

ANIMAL MODELS IN CARDIOVASCULAR RESEARCH

by

DAVID R. GROSS, D.V.M., Ph.D.

ANIMAL MODELS IN CARDIOVASCULAR RESEARCH

by

DAVID R. GROSS, D.V.M., Ph.D.

*Department of Veterinary Physiology and Pharmacology
College of Veterinary Medicine
Texas A & M University
College Station, Texas 77843-4466
U.S.A.*



1985 **MARTINUS NIJHOFF PUBLISHERS**
a member of the KLUWER ACADEMIC PUBLISHERS GROUP
POSTON / DORDRECHT / LANCASTER



Distributors

for the United States and Canada: Kluwer Academic Publishers, 190 Old Derby Street, Hingham, MA 02043, USA

for the UK and Ireland: Kluwer Academic Publishers, MTP Press Limited, Falcon House, Queen Square, Lancaster LA1 1RN, UK

for all other countries: Kluwer Academic Publishers Group, Distribution Center, P.O. Box 322, 3300 AH Dordrecht, The Netherlands

Library of Congress Cataloging Card Number: 85-2908

ISBN 0-89838-711-6

Copyright

© 1985 by Martinus Nijhoff Publishers, Dordrecht.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, mechanical, photocopying, recording, or otherwise, without the prior written permission of the publishers,

Martinus Nijhoff Publishers, P.O. Box 163, 3300 AD Dordrecht,
The Netherlands.

PRINTED IN THE NETHERLANDS

Table of Contents

	page number
Preface	1
Chapter 1: General Principles of Animal Selection, Pre- and Post-Operative Care, Preamesthesia, Chemical Restraint and analgesia	3
1. Physical examination	3
2. Normal physiological parameters	6
3. Animal behavior considerations	13
4. Special requirement considerations	13
5. The use of anticholinergic drugs for preamesthesia	14
6. Preamesthetic agents	18
7. Recognition of pain and the use of analgesics	19
Chapter 2: Cardiovascular Effects of the Opioids	21
1. Morphine	22
2. Meperidine	40
3. Methadone	43
4. Pentazocine	47
5. Fentanyl	50
6. Oxymorphone	61
7. Naloxone	62
8. Etorphine	66
9. Others	68
Chapter 3: Cardiovascular Effects of the Tranquilizers	81
1. Phenothiazine derivatives	83
2. Butyrophenones	94
3. Benzodiazepines	103
4. Rauwolfia derivatives	112
5. Xylazine	120

TABLE OF CONTENTS (Continued)

	page number
Chapter 4: Cardiovascular Effects of Other Drugs Commonly Used in Cardiovascular Research .	133
1. Combinations	134
2. Neuromuscular blocking agents	136
3. Skeletal muscle relaxants	138
4. Non-steroidal anti-inflammatory and analgesic agents	142
5. Antibiotics	168
Introduction to the Anesthetic Agents	192
Chapter 5: Cardiovascular Effects of Intravenous Anesthetic Agents	197
1. Barbiturates	199
2. Alpha-chloralose	227
3. Urethane	231
4. Alpha-chloralose and urethane combined	234
Chapter 6: Cardiovascular Effects of Inhalant Anesthetic Agents	244
1. Halothane	246
2. Enflurane	284
3. Methoxyflurane and Isoflurane	294
4. Diethyl ether	301
5. Nitrous oxide	304
6. Cyclopropane, Chloroform, Fluroxene and Trichlorethylene	306

TABLE OF CONTENTS (Continued)

	page number
Chapter 7: Cardiovascular Effects of Halucinogens, Neurolept Analgesic/Anesthetic Combinations and Steroid Anesthetics . . .	321
1. Ketamine	321
2. Ketamine-tranquilizer combinations . .	338
3. Tranquilizer - opioid combinations . .	345
4. Guaifenesin and the steroid anesthetics	355
Chapter 8: Effects of Chemical Restraint and Anesthesia on Blood Glucose Levels	366
1. Opioids	366
2. Tranquilizers	367
3. Xylazine	367
4. Barbiturates	368
5. Urethane	368
6. Inhalent anesthesia	368
7. Neurolept analgesics/anesthetics . . .	369
8. Droperidol and Fentanyl	369
Chapter 9: Normal Cardiovascular Parameters from Intact, Awake Animals	373
1. Dogs	375
2. Cats	406
3. Rats	411
4. Rabbits	417
5. Sheep	423
6. Calves	433
7. Pigs	442
8. Ponies	446
9. Primates	450

TABLE OF CONTENTS (Continued)

	page number
Chapter 10: Naturally Occurring Models of Cardio-vascular Disease	464
Chapter 11: Iatrogenic Models for Studying Heart Disease	498
1. Heart failure models	498
a. Increased ventricular workload	498
1) Pressure overload	500
2) Volume overload	504
3) Valvular insufficiencies and stenoses	506
b. Pulmonary embolism	510
c. Production of heart block and severe arrhythmias	510
d. Coronary ischemia	511
e. Cardiac tamponade	521
f. Radiation - induced	521
g. Repetitive direct current	521
h. Neurogenic stress in adrenal-ectomized subjects	522
2. Portal hypertension models	522
3. Isolated heart preparations	522
4. Microvascular studies	523
5. Organ culture	524
6. Streptococcal rheumatic carditis	524
Chapter 12: Animal Models of Atherosclerosis	536
1. Quantification techniques	536
2. Pathogenesis	537
3. Natural occurrence	538

TABLE OF CONTENTS (Continued)

	page number
4. Specific animal models	538
a. Rabbits	538
b. Birds	540
c. Dogs	541
d. Rats	542
e. Pigs	542
f. Non-human primates	543
5. Regression studies	544
Chapter 13: Animal Models of Hypertension	548
1. Renovascular hypertension	549
a. Goldblatt preparations	549
b. Aortic coarctation	550
c. Perinephritis	550
2. Spontaneously hypertensive rats (SHR)	551
3. Dahl salt-sensitive rats	552
4. Salt and DOCA - salt models	553
5. Neurogenic hypertension and the central component	553
Chapter 14: In-Vivo Measurements, Pitfalls and Problems of Instrumentation	559
1. Transduction of physiological events	559
2. Recording	561
3. Measuring electrical events	562
4. Measuring force and pressure events	564
5. Measuring interstitial fluid pressures	569
6. Measuring pressure in the micro- vasculature	570
7. Indirect techniques for measuring arterial pressures	571

TABLE OF CONTENTS (Continued)

	page number
8. Measuring flow events	573
a. Indicator dilution techniques . . .	573
b. Electromagnetic flowmeters	576
c. Ultrasonic flowmeters	579
d. Catheter - tip velocity meters . .	581
e. Hot - film anemometers	581
9. Measuring chemical events	583
10. Measuring dimension changes	583
a. Strain gauges	583
b. Ultrasonic dimension gauges	584
c. Radiographic techniques	586
11. Measuring temperature changes	587
12. Calculation and/or measurement of volumes	587
13. Use of the microsphere technique for estimating tissue blood flows	588
Index	594

Acknowledgements

For their patience, understanding, good cheer, and exceptional skill with the word processor, through constant revision and change, I thank Esther Grazioli and Tammy Cooper, without whom this work never would have been completed. For her unremitting encouragement and support, my wife Rosalie. For their faith that I could finish, my sons Ted and Jeff. For their tolerance of the time not spent helping them, my graduate students; Brian Gentile, Steve Marks, Fiona McCord, Ellen Morcum, Cynthia Snowden and Colette Wagner-Mann. For his encouragement and understanding, Dr. J.D. McCrady, head Dept. Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University.

PREFACE

Currently in the U.S.A., and in some European countries, the pressure to sharply curtail animal studies has intensified. The shrill rhetoric and horror stories of the antivivisectionists have been toned down by the animal's rights groups. A more sophisticated approach, which effectively focuses and sustains public attention on the problem, has emerged. Animal welfare groups now appear in political forums, such as congressional hearings, armed with facts and figures, sometimes regrettably true, or only slightly distorted. They are well versed in scientific terminology and capable of debating with scientists in a very calm and orderly manner. This approach has had a noticeable effect on politicians.

Regardless of pressure from animal welfare groups we, as scientists, must continue our work, but with constant appreciation and regard for the moral and ethical issues raised by animal research. The concept of "reverance for life" does not, a priori, preclude the use of animals to further scientific knowledge and understanding. It does demand an awareness of the importance and responsibility involved in using and/or taking the life of any creature. It is important to recognize that as much care must be taken in the choice and handling of animal models as in any other facet of research methodology.

It is possible, through institutional lab animal committees and the critical peer review process, to police ourselves against colleagues who violate social and professional standards for humane treatment of animals. We are now being "aided" in this task by legislated restrictions which will, certainly, become more and more stringent, unless our own policing efforts become more effective.

To become more sophisticated, more scientific and, therefore, less vulnerable to criticism in our use of animal models is a difficult task. Our information retrieval systems and/or patience to keep searching are often lacking and we are

then doomed, through ignorance, to repeat work that has been done or, worse, to repeat work that has been done better. Through ignorance of comparative physiology, a less than appropriate species may be chosen for a particular study. Through ignorance of comparative anesthesiology inappropriate chemical restraint or anesthesia may invalidate results. It is to these problems that this text is addressed.

In a statement submitted to the House Subcommittee on Science, Research and Technology, Professor Earl H. Wood, while President of FASEB, made the following observations. "...Scientists do not, by definition, walk on water. They are human and there will be those who, either from defect of instinct or indifference to social and professional values, betray the spirit of their profession and the public trust. The scientist who finds himself in this category should be prepared to live with the censure of his peers and whatever penalties society sees fit to inflict. But he or she should be dealt with as an individual aberration..." (FASEB Newsletter, Vol. 14, No. 16, Nov., 1981). There is increasing political pressure being applied to find alternatives for animal studies. Again quoting from Professor Wood: "...The so-called alternative methods -- computer simulation, mathematical models, use of cell and tissue culture, etc. -- are, in fact, aids, adjuncts, supplements or shortcuts which help an investigator decide whether an experiment on an animal is likely to produce a useful result..."

CHAPTER 1

GENERAL PRINCIPLES OF ANIMAL SELECTION, PREOPERATIVE CARE, PREANESTHESIA, CHEMICAL RESTRAINT AND ANALGESIA

It seems obvious that physiological results cannot be obtained from pathological specimens. Despite this universally accepted axiom, the number of so-called physiological experiments conducted on animals incubating disease or obviously ill can be appalling. The importance of this point cannot be overemphasized. Animals used for physiological experiments must be held long enough in approved facilities to insure that they are not incubating infectious diseases. They should be vaccinated against the diseases likely to cause a problem in that species and they should be verified free of internal and external parasites prior to use. These precautions are expensive but miniscule compared to the overall cost of conducting experiments where the results are suspect because of the condition of the subject.

Physical examination:

Prior to use, the animal should be given a thorough physical examination. One of the most neglected aspects of this examination is some history, easily supplied by observant animal care personnel, of the appetite displayed by the animal and the character of the urine and feces. It is a rare animal that eats normally when it is ill. The physical examination should include an assessment of the rectal temperature, feasible in most species commonly used. A list of normal rectal temperatures is provided as Table 1.1.

The physical examination should also include an evaluation of the mucous membranes, with particular attention to abnormal discharges from the eyes and nose. Animals with inflamed

mucous membranes should not be used. Judicious use of the stethoscope can rule out the possibility of using an animal for cardiovascular studies with a congenital or acquired heart murmur, unless that model is of particular interest. Animals which originate from areas where heartworms or parasitic blood diseases are a problem must be shown to be free of these afflictions. It is probably a good idea to take an electrocardiogram, especially if one of the giant breeds, or giant breed-crosses is being used. Although congenital and acquired arrhythmias are relatively rare in dogs they do occur (see Chapter 11).

It may not be necessary to do a complete hematological evaluation on every animal, but in studies where extensive surgical preparation is necessary and when considerable time and money are to be invested in the model, such an evaluation could be essential. Tables 1.2-1.8 provide normal hematological data for most of the species now in common use. It should be pointed out that these normal values may vary with geographical location (i.e. sea level versus high altitude) and with prevalent breeds in the particular region as well as with gender and age. The values provided are from the Texas Veterinary Diagnostic Laboratory, the Clinical Pathology Laboratory of the Texas A&M Veterinary Teaching Hospital, our Laboratory of Physiology and Applied Physics and the Texas A&M Laboratory Animal Resources Facility.

The state of hydration is an important consideration in cardiovascular studies. All animals should be well hydrated before they are used and if the procedure or preparation of the model requires extensive surgery, or is of long duration, the state of hydration must be maintained during the course of the experimental procedure. Again, these considerations seem obvious and redundant but, unfortunately, they are often ignored or forgotten.

Most procedures in most species do not require that the animal be without water prior to the anesthesia. Free choice of water to a healthy subject should insure proper hydration. As a matter of convenience, and to prevent inspiration of ingesta, it is advisable to withhold food overnight or for 12 hours prior to general anesthesia.

Recent knowledge and understanding of blood coagulation and coagulopathies have created interest in hematological parameters related to this system. Platelet counts have been done on most species and found to range from about $2-5 \times 10^5/\text{ml}$. Cats, sheep and calves range slightly higher, from

about $3-8 \times 10^5/\text{ml}$. Other commonly conducted tests include activated prothrombin time (A-PTT), prothrombin time (PT), thrombin time and Fibrinogen levels. The use of the heat precipitation method for the latter results in falsely low values. The capillary tube clotting time is insensitive and limited to detection of severe clotting defects. Results of measurements of the other parameters will vary with; the volume of sample used, the incubation time, the commercial reagent used (including freshness), the anticoagulant used for sample collection and the concentration of Ca^{++} present. There also appear to be optimal conditions for the assays which vary for each species. Most knowledgeable researchers in this field seem to agree that, if these data are required, it is best to compare subjects with normal controls at the time and in the same lab where the evaluation will be made.

Table 1.1. Range of normal rectal temperatures for some commonly used animal species (references; 1, 2)

	°C	°F
Dogs	37.9-39.9	100.1-102.8
Cats	38.1-39.2	100.5-102.5
Cattle	36.7-39.1	98.0-102.4
Sheep	38.3-39.9	100.9-103.8
Goats	38.5-39.7	101.3-103.5
Horses and Ponies	37.2-38.2	99.0-100.8
Swine	38.7-39.8	101.6-103.6
Rabbits	38.6-40.1	101.5-104.2
Monkey, Rhesus	38.4	101.1
Guinea pigs	38.6	101.5
Hamster	36.38	98-101
Rats	37.5	99.5
Mice	37.4	99.3

Table 1.2. Averages (single values) or ranges of normal blood count data.

	WBC ($\times 10^3$)	Neutro- philes (%)	Lympho- cytes (%)	Mono- cytes (%)	Eosino- philes (%)
Dogs	6-17	60-77	12-30	3-10	2-10
Cats	5.5-19.5	35-75	20-55	1-4	2-12
Rats	9.76	25.6	73.9	0.29	0.48
Mice	13.5	17.5	72.3	2.19	2.25
Rabbits	8.45	44.7	40.4	8.5	2.0
Hamsters	8.10	25.6	70.7	2.45	0.8
Guinea pigs	11.2	36.5	56.2	3.05	3.75
Pigs	14.8	34.0	55.5	4.3	0.24
Sheep	4-12	31.8	59.4	3.25	5.2
Goats	4-13	30-48	50-70	0-4	1.8
Horses	5.4-14.3	22-72	17-68	0-14	0-10
Calves	4-12	15-45	45-75	2-7	2-20
Primates (old world)	10.8	38.6	58.8	1.10	1.85
Primates (new world)	4.3-28.5	46-82	13-54	0-3	0-4

Table 1.3. Averages (single values) or ranges of normal red blood cell parameters.

	Hemato- crit	Hemo- globin	RBC	Mean Cor- puscular volume	Mean Cor- puscular Hemoglobin concn.
	(%)	(g/100ml)	($\times 10^6/\text{mcl}$)	(micron) ³	(g%)
Dogs	35-55	12-18	5.5-8.5	60-77	32-36
Cats	24-45	3-15	5-10	39-55	30-36
Rats	45.8	14.2	8.47	56.0	31.1
Mice	41.8	11.1	9.2	49.25	26.5
Rabbits	40.6	13.4	6.51	62.8	32.8
Hamsters	50.7	16.4	7.35	70.0	32.3
Guinea pigs	43.7	14.3	5.18	84	32.8
Pigs	41.0	12.4	6.99	58.5	30.2
Sheep	27-45	9-15	9-15	28-40	31-34
Goats	23-38	8-12	8.18	15-30	35-42
Horses	32-53	11-19	6.8-12.9	37-58.5	31-38.6
Calves	24-46	9.5-12.5	5.2-6.8	49.7-64.3	30.2-35.8
Primates (old world)	27-44	10-13	5.17	82.0	31.9
Primates (new world)	17-48	5-12.5	1.97-4.22	54.8-15.4	15.4-38.5