Animal Models in Cardiovascular Research

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Third Edition

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To Drs. Theodore S. Gross and Jeffrey M. Gross who continue the family obsession for the acquisition and dissemination of new knowledge.

Preface

This new edition of *Animal Models in Cardiovascular Research* describes historical and recent advances in our understanding of the cardiovascular system from studies conducted in a variety of animal models. Since the last edition, we have witnessed an explosion in the use of both congenic and transgenic animals. The use of specific knock-in and knock-out transgenic models has resulted in an avalanche of genetic, molecular, and protein-based information that, potentially, could result in an amazing new array of treatment and management options. However, the results of these studies also introduce a sometime bewildering array of redundant, overlapping, and competing molecular pathways involved in both physiological and pathological responses.

This third edition is designed to provide a better basis for understanding and using animal models in the current climate of background knowledge and information. It is significantly different than the previous two editions. Chapter 1 is updated from the previous editions addressing general principles of animal selection. It also provides expanded tables of normal physiological values for easy reference. Chapter 2 covers preoperative care, preanesthesia, and chemical restraint, and includes a significantly expanded section on pain recognition and analgesia particularly in rodents. Chapter 3 provides a summary of normal cardiovascular parameters obtained from intact, awake animals. The data have been rearranged in outline rather than the previous tabular form hopefully resulting in easier reference.

Chapter 4 addresses the techniques, problems, and pitfalls of measuring cardiac function in animals. There is an emphasis on the proper use of these measurements to develop new treatment and management strategies as well as using them to study mechanisms of disease. Chapter 5 emphasizes the techniques, problems, and pitfalls involved in the measurement of arterial function and ventricular/arterial coupling dynamics. Again the emphasis is on the use of these parameters to develop new treatment and management strategies and for studying the mechanisms of disease. Chapter 6 is an all new chapter dealing specifically with the problems and pitfalls inherent in using isolated heart preparations. The need for this chapter became apparent because so much information was published using obviously nonphysiologic preparations. The use of both pumping and nonpumping preparations is described along with techniques necessary for using hearts from larger species where oxygen-carrying capacity of the perfusate is critical. The importance of hypoxia and anoxia in the interpretation of results is discussed.

Chapter 7 focuses on the cardiovascular effects of the postoperative analgesic drugs commonly used today and how to avoid potential problems resulting from these effects when reporting experimental data. These data are also presented in outline form rather than the tabular format used in the two previous editions. Chapter 8 addresses the use of naturally occurring animal models of valvular and infectious cardiovascular disease. The information presented has been updated and expanded from the second edition. Chapter 9 examines iatrogenic models of ischemic heart disease.

Chapter 10 is new. It provides a review of iatrogenic, transgenic, and naturally occurring animal models of cardiomyopathy and heart failure. Chapter 11 includes new, updated, and revised information reviewing iatrogenic and transgenic models of hypertension. Chapter 12 contains new and updated information on iatrogenic and transgenic models of atherosclerotic disease.

Chapter 13 is completely a new material dealing with animal models for the study of neurohumeral and central nervous system control of the cardiovascular system. Chapter 14 is also new. It provides examples of cardiovascular studies involving the use of specific transgenic models that are not normally associated with the cardiovascular system, such as estrogen receptor knockouts, to study cardiovascular function.

Urbana, IL David R. Gross

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Rosalie Gross has supported, encouraged, loved and endured since April of 1960. Our journey together continues.

Special thanks are owed to Professor Gary A. Iwamoto. Chapter 13 benefited significantly from his critical review and insightful suggestions.

Introduction

Animal rights activist organizations lobbied for and obtained significantly more restrictive regulations from governments worldwide since the last edition of this text. The agenda of most animal rights groups is to stop our use of animals. If biomedical science is to advance, we must understand the complexities and interrelationships of the various physiological control mechanisms that regulate living whole animal systems. It is more essential than ever that we persevere.

"Old guard" physiologists, pharmacologists, and toxicologists, familiar with whole animal homeostatic control mechanisms, believe some "new breed" scientists working at the molecular level are so focused in their particular area of expertise they have little appreciation for the potential effects of the iatrogenic changes they induce on the whole animal. Frequently lost in the landslide of new information is an understanding of how any particular gene, molecule, and/or protein fits into our broad understanding of the basic physiology of the animal species and how that relates to the human species. A few scientists even seem unaware that most, if not all, physiological systems have multiple and redundant control mechanisms that adjust any particular organ or system behavior to the homeostatic requirements of the whole animal.

Physiological systems may have several stimulatory and inhibitory controls that operate at some constant level of activity. The same physiological response is therefore possible by increasing stimulation or decreasing inhibition or vice versa. At the molecular and cellular level, the more information we obtain the more apparent it becomes that multiple pathways are present to achieve the same response. When cells, or organ systems, are perturbed by interrupting, knocking out, or upregulating a specific molecular or genetic pathway, redundant pathway(s) are, appropriately, up- or downregulated in response to maintain homeostasis.

A significant number of the animal models described in this text have been derived using either congenic or transgenic techniques. It is therefore appropriate to provide an abbreviated description of how these animals are produced.

Congenic strains of animals are developed by mating two inbred strains and then backcrossing the descendants for at least five and up to ten or more generations with one of the original strains. At each step, selections are made for the specific phenotype or genotype of interest. This allows the phenotype or genotype to pass from the donor strain onto an otherwise uniform recipient strain. The congenic

strain can then be compared to the pure recipient strain to determine phenotypical or genetic differences. Producing large numbers of eggs via superovulation in females and then using microsatellite or nucleotide polymorphism markers to track the genes of interest can speed up specific congenic strain development.

Transgenic animals are generally created using one of two different protocols. The first uses recombinant DNA methodology to insert (knock in) or remove (knock out) a specific gene or protein from the genome. The DNA used usually includes a structural gene, as well as other sequences, that enables it to be incorporated into the DNA of the host and to be expressed by the particular cells of interest. The most common method of producing a transgenic animal model involves harvesting embryonic stem cells from the inner cell mass of blastocysts. When these cells are grown in culture, they retain their ability to produce all the cells of the mature animal. The cultured cells are then exposed to the DNA of interest and those cells that successfully incorporate the DNA are identified and separated. These isolated cells are then injected into the inner cell mass of blastocysts. The resulting embryos are transplanted into a pseudopregnant dam. It is uncommon, at least in mice, for more than a third of the embryos thus transplanted to develop into healthy offspring. The next step is to test all the offspring to identify those with the desired gene. Usually, no more than 10–20% will have that gene and they will be heterozygous for it. The next step is to mate two heterozygous mice and screen their offspring for the one in four that will be homozygous for the transgene of interest. Mating homozygous animals produces the transgenic strain.

A second method of producing transgenic animals involves the same preparation of the DNA of interest and then harvesting freshly fertilized eggs before the sperm head has become a pronucleus. The male pronucleus is injected with the DNA of interest, and when the pronuclei have fused to form diploid zygote nuclei, the zygote is allowed to divide by mitosis to form a two-cell embryo. The two-cell embryo is then implanted in the pseudopregnant foster mother and the same steps used in the embryonic stem cell method are followed.

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About the Author

Dr. David Gross entered private veterinary practice after earning the DVM degree from Colorado State University in 1960. In 1974 he was awarded the Ph.D. degree in physiology from the Ohio State University beginning a 36-year career in academics that culminated as professor and head of the Department of Veterinary Biosciences in the College of Veterinary Medicine, University of Illinois, Urbana-Champaign. Dr. Gross' research career encompassed 58 funded projects totaling over $5.5 million and 91 papers published in refereed journals using a wide variety of animal models. Ironically, his three most-cited research papers received no external funding. He and his colleagues showed that feeding dietary cholesterol to rabbits induced Alzheimer's-like lesions in the brain. Their work also showed that surgery involving cardiopulmonary bypass resulted in Alzheimer's-like brain lesions in pigs. With another group of colleagues, he helped pioneer minimally invasive coronary artery bypass grafting techniques using the pig as a model.