the cat which was identified using electrical stimulation by Borison and Wang (1949) as the vomiting centre.

The present studies resulted in an apparent paradox. After cycloheximide-induced vomiting in the ferret, a maximum physiological response to the pharmacological stimulus resulted in no increase in 2-DG uptake in the brainstem nuclei under scrutiny. Grollman and Jarkovsky (1974) cite Wang (1965) and assert that the emetic effect of emetine, a compound analagous to cycloheximide, is exerted through a central action and a

peripheral action direct on the gastric mucosa. The present work with cycloheximide indicates that the abdominal vagal afferents are indeed important, a situation also found with emetine. It was expected that 2-DG autoradiography would reveal an increase in activity, at least in the NTS, and according to Wang in the AP, and in view of the profound emetic response that was elicited, possibly in the DMVN also. None of these changes was apparent. Since cycloheximide did provoke vomiting, it must be concluded either that concurrently it prevented the uptake and retention of the 2-DG whilst permitting the neurons involved to take up non-isotopic glucose in response to the extra demand of that in this particular case these neurons are those that rely on a decrease in K^+ conductance for activation which Carpenter has suggested do not require increases in 2-DG uptake. Cycloheximide is an inhibitor of protein synthesis and work by Grahame-Smith (1975) showed originally that such inhibition can affect CNS function and modify the release and action of neurotransmitters, irrespective of any other action on neurons. Emetine has also been reported as having a direct effect on neuronal transmission; for instance it blocks activity in sympathetic nerve endings. Now, although such actions as blocking the production of critical proteins or, directly affecting neuronal transmission might explain its action as an emetic substance (see Harris, 1985 and 1986), none of this seems to account for the ability of cycloheximide to prevent the uptake of 2-DG in response to the vomiting stimulus unless possibly it interferes with the synthesis of a rapidly turning-over protein constituent of the neuronal membrane which functions as a

carrier in membrane transport (e.g. Oldendorf, 1971). Since the 2-DG represents such a minute fraction of total glucose available in the extra-cellular pool, this might explain why metabolic work occurs and physiological functions take place but at the same time the events go unrecorded by 2-DG autoradiography. A partial transport block would under these circumstances, have proportionately a much greater affect on the 2-DG tracer than indigenous plasma glucose. Lastly, it may be possible that at the dose used (i.e. twice that used to inhibit brain protein synthesis in the rat by 100%) cycloheximide, like emetine, can act as an inhibitor of certain enzymes (Hanische et al., 1966). One could then postulate that inhibition of hexokinase-catalysed phosphorylation might be taking place (Sols and Crane, 1954). However, because 2-DG and glucose are competitive substrates for the enzyme system, phosphorylation of 2-DG and its accumulation in the neuron would take place on only a very minor scale by comparison to glucose itself. This might allow sufficient glucose for neuronal metabolism to progress, but to do so undetected by the ^{14}C-2-DG probe. The block induced by cycloheximide may even be specific to the high-affinity glucose transport system in the synaptosomes which are located in the terminals where it is thought the greater amount of neuronal cellular energy is required for synaptic activity.

5.7 CONCLUSIONS

The present work has clearly established that the ferret responds to a wide variety of emetic stimuli acting via several different mechanisms. Some of these stimuli are of current clinical interest and others have a bearing on clinical problems yet to be addressed.

Analysis of the dose-response sensitivity data to emetic agents reveals that the ferret displays a unique combination of responses, many of which are similar to man and the other commonly used emetic model species, e.g. dog, which makes it a very suitable alternative for the study of the mechanism of this reflex and for the trialling of drugs. Presently, the major omission in our knowledge of the emetic profile of the ferret is that we do not know its response to motion, although in view of its record with other stimuli it would be surprising if it did not display emesis to this stimulus.

The studies reported here together with the literature review have revealed a number of things which have not previously been very obvious. The most important of these is that the mechanism of action of one cytotoxic drug does not predict that of another, even in the same species. Nor, apparently, does an individual cytotoxic agent necessarily use the same mechanism from species to species. The latter conclusion applies also to the pathways for induction of X-radiation emesis. Indeed not only does the balance of dominance between the various mechanisms seem to vary but there are indications that the system is to an extend plastic. Figure 55 is an attempt to synthesize our current knowledge of

emetic pathways emphasizing the interaction beween the role of the vagal afferents and that of the AP.

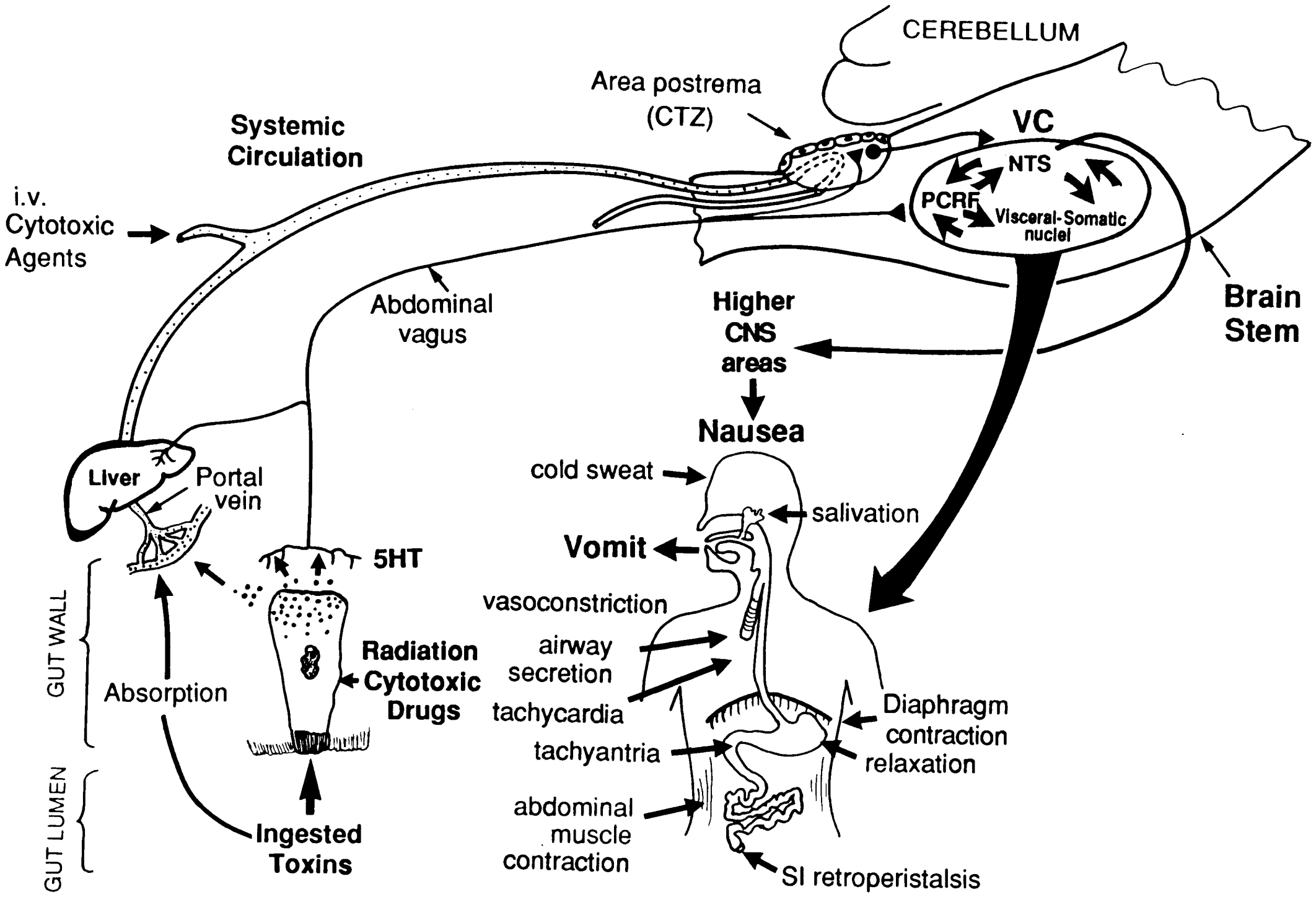
Prior to this study most of the work had been qualitative with little distinction being made between retching and vomiting and little attention being paid to the pattern of emetic response. One of the aims of the present study was to remedy that situation at least in respect of one animal model. The results have revealed that each has an individual 'fingerprint' of emetic response which clearly must reveal something of the mechanisms involved in each case.

Studies using radioactively labelled 2-DG have shown that this technique, using both ^{3}H and ^{14}C isotopes, can be used successfully in the ferret and specifically to investigate the emetic process. Using a limited number of emetic stimuli we have demonstrated that areas involved in emesis such as the AP do indeed increase relative metabolic activity in response to emetic stimuli. One of the most important of these results was that stimulation of abdominal vagal afferents activates the AP, providing for the first time functional evidence for the anatomical links suggested by Leslie's work (Gwyn and Leslie, 1979 and Leslie and Gwyn, 1984). The particular significance of this result is that it raises the possibility that many emetic stimuli which have been suggested to act via the AP, based on the results of lesion studies, may instead be acting via activation of abdominal vagal afferents which project to the AP.

One of the problems with the use of 2-DG in conscious animals is that of separating cause and effect in the resultant

Figure 55 A Schematic Representation of the Components of the Emetic Reflex in Man illustrating the Balance between Nervous and Humoral Pathways to and from the "Vomiting Centre"

Diagram summarizing the major visceral and somatic motor changes during nausea and vomiting, the brain stem nuclei involved and the major pathways by which emesis may be triggered i.e. abdominal vagus and AP
Key: AP = Area Postrema, CTZ = Chemoreceptor Trigger Zone, VC = Vomiting Centre, NTS = Nucleus Tractus Solitarii, PCRF = Parvicellular Reticular Formation, CNS = Central Nervous System


CEREBELLUM
Area postrema
(CTZ)
VC
NTS
PCRF
Visceral-Somatic
nuclei
Systemic
Circulation
i.v.
Cytotoxic
Agents
Abdominal
vagus
Brain
Stem
Higher
CNS
areas
Nausea
cold sweat
salivation
Vomit
vasoconstriction
airway
secretion
tachycardia
tachyantria
abdominal
muscle
contraction
Diaphragm
contraction
relaxation
SI retroperistalsis
Liver
Portal
vein
5HT
Radiation
Cytotoxic
Drugs
Absorption
Ingested
Toxins
GUT WALL
GUT LUMEN

autoradiograph, especially in the study of vomiting. This could be overcome by the use of decerebrate paralysed animals in which emesis is monitored by recording the neurophysiological correlates of vomiting from the phrenic nerve and the nerve supply to the muscles of the abdominal wall.

Whilst, like many others, we have sectioned nerves in order to determine the pathways from the gut which are involved in the emetic response, we have obtained preliminary data that adaptive changes may occur in response to these lesions. For example, whilst in the normal animal emetic signals are conveyed via the vagus, when the vagus is cut, other mechanisms may be recruited either involving the splanchnic afferents, the release of humoral agents from the gut, or re-organisation of pathways in the medulla. This has most important implications for understanding the genesis of emesis in disease states e.g. post-vagotomy or diabetic gastroparasis

A major area of progress has come with the advent of the $5HT_3$ receptor antagonist class of drugs. Our studies have shown that these compounds are potent anti-emetics with high efficacy against cytotoxic drug-induced and X-radiation-induced vomiting. Their site(s) of action is of particular interest because the evidence to date suggests that this is peripheral rather than central as had previously been assumed to be the case for anti-emetics. The high degree of parallelism between the effects of abdominal vagotomy and the $5HT_3$ receptor antagonists together with the data on the distribution of $5HT_3$ receptors clearly implicates peripheral vagal afferent

terminals as being their locus of action as anti-emetics. However, although we have demonstrated a role for the vagus in the genesis of radiation, cytotoxic, and intragastric induced emesis, it is intriguing that the $5HT_3$ antagonists only block emesis due to the first two stimuli. This preliminary observation may well indicate that the neuropharmacology of afferents differs from gut region to gut region or alternatively that different groups of emetic agents cause activation of afferents by the release of different neuroactive chemicals, 5-HT being only one of these.

The role of the AP in the control mechanism of the vomiting reflex has dominated thought for a number of years and drawn attention away from the upper gastrointestinal tract which can be regarded as the "first line of defence" in the body's defence against ingested toxins. In this respect it is particularly ironic that $5HT_3$ receptor antagonists, the first new class of potent anti-emetics to be discovered for some time, appear to have their principal site of action in the gut. At this stage of our understanding this does not of course rule out a central site of action for these compounds as well and hence a role for the AP. Nevertheless the results contained herein have helped to broaden the possibilities under consideration by focussing on the gut as a potential site of action for systemic toxins like cytotoxic drugs and ionizing radiation.

TECHNICAL APPENDICES

APPENDIX 1

A.1 TECHNICAL CONSIDERATIONS IN THE CHOICE OF ISOTOPE FOR 2-DG AUTORADIOGRAPHY

A.1.1 Autoradiographic Image Resolution and Contrast

Resolution-limiting factors in autoradiography fall into two broad classes (a) those that determine how far the radioactive tracer diffuses away from its in vivo loci during tissue preparation and (b) those that determine the size of the spot produced by a point source of radiation during exposure, development and densitometric scanning of the film. Factors including film type, section thickness and power of isotope are important. Of these the power of the isotope is the dominant factor in conventional 2-DG autoradiography. Even the fastest (and hence poorest resolving film) permits higher levels of resolution than are usually in practice obtained. In principle, near cellular (20 m) levels of resolution are obtainable in conventional 2-DG autoradiography using [^{3}H] instead of [^{14}C] and film without an anti-scratch protective layer (LKB ^{3}H Ultrofilm) (Gallistel and Nichols, 1983).

Neither the delay betwen sacrifice of the animal and the freezing of the brain nor the perfusion (e.g. with 4% Phosphate-buffered paraformaldehyde) or non-perfusion of the brain has any effect on the sharpness of the images obtained, suggesting that the radioactive 2-DG-6-phosphate is confined within the cell membrane so long as this membrane remains intact (Gallistel and Nichols, 1983).

If so, whatever diffusion occurs must occur either during the slow freezing of the intact brain which can rupture cellular membranes by the build-up of severe osmotic gradients or during thawing and drying of the tissue after sectioning.

The two features contributing to the gain in resolution obtained by [^{3}H]-2-DG are (a) the shorter range of low energy β-particles emitted by tritium and (b) the inrease in O.D. contrast between grey matter and white matter caused by the quenching by lipids of low energy emissions (Herkenham and Sokoloff 1983). The lower energy of the tritium -particles (Emax = 18.6 KeV for Tritium versus 156 KeV for Carbon-14) means that emissions from a molecule of [^{3}H]-2-DG will only be able to expose silver grains in the emulsion located within the few micrometers or less from the source. The distance that [^{14}C] emissions will travel is much greater. The longer path length results in a loss of resolution in the autoradiographic image (Gallistel and Nichols, 1983). However, it should be noted here that quantitative autoradiographic resolution is often limited by the resolving power of the densitometer itself rather than the resolution of the isotope used; indeed the autoradiographic resolution achieved with tritium is greater than that detectable by most microdensitometers. The best opportunity for optimising the possible increase in resolution achievable for example by using [^{3}H] 2-DG and LKB^{3}H Ultrofilm, is in the use of a digital video-based densitometer in which the optical system performance is maximised (Ramm et al., 1984) The sharp contrast that appears between grey matter and white

matter in tritium 2-DG autoradiographs relative to [^{14}C] autoradiographs results from the greater attenuation of the low energy tritium β-particle by white matter than grey matter (Alexander et al., 1981). This artefact can be corrected if tritium standards made from grey white and mixed grey/white matter areas are used for calibration in quantitative densitometry. The qualitative appearance of [^{3}H]-2-DG autoradiographs cannot be corrected for this differential quanching. However, this does not detract from the usefulness of [^{3}H]-2-DG in the study of manipulation-induced changes in local cerebral glucose utilisation. When the same regions of experimental and control brains are compared, the application of normalisation by the construction of O.D. ratios, means that differential quenching is not a factor.

A.1.2 Microtome-induced Section - Thickness Variation

The two isotopes are also distinguished by the consequence of variations in section thickness; whereas tritium autoradiographs will not reflect a difference in thicknesses greater than 5μm, ^{14}C autoradiographs will. As a result, for the weak emitter, section thickness is not an important factor, but for ^{14}C differences in thickness resulting from imperfect microtome cutting will be reflected in artifactual variations in the measured concentrations of the isotope giving rise to minor variability in the density of the autoradiographic image (Gallistel and Nichols, 1983). These variations, which can be substantial, are not corrected for in the conventional quantification procedure. They are, however,

irrelevant when appropriate normalised indices of functional activity are used (e.g. O.D. ratios) because normalisation by reference to within-image norms e.g. white matter density, removed the effect of factors such as section thickness, that operate equally over the entire image (Gallistel et al., 1982).

A.1.3 Film Type, Exposure Time and Photographic Processing

(see Table 23)

2-DG autoradiography with [^{14}C]-2-DG has employed a variety of x-ray film types e.g. Kodak SB5, all having the basic qualities of being suitably sensitive to the β-particles emitted by ^{14}C and of having adequate resolving power. Spatial resolving power depends on the thickness of the antiabrasion coating that overlies and protects the emulsion, the thickness of the emulsion layer and the size of the silver grains. All of these (as well as the tightness of contact between film and tissue) help determine the distance that an emitted β-particle can travel away from its source and still cause conversion of a silver grain in the emulsion. The greater this distance, the greater the size of spot produced by a point source, hence the poorer resolution. The low power β-particle radiation from tritium cannot penetrate an antiabrasion layer to any appreciable extent and only one film currently exists which lacks this anti-scratch layer because it has been specially fabricated for use in [^{3}H]-autoradiography i.e. LKB ^{3}H-Ultrofilm (Ehn and Larsson, 1979).

As has already been explained, even the fastest and therefore lowest resolution film used in [^{14}C]-autoradiography

TABLE 23

Summary of Processing Considerations for Isotopes used in 2-DG Autoradiography

		$[^{3}H]$-2-DG$_{-1}$ [Dose=1mCikg^{-1}]	$[^{14}C]$-2-DG$_{-1}$ [Dose=125μCikg^{-1}]
1.	Film Type	LKB Ultrofilm3H	KODAK X-OMAT AR5
2.	Film sensitivity	High to Tritium-β (large silver grains, high silver to gelatine ratio and absent anti-scratch layer)	High to ^{14}Carbon-β (Double emulsion tnin anti-scratch layer)
3.	Film resolutions	Best possible with low power β	Excellent witn higher power β
4.	Film Background	Clear base	Clear base
5.	Tendency to underestimate nigh isotope concentration	Minimized by short exposure ($\simeq$21 days)	Absent
6.	Film antiscratch layer	Absent	Thin
7.	Film handling	Difficult - physically delicate	Easy - robust format
8.	Film exposure time	Long - 28 days	Short - 5 days
9.	Film fade tendency	Absent in exposures $\leq$25 days	Absent in experimental time scale
10.	Film development system	Manual (total of 60min)	Automatic (90sec)

permit higher levels of resolution than can at present be obtained by the techniques widely in use today (but see Hokfelt et al., 1983). Therefore, we were left with the clear decision of choosing one X-ray film suitably sensitive for ^{14}C radiation and the LKB Ultrofilm-^{3}H for the ^{3}H radiation. Table 23 summarises the characteristics of the films used. It is important to note that clear advantages are offered by both systems. X-OMAT AR5 (Eastman Kodak, Rochester, N.Y.) was specially designed for high sensitivity to β-particles (and use in autoradiographic procedures) but in particular, at the tracer dose used (125μCi/kg) it allowed exposure time to be limited to 5 days, thus decreasing potential experimental "turnaround" time. Secondly this film can be processed automatically in a 90 second processor (Kodak RPX-OMAT). This means that the process of development can be standardised from film to film and goes some way to remove inter-animal variability. The process is also very quick (90 seconds) by comparison to manual methods.

For the LKB Ultrofilm-^{3}H the advantages lie in not having to use emulsion which is otherwise the only alternative to using a film with an anti-abrasion layer; the best sensitivity that could be considered with such a system is 1/12th of that possible with LKB film. This greatly increases the reliability and ease of using the low power β-emitter, tritium, which is approximately twice as cheap as the ^{14}C isotope (as used by the methods in this thesis) and holds the best hope of maximising the resolving power of the system.

APPENDIX 2

A.2 COMPUTER-ASSISTED HIGH RESOLUTION DENSITOMETRIC IMAGE ANALYSIS

A.2.1 Introduction

The routines described are orientated towards the use of the 2-DG technique to detect and numerically index localised alterations in the metabolically coupled functional activity of neural systems rather than toward the measurement of the local rate of glucose utilisation i.e. towards the semi-quantitative 2-DG technique. The system described provides for:-

a. Very rapid point-by-point digitisation of the darkness of picture points or pixels in the autoradiograph by means of a Television Camera (TV) which displays an image on a TV screen to facilitate interactive analysis of the autoradiographic image.

b. Superimposition of the autoradiographic image upon the histological image of the section from which the autoradiograph was taken so that effects seen on the autoradiograph may be accurately related to histologically defined neural structures.

c. The computation of normalised indices of functional activity i.e. a system whereby darkness of the autoradiographic image over a given neural structure is translated into a class rank by reference to a norm such as the darkness of a white matter structure in the same structure.

d. The conversion of the autoradiographic data to numerical data suitable for statistical treatment.

e. Pseudo-colour coding of the black and white autoradiographic image that makes it easier to detect differences in the relative darkness of a structure in control and experimental brains and easier to determine the limits of regions of abnormal darkness. It also allows for provision of hard copy records in colour.

f. The provision of black and white hard copy records of autoradiographic images.

Computer-assisted imaging devices consists four major components i.e. an image acquisition device, a general purpose processor, an image processor and image display monitors (Ramm et al., 1984). Before commencing the analysis of 2-DG autoradiographs it was necessary for a system comprising each of these elements to be constructed. There follows a description of the individual elements of this sytem and the way in which they were integrated to perform the tasks already delineated.

A.2.2 Hardware Configuration and Characteristics

A.2.2.1 Image Input System

A.2.2.1.1 Image Acquisition Device - Camera and Tube

Three important factors dictate the performance of such devices, viz.,

a. Dynamic Range: The difference in O.D. between the dark threshold (zero output) and a saturation threshold (maximum output), is the dynamic range and this should contain the range of densities to be found in the material to be analysed.

b. Sensitivity: A scanner should be capable of producing at least 6 bit (64 grey level) densitometric resolution when noise reduction is taken into account.

c. Shading: Shading is a measure of photometric non-uniformity observed in scans of a homogeneous image.

Various devices for image acquisition have advantages and disadvantages and perform more or less efficiently any given task (Ramm et al., 1984). Thus:-

i. Scanning Microscope Photometers have wide dynamic range, high sensitivity and high precision but they are very slow (20min for a 512 x 512 pixel image), and image processing and display capabilities are limited.

ii. Scanning Microdensitometers (Flat Bed or Rotating Drum) are accurate, have wide dynamic range, high sensitivity and excellent spatial linearity. Unfortunately, they are very costly, have a slow scan rate, and fixed ultimate resolution.

iii. Line-scan and Area Array Charged Couple Devices (CCD) exhibit wide dynamic range, good response to low light levels and a linear respons to incident illumination (i.e. unity gamma). They can produce high precision images e.g. (1280 x 1280 pixels in 20 seconds) and will soon approach the speed of video cameras. Although the array type CCD permits real time imaging, the resolution is limited and those designed for densitometry are expensive and unproven technically.

iv. Video Cameras (Traditional thermionic tube-based cameras) come in a range of special forms, all variants being based on the original Vidicon tube which has become the generic term.

The most important characteristics of an autoradiographic densitometer is its ability to perform consistent measurements at a level of precision equal to or greater than that of the autoradiograph. This essential performance characteristic is exceeded by the Vidicon-based camera system that has been suitably corrected. Vidicons show a dynamic range, accuracy and a sensitivity suitable for quantitative autoradiographic densitometry at a price that is a fraction of that for the other systems. Moreover, autoradiographic procedural errors ultimately limit the discriminability of density differences and the Vidicon's 8-bit density resolution easily exceeds this limiting factor. Shading imposes a major limitation upon ultimate accuracy and after correction of physical causes residual shading must be minimised by application of computer software-based correction algorithms in order to limit maximum shading variation in any area of the raster throughout the density range to < 1%. Bearing these arguments in mind it was decided to choose a Vidicon-based camera as the image acquisition device. Of those available, the BOSCH TYK 9B1 camera with 2.5cm Plumbicon XQ 107L tube (Robert Bosch GmbH, Darmstadt, F.R.G.) displayed characteristics best suited to the particular task of quantitative autoradiographic densitometry (see Table 24) (Bosch technical data).

Post-correction performance of the Bosch Plumbicon can be tested to check linearity of response and measurement repeatability by scans of standard neutral density filters.

TABLE 24

Summary of the Characteristics and Specification of the Video Camera and CRT

Camera: BOSCH TYK 9 B1

	Characteristics	Specification
1.	Television Standard (horizontal resolution)	800 lines/50Hz at centre
2.	Signal to Noise Ratio	55dB at signal current 200mA
3.	Linearity	0.95 or better
4.	Automatic Gain Control	Can be disabled (requirement for densitometry)
5.	Gamma Correction (Signal current/incident illumination)	0.45 - 1.0 continuously adjustable
6.	Shading Signal Compensation	Up to 20% of edge loss
7.	Modulation Transfer Function (Amplified Response)	>50% @ 5 MHz
8.	Geometric Distortion	0.5 - 1.0%

Tube: PLUMBICON XQ107L (2.5cm)

	Characteristics	Specification
1.	Spectral (wavelength) Response	Similar to human eye (good visual impression and least interference from infra-red)
2.	Gamma response; signal input/ output relationship or photometric linearity	Very good; λ = unity
3.	Geometric resolution	Excellent (limited only by optics)
4.	Sensitivity (a combination of overall light requirements and uniformity of response to light)	High
5.	Burn-in sensitivity (Lag)	Minimal (x2 scan times)

A.2.2.1.2 Optical System

The camera was fitted with a television camera lens (Dallmeyer, London) having a fixed focal length of 76mm, variable apperture (1.9 - 16.0), and variable focus capability. To give suitable initial magnification for ferret brain sections, C-mount tube extensions of 3cm and 8cm were interposed between the camera and the lens system.

This allowed the medulla oblongata (approx. 8mm x 4mm) to be magnified to fill the RGB video image monitor (14 inch diagonal) and corresponds to a rectangular image of 768 x 512 pixels (picture points) before digital zooming was carried out.

A.2.2.1.3 Light Input and Control

A source of illumination was provided by a Durst BWL450 diffused light unit (Durst AG, Italy) with integral infra-red absorbing filter and cooling unit. The light source component of this device is a 240 volt, 250 watt, tungsten-halogen lamp with diathermic reflector, powered from the mains supply via a Durst TRA450 transformer (300 watts, 240 volts). The mains source itself was stabilised using a Reguvolt Mains Conditioner, Model 500D (Cetronic Components Ltd., Hertfordshire). The light produced was passed through a further diffusing box, containing a 0.40 density diffuser, (Durst Laborator 1000 Enlarger and Taunodap 450 adaptor) thence through an additional 5mm thick pearl glass diffusor plate, followed by attenuation using a Wratten Neutral density filter (O.D.0.6) before emerging through 5cm diameter window in a matt

black viewing platform facing the camera lens. This system provides light of high field uniformity and homogeneity and high stability. The output of the system is finely controlled by interposing a VARIAC variable output transformer (Zenith Electrical Company Limited, Milton Keynes) in the circuit to control the power to the lamp assembly. This allows precise re-setting to a standard output of the white light level for each series of densitometric measurements undertaken.

A.2.2.2 Computerised Image Processing System (See Fig. 56)

The KONTRON IBAS system consists of IBAS 1, a semi-automatic evaluation unit and host computer capable of independent operation and IBAS 2, a special image analysis processing unit. This distribution of computing capability results in maximum efficiency for image processing, easier handling and greater flexibility (Kontron 1984 and 1985).

A.2.2.2.1 The Host Computer; IBAS 1

This is a universal microcomputer system based on the Zilog Z80 (4MHz) series chip with 64KBytes of RAM, 16KBytes of graphic memory and a cycle time of 250msecs. IBAS 1 can operate alone as a semi-automatic data acquisition and data-processing unit, but is normally connected to IBAS 2, for which it acts as the host computer, assisting in the fully automatic image analysis evaluations. Used together with the digitiser tablet, the integrated X/Y - co-ordinate measuring system enables the manual recording of object geometry by tracing structure contours with the crosshair cursor. The calculation of the geometric parameters and the evaluation and

storage of data follows automatically. When used in combination with IBAS 2 in the normal configuration the digitiser tablet can also be used for interactive image editing as well as for the control of various menu operations. The keyboard connected to IBAS 1 is used for any alphanumerical input. IBAS 1 is served by twin floppy disk units and an OKI DP125 printer/plotter for data storage and the black and white graphics data monitor for data display.

A.2.2.2.2 The Image Analysis Processing Unit; IBAS 2

This is connected to the IBAS 1 host computer by a 16 bit DMA interface to complete the system. The most essential components of IBAS 2 are the programmable video input module, the digital image memory, the microprogrammable image array processor and the memory address controller. Thus:-

Video Input/Output Unit; This programmable unit provides the analogue-to-digital (A/D) 8 bit conversion of the video input signal as well as the digital-to-analogue conversion of the image data back to the monitor. It is also responsible for synchronisation of external signals and devices supplies appropriate software instructions. It also includes the look-up tables for real-time grey level and psuedo-colour transformation as well as the special logic unit for the display of real colour images. It is capable of acquiring a complete image in one TV cycle (40ms).

Digital Image Memory (Framestore or Videomemory Board); The digital grey level image memory forms the core of the IBAS 2 system. It is modular and extendable to a maximum of

128MBytes but in this configuration has 2MBytes of image memory - equivalent to 3 images, each with a geometric resolution of 768 x 512 pixels. The grey level resolution consists of 256 intensities (8 Bits) plus an additional Bit per pixel, serving independent graphic overlay (i.e. 9 Bits per pixel).

Microprogrammable Image Array Processor; This pipeline structured processor was especially developed for fast image analysis operations and has a cycle time of 100nsec providing a processing speed of 10 million operations per second (MIPS), with multiple operations performed in parallel. Its operation is under the control of its own dedicated microprogrammes, microprogramme memory and sequencer. Extremely efficient processing of image data and data transfer to and from the image memory is achieved in this manner. Loading, starting and control of the microprogrammes is carried out automatically by IBAS 1.

Memory Address Controller; This unit is responsible for the geometric and logical alignment of picture information (pixels) and computes the position of the cursor and displays it on the screen. It also generates a refresh signal for the image memory.

A.2.2.2.3 Monitors, Keyboard and Digitizer Tablet

The interaction between the user and the digitised representation of the image is mediated via a 14 inch diagonal 25MHz band width RGB monitor giving 800 lines at 50Hz with a geometrical distortion <3%. The guns in the TV monitor are modulated by a signal derived either from the image refresh

memory or the TV camera. In either case the beam modulating signal passes through an electronic "Look-up table" which can create special modulations for a given pixel. The modulations are triggered by the grey scale value for each pixel when colour windows are used; when graphic information is written on the display, the modulations are triggered by the address of the pixel.

The user directs the analysis of the displayed image via the 14 inch black and white data monitor with 15MHz band width. This is addressed by the alphanumeric keyboard (ASC II with a separate pad for numerical inputs) and by the digitiser tablet (280mm x 280mm with 0.2mm resolution and accurate to 0.1mm), and its cursor which is also used to interact with images on the RGB monitor.

To this system was also added a third CRT monitor "slaving" directly off the video signal from the Bosch camera and by-passing the IBAS system. (Model VM/906AE/K Videomonitor, Hitachi & Denshi Ltd., Japan).

This allows simultaneous reference to be made to the original image on a dedicated high resolution black and white monitor during the period of image processing when the RGB monitor may be occupied with processed and/or colour images. This is a useful facility that can aid the interactive analytical process because the quality of this black and white 'reference' image is much higher than that achieved on the RGB monitor.

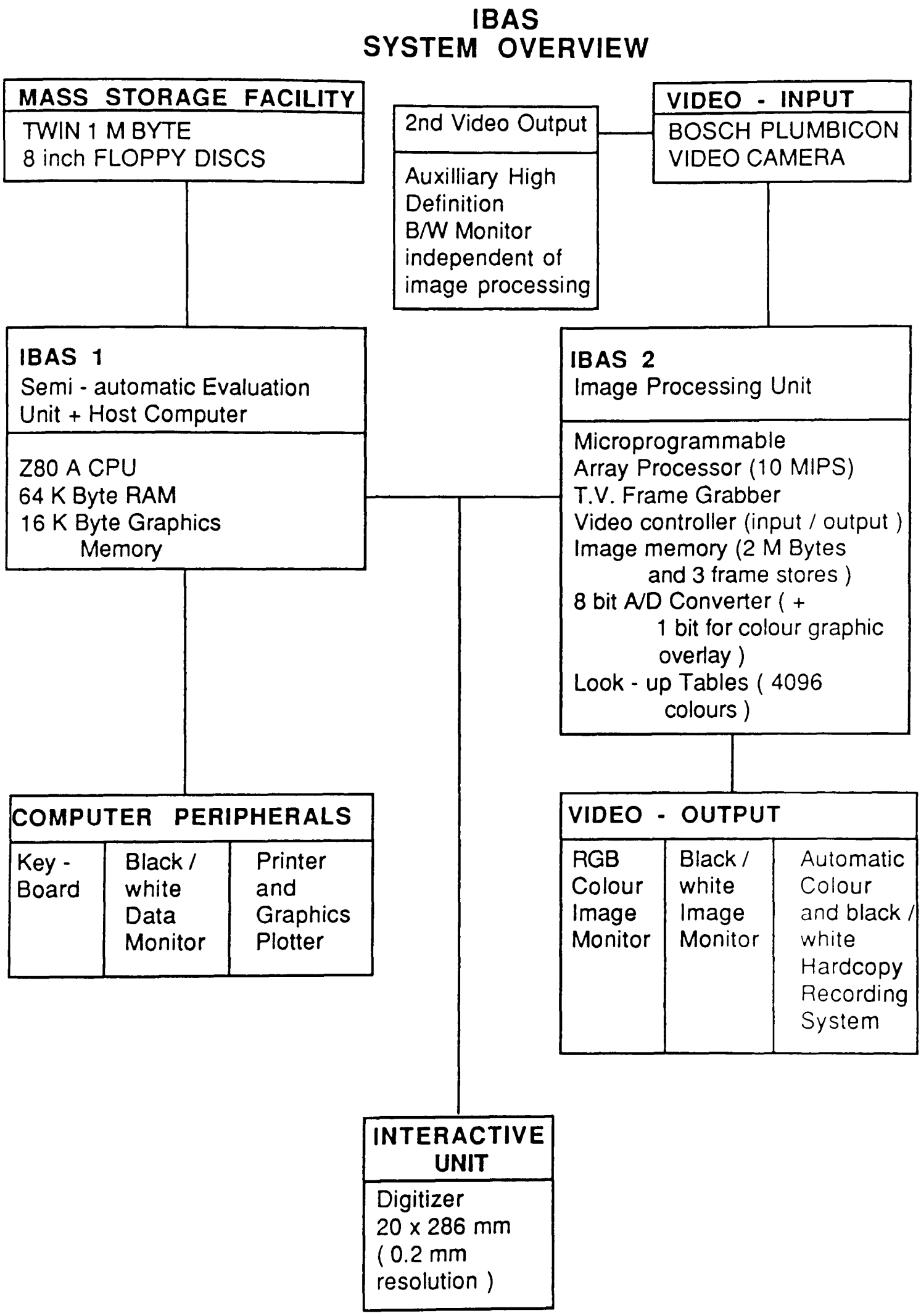


Figure 56 A Structural Overview of the KONTRON IBAS II Computerized Image Analysis System

A.2.2.3 Data-recording Systems

A.2.2.3.1 Numerical and Graphics Data

These data can be stored on 8 inch floppy discs, each with a storage capacity of 1MByte. Hardcopy was also obtained by interfacing to an OKI, DP125 printer/plotter (Electric Industry Company Limited, Japan).

2.7.2.3.2 Image Data

Imaga data can be readily stored on the 8 inch floppy discs. (2 images maximum per disc each of 768 x 512 pixels). However, it is also desirable to be able to obtain permanent photographic records of the autoradiographic and histological images. For this purpose an Image Corder 4500 (Focus Graphics, California) was interfaced with the Kontron IBAS 2 host. By this means any image capable of being displayed on the RGB monitor could also be recreated on the slave CRT of the Image Corder 4500 and photographed using 35mm or Instant Polaroid film systems, giving the opportunity of recording images on slides, as prints and instant prints, in black and white or colour.

A.2.3 Software Routines

A.2.3.1 System Capability

By definition the IBAS system is software-orientated and a very wide variety of relevant programmes for measurement, manipulation and evaluation are already implemented in the basis configuration of IBAS 1 and IBAS 2. These functions may be called by the user addressing the menu displayed on the black and white data monitor via the keyboard or the

digitising-tablet cursor. When the user calls a function from one of the 'function groups' the corresponding programmes are loaded and implemented on activation of the carriage return key. Thus the IBAS may be 'programmed' from the options in the menu; this is known as procedure editing. These programmes or sequences of functions can be stored and, if required, can then be re-read from disc. However, those familiar with the computer language FORTRAN can manipulate the large number of sub-routines available in order to programme at a deeper level. This method was employed to optimize the routine created by me via the procedure editor for densitometric analysis of the autoradiographs. In this way, it was possible to greatly speed up the analysis time for any single image by sacrificing the flexibility of access to the menu field system in return for obtaining a highly dedicated sequence of single functions entirely specific to the analysis procedure to be carried out. A facility of the free programming capability which was not used was the possibility of programming the array processor itself. Such manoeuvres require extensive programming ability and a knowledge of Microcode Assembler and Processor Architecture, skills not possessed by the author.

A.2.3.2 Individual Procedures

A.2.3.2.1 Signal Averaging: The analogue output of the camera contains a signal and a white-noise component generated in the photonconductor and electronics of the camera. For accurate quantification signal amplitude must be sufficiently large to negate the effects of this noise. The greater the

number of grey levels into which the analogue signal is digitised, the greater is the signal-to-noise (S/N) ratio required. The S/N ratio of the Bosch Plumbicon camera is greater than the 55 dB already adequate for 256 grey level resolution.

However, it was deemed advantageous to optimize the S/N ratio by implementing the software function AVERNI, which is specifically designed to reduce camera noise. The Averni Function provides additional'depth' to the frame store by putting two 8 frame stores together to give 16 bits (1024 grey levels). The 8 least significant bits are then discarded and the eight most significant bits used. The number of additions of the image and the dividing factor can be independently set to any figure up to 256. Ten additions with a dividing factor of ten were used to achieve an appropriate improvement on a reasonable time scale, i.e. without slowing down the densitometric quantification to any appreciable extent (Ramm et al., 1984).

A.2.3.2.2 Shading Correction: Sources of shading error include the illumination source, camera lens, electronics and tube characteristics, and extraneous study light variations. After the errors induced by those factors have been minimized by suitable choice of equipment and optical adjustments thereto, there is still an element of shading variation in the 'white light' or blank image. These non-geometric image errors can only be compensated for by implementation of appropriate software correction (Porro et al., 1984 and Ramm et al., 1984).

Shading error was established on a focussed blank field at 100% transmission. This reference image (or error matrix) is reduced to a quarter of its original size to minimize noise and then stored in the image memory. The grey level occurring with the greatest frequency (reference grey value or RGV) is then calculated from this reference image. This value is then used to calculate the factor by which all other pixels in the reference image which differ from this reference value, must be adjusted in order to bring them back to the RGV. This adjustment can then be carried out multiplicitively to correct for errors. After this process the residual shading at 100% transmission was found to be minimal over the entire image and in the central (most used) region of the field was not significant. This shade-corrected image is then applied to each new incoming 'working' image and the final shade corrected working image displayed on the RGB monitor for interactive analysis or manipulation.

A.2.3.2.3 Calibration

The system is set to the maximum range of values to which the camera is sensitive. First the 'black level' is recorded by closing off the light to the camera completely. This corresponds to 0% transmission and 0, grey levels. Second, the maximum tolerated white light level is recorded by adjusting the light output of the illuminator to 200grey levels. This corresponds to 100% transmission. These two levels are the anchor points for all calibrations. Then the routine requests a series of inputs of specified standard

optical density (Wratten No. 96 Gelatine Neutral Density Filters, Eastman Kodak Company, Rochester, N.Y.) with values between 0.1 and 1.0. These filters contain a dispersion of fine carbon particle in gelatine and are particularly suitable for repeated temporally separate comparisons, as they exhibit very low light scatter (Ramm et al., 1984).

The machine then reads out its assessment of these external standards in units of optical density (O.D.). It does this by mapping the available incoming grey level range (0 - 200) to the available optical density scale 2.55 - 0 (low grey values being associated with high density values). This optical density scaling exercise produces a grey level transformation table. This relates grey levels to transmission (T) then uses an algorithem which applies the formula O.D. = $\log^{10}$ $(\frac{1}{T})$ to convert each measurement so that it can be expressed in optical density units. This exercise calibrates the system as a densitometer and checks the linearity of its performance. If performance drops below acceptable levels throughout the range of optical densities relevant to the autoradiographs being analysed, then the response can be adjusted by application of a correction factor. A typical machine response to a series of O.D. standards is illustrated in Fig. 57 taken during a calibration run for a ^{14}C-2DG autoradiograph where the range of O.D's to be measured would be of the order 0.08 - 0.30.

A.2.3.2.4 Image Magnification (Digital Zooming)

Digital zooming involves the magnificaton of an area of

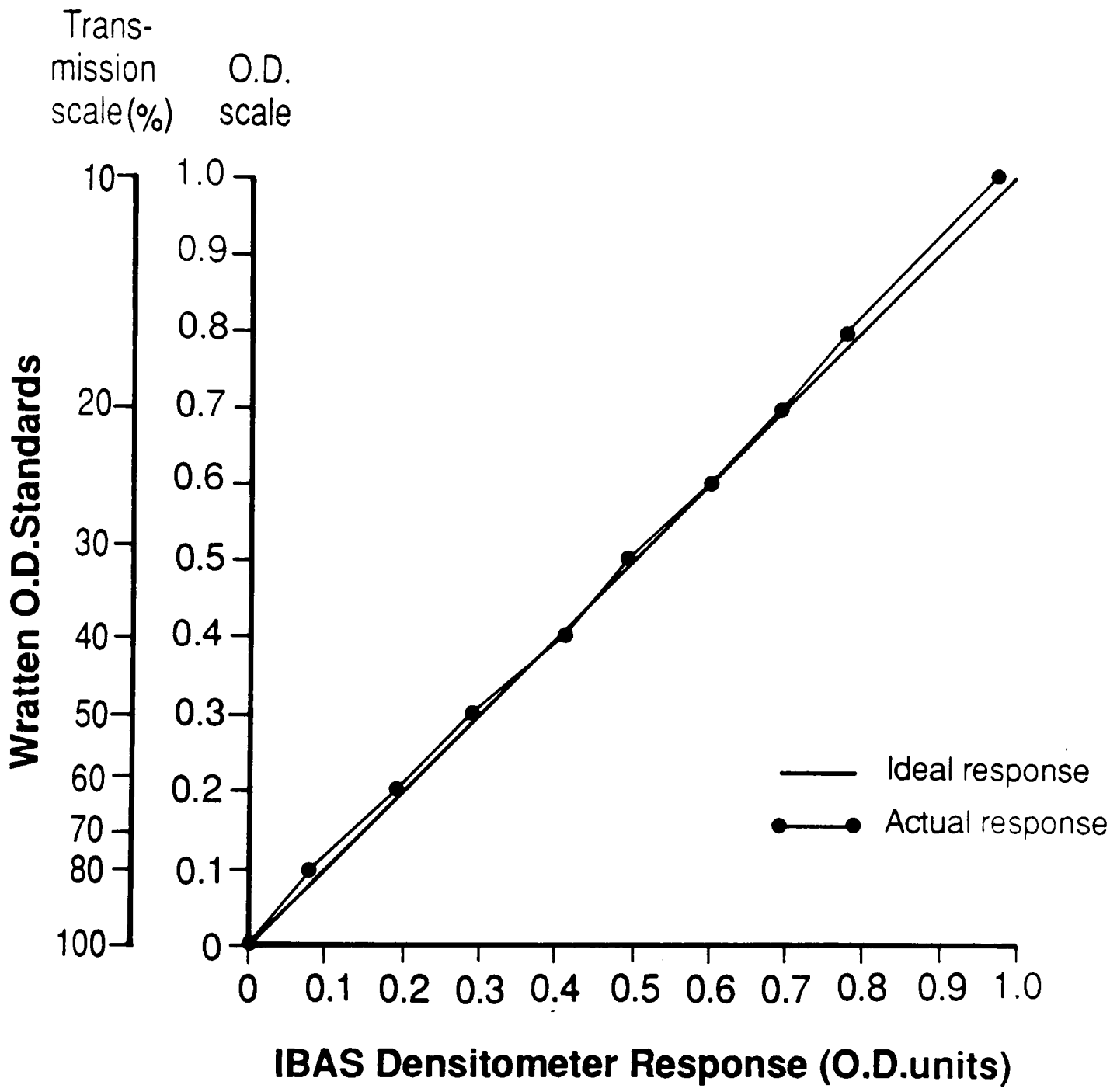


Figure 57 The Performance of the IBAS II as a Video-based Densitometer

An example response of the IBAS II Image Analysis Computer (Calibrated as a densitometer in Optical Density Units (O.D.)) to a series of Wratten O.D. Standards

interest in the image displayed on the RGB image monitor by a linear factor of 2. By this means any 380 x 256 pixel image-display memory (one quadrant) can be expanded to fill the entire 760 x 512 memory array, each of the elements in the original data becoming four elements in the new 760 x 512 image. This magnification process can be repeated sequentially as many times as desired. For these studies magnification was performed only once in order to aid the interactive measuring process especially in the medulla of the ferret. It is important to note that although the process itself does not result in any increase in resolution, it is very useful for studying fine detail (Goochee et al., 1980).

A.2.3.2.5 Pseudocolour Coding

Pseudocolour coding is a technique for transforming a monochrome film image into an enhanced colour image. This technique involves the division of a broad range of optical densities into subranges of optical density that are each assigned a distinct colour for presentation. Pseudocolour presentation takes advantage of the human visual system's exquisite sensitivity to colour variation as compared with its very limited ability to resolve shades of grey (Goochee et al., 1980 and Joyce-Loebl, 1985). It may be employed to represent optical densities in colour and thus help in the identification of the position and extent of specific neural structures. Alternatively, if data on radioactive standards and plasma radioactivity and glucose are available O.D. values can be translated into local cerebral glucose

utilization and the autoradiographs can be transformed into quantitative colour coded maps of local rates of cerebral glucose utilization distributed anatomically throughout the brain section. This can be carried out when the fully quantitative 'Sokoloff technique' is used. Because the densitometer system used can discriminate and store 256 grey levels, it is possible to assign an individual colour for presentation to each of the 256 density levels stored in the image memory giving rise to 10^7 possibilities. However, the RGB monitor can display only 256 of these at any one time because frame store resolution is 256 grey levels. IBAS provides for the construction and storage of 999 colour transformation (or 'Look-Up') tables each consisting of a maximum of 256 separate colours. Once stored on disc a colour table may be implemented at any point in a procedure. In practice, colour schemes in which the full range of density levels is divided among 10 to 20 colours have proved the most appropriate.

Construction of such colour schemes or look-up tables was based on (1) allowing enough colours for small changes or differences on the autoradiograph to be displayed, (2) being easily interpretable by the observer (i.e. bright colours starting at the red end of the spectrum representing areas of high optical density and dark colours, at the blue end representing areas of low optical density) and (3) being free from discontinuities in assigning a group of grey levels to a colour. This exercise of constructing a colour look-up table

is therefore an arbitrary one in which colours can be assigned to equal intervals of the full range or colours can be assigned to intervals that increase in some continuous way (Goochee et al., 1980).

The pseudocolouring used in these studies assigned proportionately narrower ranges of optical density to colours representing areas of high optical density thus providing greater sensitivity in the higher ranges and making discrimination within the densely-packed dorsal medulla much easier.

A.2.3.2.6 Superimposition and Geometric Transformation of Images

One application of this routine is to measure optical densities from an area of the autoradiograph corresponding to an histologically defined structure; this can be important for anatomical areas that occupy a small area of the total section.

Using a function clled Image Positioning this superimposition of histological and autoradiographic image can be carried out, eliminating in the process linear differences such as size, orientation and position. It is executed by placing both images in the image memory then recalling each to occupy the left and right upper quandrants of the RGB display respectively, prior to the selection of a number of matched key 'control' points within either image that correspond in geographical location. Transformation is then carried out with reference to these points and the histological image becomes an overlay for the autoradiographic one and can be used to delineate specific anatomical areas using the interactive cursor as previously described.

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