The 3R's Approach in Preclinical Pharmacology

The 3R's Approach in Preclinical Pharmacology:

Promoting Animal Welfare and Scientific Ethics

Edited by

Prashant Tiwari and K Sunil Kumar

Cambridge
Scholars
Publishing

The 3R's Approach in Preclinical Pharmacology:
Promoting Animal Welfare and Scientific Ethics

Edited by Prashant Tiwari and K Sunil Kumar

This book first published 2025

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-1-0364-4812-7
ISBN (Ebook): 978-1-0364-4813-4

TABLE OF CONTENTS

CHAPTER ONE

HISTORICAL PERSPECTIVES OF ANIMAL UTILIZATION IN PHARMACEUTICAL RESEARCH

SUNNY RATHEE[1,2], SAKSHI SONI[2], SUNIL KUMAR KADIRI[1], PRASHANT TIWARI[1], DEBASIS SEN[2], SINA ANDALIB[3], DEENANATH JHADE[4]

[1]College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru, Karnatka-560111, India.
[2]Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar, Madhya Pradesh, 470003, India.
[3]Department of Pharmacology, Zanjan University of Medical Sciences, Zanjan, Iran.
[4]School of Pharmacy, Mody University of Science and Technology, Lakshmangarh, Rajasthan-332311, India.
.

Abstract

The use of animals in pharmaceutical research has a long and complex history, deeply intertwined with the advancement of medicine and scientific discovery. This chapter, Historical Perspectives of Animal Utilization in Pharmaceutical Research, traces the evolution of animal experimentation from ancient civilizations to the modern era, highlighting its pivotal role in the development of pharmacological knowledge. Beginning with early dissection and physiological studies by classical thinkers like Galen and Aristotle, animal models have contributed significantly to the understanding of human biology and disease mechanisms. As experimental pharmacology took shape in the 17th and 18th centuries, animal testing became a cornerstone in drug discovery and safety assessment, leading to breakthroughs in

anesthesia, antibiotics, and other life-saving treatments. However, the growing standardization of these methods was not without ethical concerns, and by the 20th century, animal welfare movements gained traction, culminating in the introduction of the 3 R's principle i.e., Replacement, Reduction, and Refinement in 1959. This ethical framework revolutionized the approach to animal experimentation, encouraging the use of alternatives, minimizing the number of animals used, and improving animal welfare in research settings. In this chapter, we explore the implementation of the 3 R's principle in modern preclinical pharmacology, examining how regulatory guidelines, ethical considerations, and technological advancements have shaped current practices. Through a series of historical case studies, we illustrate how animal models have facilitated major pharmaceutical breakthroughs while also addressing the ethical challenges that arise in balancing scientific progress with animal welfare. Looking ahead, we discuss the future of preclinical pharmacology, focusing on emerging alternatives to animal models, such as *in silico* and *in vitro* methods, and their potential to transform drug development while maintaining high ethical standards. This chapter offers a comprehensive understanding of the historical role of animals in pharmaceutical research, emphasizing the ongoing need to integrate innovation with scientific ethics in the pursuit of therapeutic advancement.

Keywords: Animal experimentation**;** Preclinical pharmacology**;** 3 R's principle**;** Drug discovery**;** Ethical guidelines**.**

1. Introduction

Animal use in pharmaceutical research has played a foundational role in the discovery, development, and testing of drugs, allowing scientists to understand disease mechanisms, explore potential treatments, and ensure the safety of new therapies. This practice, dating back to ancient times, has significantly contributed to the growth of modern medicine. However, it has also raised complex ethical questions about the treatment of animals in experimental settings. Balancing scientific advancement with the humane treatment of animals remains a core challenge in pharmaceutical research today (Sparrow et al., 2011, Nassar, 2018).

1.1. Overview of Animal Use in Pharmaceutical Research

The utilization of animals in pharmaceutical research has a long-standing history, as animals have served as crucial models for understanding human

physiology, pathology, and pharmacology. In the absence of in vitro models and advanced computational tools, researchers relied heavily on animals to replicate the biological processes seen in humans. Animal studies allowed researchers to simulate diseases, test drug efficacy, and assess toxicological effects, forming the bedrock of preclinical drug development. Early experiments laid the foundation for breakthroughs in various areas, including the discovery of antibiotics, anesthetics, vaccines, and life-saving treatments for chronic diseases. The information gleaned from these studies has been indispensable in shaping modern pharmacology and the creation of effective therapeutic interventions. Historically, animal models have been used for their ability to provide insights into human-like biological responses, offering invaluable data for drug discovery and safety assessments. Rodents, primates, dogs, and other species have been integral to understanding how a drug interacts with complex living systems. The development of novel drugs and the identification of adverse effects before human trials remain primary goals of preclinical studies involving animals. Despite this, concerns regarding the ethical treatment of animals, their well-being, and the necessity of using animals in certain experiments have prompted significant debate in scientific and public spheres alike (Kinter et al., 2021, Mukherjee et al., 2022).

1.2. Evolution of Ethical Perspectives in Animal Experimentation

As animal experimentation became more common, ethical concerns about the welfare and rights of animals gradually emerged. In the early days of animal research, there were few formal guidelines or laws governing the treatment of animals in scientific studies. However, by the late 19th and early 20th centuries, awareness of the moral implications of animal testing began to grow. Prominent figures in science and philosophy, as well as animal welfare activists, began to question the necessity of using animals in research and called for more humane practices (Grandin and Whiting, 2018; Huxley and Ruse, 2024; Garner, 2024).

The turning point in ethical discourse came in 1959 with the introduction of the 3 R's principle; Replacement, Reduction, and Refinement; proposed by Russell and Burch. This principle aimed to provide an ethical framework for animal research, encouraging scientists to seek alternatives to animal models where possible (Replacement), reduce the number of animals used in experiments (Reduction), and refine experimental techniques to minimize suffering and improve animal welfare (Refinement). The 3 R's principle has

since become a cornerstone of ethical guidelines for animal experimentation worldwide, leading to the development of stringent regulations governing the use of animals in pharmaceutical research.

In recent decades, technological advancements such as in vitro testing, computational modeling, and advanced imaging techniques have offered viable alternatives to animal testing. These innovations, in conjunction with regulatory frameworks and ethical codes, have helped reduce the reliance on animal models while still ensuring the reliability and efficacy of preclinical drug testing. However, challenges remain in fully replacing animal models, particularly in complex biological systems where alternative methods may not yet replicate the intricacies of living organisms (Navalta et al., 2019).

Today, the ethical landscape of animal research continues to evolve, with a growing emphasis on promoting humane treatment, reducing animal use, and developing scientifically sound alternatives. International organizations and regulatory bodies, such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the Organisation for Economic Co-operation and Development (OECD), have integrated these ethical considerations into guidelines that emphasize both scientific rigor and animal welfare. **Figure 1** presents a timeline tracing the evolution of animal use in medical research, from early anatomical studies in 3000 BCE through significant milestones such as Galen's dissections and modern advancements in COVID-19 vaccine development. It emphasizes key discoveries in various fields and highlights the ethical and regulatory developments shaping animal research practices over millennia (Kropotkin, 2021).

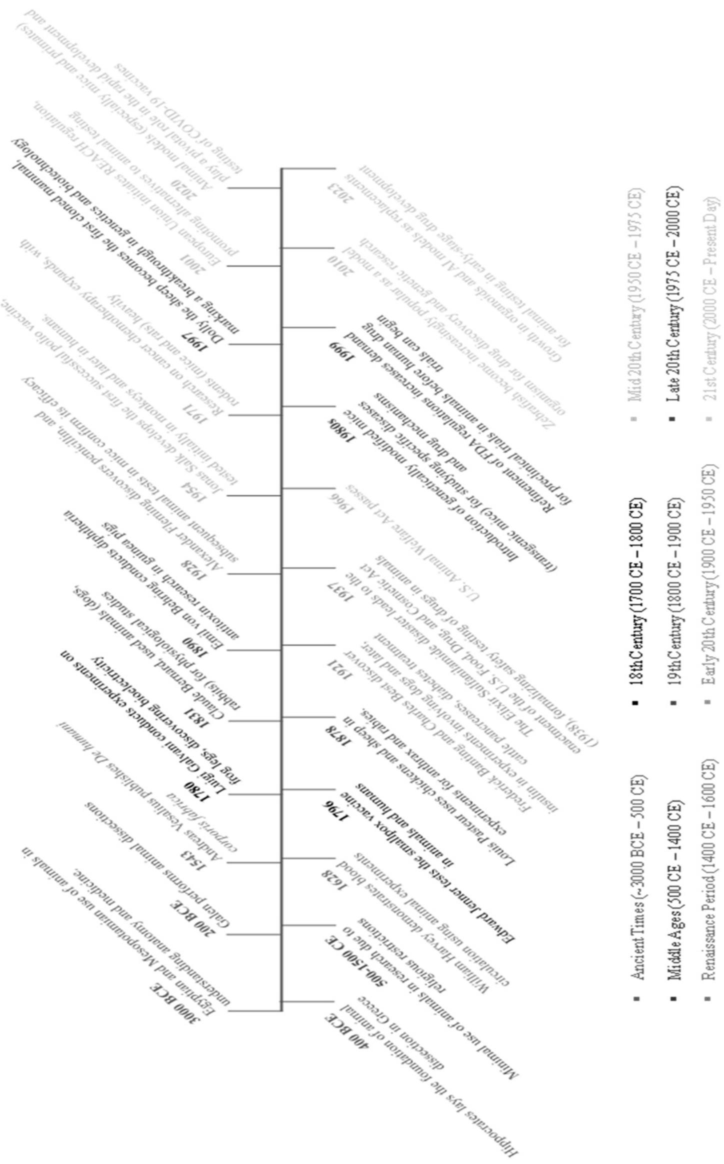

Figure 1. Timeline of Animal Use in Medicine: Key Milestones from Ancient Times to the Present Day

2. Early History of Animal Use in Medicine

The history of animal use in medicine is deeply rooted in the earliest attempts to understand the human body, disease, and the therapeutic potential of natural remedies. Across various ancient civilizations, animals were used not only for food and clothing but also as models for studying human physiology and developing medicinal practices. The evolution of medical knowledge, driven by curiosity about the natural world, inevitably led to the use of animals for experimentation and observation. From ancient Egypt to Greece, and later into the medieval European period, animals played a key role in advancing medical practices, particularly in areas where human dissection was limited by cultural and religious taboos. By experimenting on animals, early physicians and scholars began to unlock the mysteries of the human body, setting the stage for modern medical and pharmaceutical research. However, these practices were not without controversy, even in ancient times, as debates about the ethical implications of using animals in experimentation started to take shape (Castiglioni, 2019, Serpell, 2025, Kirchhelle, 2018).

2.1. Animal Experimentation in Ancient Civilizations

The use of animals in medicine can be traced back to some of the world's earliest civilizations, including ancient Egypt, Mesopotamia, India, and China. In these cultures, animals were used for both symbolic and practical purposes in medical practice. For instance, in ancient Egypt, animals such as cats, dogs, and birds were often associated with gods and goddesses of healing, and their bodies were occasionally dissected to learn about anatomy. Egyptian papyri, such as the Ebers Papyrus, contain some of the earliest known references to medicinal treatments that were tested on animals before being used on humans. In ancient India, the Ayurvedic system of medicine involved the use of various animals in understanding disease processes and developing treatments. The Charaka Samhita and Sushruta Samhita, two foundational texts of Ayurveda, include discussions on the importance of animals for medical research and experimentation. Similarly, in China, animals were often used in early medicinal practices, with texts like the Huangdi Neijing (The Yellow Emperor's Classic of Medicine) discussing the use of herbal remedies and their effects on animals as a preliminary to human application. One of the most influential civilizations in the use of animals for medical purposes was Ancient Greece. Greek physicians, such as Hippocrates, emphasized the importance of empirical observation in medical practice. This led to the early use of

animals as models for human biology, where observation of animal anatomy and physiology helped to form the basis of medical treatments and procedures (Akpan et al., 2020; Merheb et al., 2019; Adams, 2018).

2.2. Contributions of Classical Thinkers (Galen, Aristotle, etc.)

The contributions of classical thinkers such as Aristotle and Galen were monumental in shaping the early use of animals in scientific experimentation. Aristotle (384–322 BCE), often regarded as the father of biology, conducted extensive studies on animals, particularly in his work Historia Animalium (History of Animals). He was one of the first to systematically categorize animals and make detailed observations of their anatomy and behavior. Although Aristotle's conclusions were sometimes speculative, his studies laid the groundwork for future biological and medical research (Willingham and Riener, 2019; Kirk, 2018).

Aristotle believed that animals, as complex living organisms, shared many physiological characteristics with humans. This belief justified his use of animals in dissecting and studying their internal structures. His observations contributed significantly to the understanding of cardiovascular systems, reproductive systems, and other biological processes. Aristotle's emphasis on empirical observation over purely theoretical speculation helped establish animals as vital subjects for scientific research (Lennox, 2019; Yack, 2023).

Galen of Pergamon (129–216 CE), a physician in ancient Rome, made perhaps the most significant contributions to early animal experimentation, particularly in the fields of anatomy and physiology. Since human dissection was forbidden in Rome at the time, Galen used animals especially monkeys, pigs, and dogs for his anatomical studies. Through these dissections, he made groundbreaking discoveries about the circulatory and nervous systems, and his work was foundational in the development of medical science in both the Islamic world and medieval Europe. Galen's approach to animal experimentation was systematic and scientific, laying the groundwork for modern experimental physiology. His observations on animal organs and systems were extrapolated to humans, and though some of his ideas were later disproven (such as his view of the circulatory system), his methodology had a profound and lasting influence. Galen's work was considered authoritative for over a millennium, forming the cornerstone of European medical thought well into the Renaissance (Muraru, 2018; Nutton, 2020; Clements, 2022). **Table 1** highlights the role of animals in early medical practices across different civilizations, showcasing their

contributions to anatomical studies, traditional medicines, and healing rituals.

Table 1. A Historical Overview of Animal Use in Medicine: From Ancient Civilizations to the Renaissance

Period/ Date	Civilization/ Region	Animals Used	Purpose in Medicine	Key Contributions/ Notes
Ancient Egypt (c. 3000 BCE)	Egyptian Civilization	Cats, snakes, ibises	Worship and healing practices, snake venom for ailments	Cats revered for protection against evil spirits; animal sacrifices for health and healing
Ancient India (c. 2500 BCE)	Indus Valley Civilization	Cows, horses, snakes	Ayurveda used animal parts and products for treatment	Ayurveda recognized the importance of cows' milk, ghee, and snake venom in therapies
Ancient China (c. 2000 BCE)	Chinese Civilization	Tigers, deer, bears, snakes	Use in Traditional Chinese Medicine (TCM), snake venom, animal organs	Animal parts believed to restore balance of Yin and Yang; deer antler used for vitality
Ancient Greece (c. 500 BCE)	Greek Civilization	Pigs, monkeys, snakes, bees	Dissection for anatomical studies, use of honey for healing	Hippocrates advocated animal dissections for learning; bee products used for wound treatment
Roman Empire (c. 200 BCE)	Roman Civilization	Pigs, horses, dogs, goats	Animal dissections, use in veterinary medicine	Galen’s work on pig dissections laid foundation for human anatomy; military animal medicine

Middle Ages (c. 500–1500 CE)	European Civilization	Cats, birds, leeches, horses	Leeches for bloodletting, animal products for medicine	Use of animal fats and blood for medicinal salves; leech therapy common in medieval Europe
Early Islamic Medicine (c. 700–1300 CE)	Islamic Civilization	Horses, camels, goats	Veterinary medicine and animal experimentation	Pioneering veterinary sciences; Al-Jahiz wrote on animal psychology and behaviors
Renaissance (c. 1400–1600 CE)	European Renaissance	Monkeys, pigs, dogs, birds	Anatomical research, study of physiology through dissection	Vesalius and Harvey used animals to advance knowledge of human circulatory systems

2.3. Animal Dissection and Physiology in Medieval Europe

The practice of animal dissection continued into the medieval period, although it was significantly shaped by the cultural and religious contexts of the time. During the early Middle Ages, the dissection of human bodies was strictly prohibited by both the Christian Church and Islamic scholars, largely for religious and ethical reasons. However, the dissection of animals was permitted and became a primary method for physicians to learn about anatomy and physiology. Scholars in both the Islamic world and Europe relied heavily on animal dissections to expand their knowledge of medicine. In the Islamic Golden Age (8^{th} to 14^{th} century), scholars like Avicenna (Ibn Sina) built upon the knowledge of Galen and Aristotle, conducting detailed anatomical studies on animals. Avicenna's The Canon of Medicine, which synthesized Greek, Roman, and Islamic medical knowledge, incorporated findings from animal experimentation and dissection, becoming a cornerstone text in both Islamic and European medical education. In medieval Europe, knowledge of anatomy remained limited until the Renaissance. However, certain monasteries and medical schools began incorporating animal dissections into their curricula as a way to understand

human anatomy by proxy. Although human dissection was officially prohibited, the medical schools of Bologna and Padua started to cautiously perform human dissections in the 13th and 14th centuries. Nevertheless, animal models continued to play a crucial role in understanding basic physiological processes during this period. The dissection of animals, particularly pigs and dogs, became standard practice in the medical schools of Europe. These dissections allowed scholars to advance their knowledge of the circulatory and nervous systems, preparing the way for the breakthroughs in human anatomy that would come with the Renaissance. By the end of the medieval period, dissection had evolved from a forbidden practice to a crucial tool in the medical sciences, setting the stage for a new era of scientific inquiry that would incorporate both animal and human studies (Conti and Paternostro, 2019; Brenna, 2022; McCall, 2018; Irschick et al., 2019; Geller, 2019).

3. The Birth of Experimental Pharmacology

The birth of experimental pharmacology marked a significant shift in the way scientists approached the study of drugs and their effects on living organisms. Before the 17th century, the understanding of drugs was largely based on anecdotal evidence, traditional medicine, and observational studies. However, the rise of the scientific method, coupled with a growing interest in the precise mechanisms of drug action, led to the development of experimental pharmacology as a discipline. This transition from theoretical medicine to a science based on empirical evidence and experimentation brought animal models into the forefront, as researchers needed reliable and controlled environments to test hypotheses and study the effects of substances on biological systems (Kenakin, 2024; Upadhyay et al., 2020; Neal, 2020).

3.1. Rise of Scientific Methods in the 17th and 18th Centuries

The 17th and 18th centuries were transformative periods in the history of science, marked by the emergence of the scientific method as a systematic approach to inquiry. This method emphasized the importance of experimentation, observation, and the replication of results, which contrasted sharply with the speculative and philosophical methods that had dominated earlier medical practices. Key figures in this period, such as Galileo Galilei, René Descartes, and Francis Bacon, championed the idea that knowledge should be derived from empirical evidence rather than abstract reasoning. Their influence laid the groundwork for modern

experimental sciences, including pharmacology (Shapin, 2018; Davies et al., 2020; Heilbron, 2023).

In the realm of medicine, the application of the scientific method led to a more rigorous examination of how substances, including plants and minerals, affected the body. Physicians and early pharmacologists began conducting controlled experiments, often using animals to observe the physiological effects of drugs. This period also saw the development of more sophisticated techniques for studying biological processes, including dissection, microscopy, and chemical analysis, which allowed researchers to better understand the mechanisms through which drugs exerted their effects.

The use of animals in these experiments became a critical part of pharmacological research, as it provided scientists with a living model to observe the interaction between drugs and biological systems in real-time. Rodents, dogs, and rabbits were commonly used during this period, as their physiological responses often closely resembled those of humans. These early experiments laid the foundation for modern pharmacology by establishing the principles of dose-response relationships, drug metabolism, and the concept of drug toxicity (Tsatsakis et al., 2018; Borgert et al., 2021; Gupta and Gupta, 2019).

3.2. Pioneers of Experimental Pharmacology (e.g., Claude Bernard)

One of the most influential figures in the development of experimental pharmacology was Claude Bernard (1813–1878), a French physiologist who is often credited as one of the founding fathers of the discipline. Bernard was a pioneer in the use of animals for scientific experimentation, and his work greatly advanced the understanding of how drugs affect the body. His famous experiments on curare (a plant-derived poison used in South American hunting) demonstrated how the substance caused paralysis by interfering with nerve signals to the muscles, a discovery that had profound implications for both pharmacology and medicine (Supuran, 2020).

Bernard's scientific approach was based on the idea that the effects of drugs could be studied in a controlled, experimental setting. He believed that by using animals as models, researchers could isolate and observe specific physiological responses to drugs. His book Introduction to the Study of Experimental Medicine (1865) articulated the principles of the scientific

method in biological research, emphasizing the importance of hypothesis testing, controlled experiments, and reproducibility. This text became a cornerstone for the burgeoning field of experimental pharmacology, inspiring future generations of scientists to adopt similar methods (Barber, 2018; Bédécarrats et al., 2020).

In addition to his work on curare, Bernard also made significant contributions to the understanding of the role of the liver in drug metabolism, the regulation of body temperature, and the effects of anaesthetics on the nervous system. His use of animals in these experiments was critical to his discoveries, as it allowed him to explore the complex interactions between drugs and physiological processes in a way that was not possible with human subjects. Bernard's research also underscored the ethical considerations of using animals in research, as he advocated for humane treatment and minimizing suffering in experimental subjects, laying an early foundation for later ethical frameworks like the 3 R's principle (Mancini and Nannoni, 2022; Beauchamp and DeGrazia, 2019; Petkov et al., 2022).

3.3. Early Animal Models and Their Contributions to Drug Discovery

The use of animal models in pharmacological research became increasingly sophisticated during the 19th century, as researchers developed more precise methods for studying the effects of drugs on living organisms. Early animal models were chosen based on their physiological similarities to humans, and they became essential tools for understanding drug action, toxicity, and therapeutic potential. By the mid-19th century, experimental pharmacologists were using a range of animals, including rodents, dogs, frogs, and rabbits, to conduct systematic studies on the effects of various substances (Dawson et al., 2018; Ingber, 2022).

One of the most significant early contributions of animal models to drug discovery was the development of anesthetics. In the early 1800s, scientists used animals to test substances such as ether and chloroform, which eventually led to their widespread use in human surgeries. Animal studies provided crucial data on the dosage and safety of these anaesthetics, as well as their effects on the nervous system, respiratory system, and cardiovascular system. The success of anesthetic drugs demonstrated the value of animal models in translating research findings into clinical applications (Van Norman, 2019; Müller et al., 2022).

Another key area where animal models proved invaluable was in the study of infectious diseases and the development of vaccines. Louis Pasteur, another giant in the history of medicine, used animal experiments to develop vaccines for rabies and anthrax. His experiments on rabbits and sheep were crucial in demonstrating the efficacy of these vaccines, which laid the groundwork for modern immunology and vaccine development.

In addition to anesthetics and vaccines, early animal models also contributed to the discovery of many other therapeutic drugs, including antibiotics, insulin, and chemotherapy agents. These breakthroughs were made possible through careful experimentation on animals, which allowed researchers to understand the pharmacokinetics (absorption, distribution, metabolism, and excretion) and pharmacodynamics (drug action and effect) of these compounds before moving to human trials (Dugger et al., 2018; Hedaya, 2023).

The refinement of animal models in the late 19th and early 20th centuries led to more accurate predictions of how drugs would behave in humans. As a result, animal testing became a standard component of drug development, regulated by emerging ethical guidelines and scientific protocols. This period set the stage for the modern drug discovery process, where animal models continue to play a crucial role in ensuring the safety and efficacy of new therapeutic compounds, even as alternatives to animal testing are being developed (Sinha and Vohora, 2018; Patil et al., 2019).

4. Animal Models in the 19th and Early 20th Centuries

The 19th and early 20th centuries saw significant advancements in the use of animal models in scientific research, particularly in pharmacology. During this period, animal experimentation became more standardized and systematic, paving the way for key discoveries in drug development, toxicology, and safety testing. The use of animals in research played a crucial role in shaping modern pharmacology by providing insights into drug mechanisms, efficacy, and safety, and by enabling the discovery of life-saving drugs like anesthesia and antibiotics. As the field advanced, ethical considerations began to emerge, but the focus remained on refining experimental models to produce reliable, reproducible, and translatable results. **Table 2** shows how animal models were central to major scientific breakthroughs in medicine during the 19th and early 20th centuries, particularly in physiology, immunology, neurobiology, and disease research (Mukherjee et al., 2022; Bresalier et al., 2021; Lewis, 2019; DeMello, 2021).

Table 2. The Role of Animal Models in Advancing Medical Research: Key Developments in the 19th and Early 20th Centuries

Period/ Date	Scientist/ Researcher	Animal Model	Purpose/ Area of Study	Key Contributions/ Notes
Early 1800s	François Magendie	Dogs, rabbits	Physiology, nervous system research	Pioneered experiments on the nervous system; studied reflexes and nerve functions in animals
1830s-1850s	Claude Bernard	Dogs, frogs, rabbits	Experimental medicine, physiology	Developed the concept of homeostasis; studied pancreas and liver function using animal models
1860s	Louis Pasteur	Sheep, chickens, rabbits	Microbiology, germ theory, vaccination	Developed vaccines for anthrax and rabies; used sheep and rabbits for vaccine trials
1870s	Ivan Pavlov	Dogs	Classical conditioning, digestive physiology	Pavlov's experiments on conditioned reflexes in dogs became foundational in psychology and physiology
1880s	Robert Koch	Mice, guinea pigs	Infectious disease research, tuberculosis	Demonstrated the link between microbes and diseases using animal models, leading to Koch's postulates

Late 1800s	Emil von Behring	Horses	Immunology, diphtheria antitoxin development	Used horses to develop diphtheria antitoxin, revolutionizing treatment of bacterial infections
1890s	Camillo Golgi & Santiago Ramón y Cajal	Birds, rabbits	Neuroanatomy, structure of the nervous system	Used animal models to map the structure of neurons, pioneering the neuron doctrine
Early 1900s	Alexis Carrel	Cats, dogs	Organ transplantation, vascular suturing techniques	Developed surgical techniques for organ transplantation, used animal models to test grafting
1910s-1920s	Frederick Banting & Charles Best	Dogs	Diabetes, discovery of insulin	Conducted experiments on pancreatic removal in dogs, leading to the discovery of insulin
1920s	Otto Loewi	Frogs	Neurotransmission research	Demonstrated chemical transmission of nerve impulses using frog hearts
1930s	Hans Spemann	Amphibians (newts, frogs)	Embryology, developmental biology	Pioneered research on embryonic development using amphibians, winning the Nobel Prize in 1935

4.1. The Role of Animals in the Discovery of Major Drugs (e.g., Anesthesia, Antibiotics)

Animal models were instrumental in the discovery and development of some of the most important drugs in medical history, particularly anaesthesia and antibiotics. The discovery of anaesthesia revolutionized surgery and medical procedures in the 19th century. Scientists like Crawford Long, William Morton, and James Simpson experimented with ether and chloroform on animals to determine their effects on the nervous system, enabling the safe and effective use of these substances in human surgeries. These early animal experiments revealed critical data on dosage, safety, and mechanisms of action, which laid the groundwork for anesthesiology as a medical discipline (Calixto, 2019, Süntar, 2020).

Similarly, the discovery of antibiotics in the early 20th century relied heavily on animal experimentation. Alexander Fleming's discovery of penicillin in 1928 was followed by extensive testing on mice, which demonstrated the drug's efficacy in treating bacterial infections. This breakthrough led to the widespread use of penicillin, saving millions of lives during World War II and beyond. Animal models played a key role in validating the safety and therapeutic potential of antibiotics, ensuring that they could be used safely in human populations. These experiments showcased the essential role of animal models in bridging the gap between discovery and clinical application (Anand et al., 2019) (Zhang and Tang, 2018).

In both anaesthesia and antibiotics, animal models helped researchers understand the pharmacodynamics and pharmacokinetics of these drugs, contributing to their success in clinical settings. The lessons learned from these early experiments continue to influence drug discovery and development today.

4.2. Increasing Standardization of Animal Experiments

As the use of animal models in research became more prevalent in the 19th and early 20th centuries, scientists recognized the need for standardization to ensure consistency and reliability in experimental results. This period saw the introduction of standardized protocols, such as controlling variables like species, strain, age, and health status of the animals used in experiments. These efforts aimed to reduce variability in experimental outcomes, enabling more accurate comparisons between studies and more reliable predictions of how drugs would behave in human systems.

The development of laboratory animal breeding programs also contributed to standardization by producing animals with known genetic backgrounds and health profiles. For example, the Wistar rat, developed in the early 20th century, became one of the first standardized laboratory animal models and was widely used in pharmacological research. Standardized animal models allowed scientists to systematically study the effects of drugs and other substances, minimizing confounding factors and increasing the reproducibility of results across different research laboratories (Voelkl et al., 2018; Smith et al., 2018).

In parallel, researchers began developing more sophisticated methodologies for dosing, measuring drug effects, and analyzing data. The establishment of standardized units of measurement, such as the use of the LD50 (lethal dose for 50% of the population) to assess drug toxicity, further enhanced the precision of animal experimentation. These efforts contributed to the maturation of pharmacology as a science, where animal models became essential tools for understanding the complex interactions between drugs and living organisms (Landi et al., 2021).

4.3. Emergence of Toxicology and Safety Testing

The 19th and early 20th centuries also marked the emergence of toxicology as a distinct scientific discipline, closely linked with the development of pharmacology. Toxicology focuses on understanding the harmful effects of chemicals and drugs on living organisms, and animal models played a pivotal role in this field's early development. The increasing use of chemicals in industrial, agricultural, and pharmaceutical settings raised concerns about their potential risks to human health, necessitating systematic safety testing (Krewski et al., 2020).

Animals, particularly rodents and rabbits, became the standard models for toxicological studies, allowing researchers to assess the safety of new compounds before they were introduced to human populations. Early toxicological experiments involved testing for acute, chronic, and reproductive toxicity, as well as determining safe dosage ranges. The development of the LD_{50} test, which quantified the dose of a substance that would cause death in 50% of a test population, was one of the earliest methods for assessing the toxicity of chemicals and drugs. Though later criticized for ethical reasons, this method was widely used throughout the 20th century and became a benchmark for toxicology research (Pognan et al., 2023; Avila et al., 2020).

The emergence of regulatory bodies, such as the U.S. Food and Drug Administration (FDA) in the early 20th century, formalized the requirement for toxicological testing as part of the drug approval process. Animal models became the cornerstone of this safety testing, ensuring that drugs were thoroughly evaluated for potential adverse effects before reaching the market. The use of animals in toxicology provided crucial data on the safety margins of drugs, their potential side effects, and long-term risks, thus safeguarding public health while advancing scientific knowledge.

In summary, the 19th and early 20th centuries saw animal models evolve into essential tools for pharmacological research, toxicology, and drug safety testing. These advancements laid the foundation for modern pharmacology and toxicology practices, establishing the principles and standards that continue to govern the use of animals in scientific research today (Beilmann et al., 2019; Pridgeon et al., 2018).

5. Evolution of Animal Welfare Movements

The evolution of animal welfare movements is a significant part of the history of biomedical research and pharmacology. Over time, growing awareness and concern for the ethical treatment of animals used in scientific research have led to the development of movements advocating for their rights and welfare. These movements, driven by both philosophical and scientific perspectives, have shaped the current frameworks for the ethical use of animals in research. The mid-20th century saw the emergence of the 3 R's principles; Replacement, Reduction, and Refinement; which provided a clear, actionable path for balancing scientific progress with the humane treatment of animals. The adoption of this principle, along with other legislative and ethical guidelines, has influenced not only how animals are used in research but also how we understand our moral responsibilities toward them (Freire and Nicol, 2019; Buller et al., 2020; Sekar and Shiller, 2020).

5.1. Early Advocacy for Animal Rights

The roots of the animal welfare movement can be traced back to the 18th and 19th centuries, when philosophers, writers, and activists began questioning the moral and ethical implications of animal suffering. Early thinkers such as Jeremy Bentham, a prominent utilitarian philosopher, argued that animals' capacity for suffering should grant them moral consideration. His famous quote, "*The question is not, Can they reason?*

nor, Can they talk? but, Can they suffer?" became a foundational argument for the emerging animal rights movement(Stucki, 2020; Vea, 2020; Menely, 2019).

Throughout the 19th century, animal rights advocates began organizing campaigns against practices such as vivisection (the dissection of live animals), which was a common method of experimentation. The rise of this movement was not solely based on moral grounds but was also influenced by public outrage over the perceived cruelty of scientific experiments on animals. One of the first organized efforts to advocate for animal welfare was the founding of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) in 1824 in the UK, followed by similar organizations worldwide. These groups pressured governments to introduce laws that would protect animals from unnecessary suffering, laying the groundwork for future legislation (Hopwood et al., 2020).

Public awareness of animal welfare issues grew with the publication of books and essays highlighting the plight of laboratory animals, including Anna Sewell's Black Beauty (1877), which, though focused on horses, contributed to the broader discourse on animal welfare. By the early 20th century, the animal welfare movement had gained significant traction, influencing both public opinion and scientific practices.

5.2. The Impact of the 1959 3 R's Principle (Replacement, Reduction, Refinement)

A major turning point in the ethical treatment of animals in research came with the publication of The Principles of Humane Experimental Technique in 1959 by British scientists William Russell and Rex Burch. In this work, they introduced the concept of the 3 R's: Replacement, Reduction, and Refinement. This principle became a cornerstone for ethical guidelines in animal research, emphasizing the importance of minimizing the use of animals while maximizing the scientific value of experiments.

Replacement refers to methods that replace the use of animals in research with alternative techniques, such as *in vitro* (cell culture) models, computer simulations, or the use of lower organisms like bacteria and fungi that do not experience suffering in the same way as higher animals (Strech and Dirnagl, 2019).

Reduction focuses on minimizing the number of animals used in experiments without compromising the quality of research results. This can be achieved

through better experimental design, statistical methods, and improved data collection, ensuring that fewer animals are used but that the research remains robust and meaningful (Jean-Quartier et al., 2018).

Refinement involves improving experimental techniques and animal care protocols to minimize pain, distress, and suffering. This includes using anesthesia, analgesics, and humane endpoints, as well as improving the living conditions and overall welfare of the animals involved in research (Fleischer et al., 2022).

The 3 R's principle revolutionized the ethical landscape of animal research by offering a pragmatic and scientifically grounded approach to balancing animal welfare with the need for experimentation. It has since become widely adopted in research guidelines, regulatory frameworks, and institutional practices globally, and is considered a moral imperative in modern preclinical research (Eggel and Würbel, 2021).

5.3. Key Legislation and Ethical Guidelines in the 20th Century

The 20th century saw a marked shift in how animal welfare was legislated and regulated, particularly in the context of scientific research. In response to increasing public concern over the ethical treatment of animals, several key pieces of legislation and ethical guidelines were introduced, setting standards for animal welfare and use in research (Lundmark et al., 2014).

The Cruelty to Animals Act of 1876 (UK) was one of the first laws that directly regulated animal experimentation. It required researchers to obtain licenses and ensured that experiments were conducted in a manner that minimized unnecessary suffering. Although it was limited in scope, it laid the foundation for future legal frameworks (Mellor et al., 2009).

The Animal Welfare Act (AWA) of 1966 (USA) became a landmark piece of legislation that set minimum standards for the care and use of animals in research, exhibition, transport, and by dealers. The AWA was expanded several times to include more species and to strengthen regulations on how animals were housed, treated, and used in experiments. It remains the primary law governing animal welfare in research settings in the United States (Haynes, 2008).

The 1986 Animals (Scientific Procedures) Act (ASPA) (UK) was another significant legislative milestone. ASPA introduced strict guidelines for the care and use of animals in scientific procedures and mandated ethical review

processes for all experiments. Researchers were required to demonstrate that their studies adhered to the principles of the 3 R's and that any potential harm to animals was justified by the scientific benefits (Kean, 1998).

The Declaration of Helsinki (1964) and Good Laboratory Practice (GLP) Guidelines also had an indirect impact on animal research by enforcing ethical standards and rigor in clinical and preclinical studies. These guidelines emphasized the need for ethical oversight and the responsible use of animals in experiments designed to inform human health and safety (Mench, 2008).

Furthermore, the formation of ethical committees such as the Institutional Animal Care and Use Committees (IACUCs) in the U.S. and Animal Ethics Committees (AECs) in other countries formalized the ethical review of animal research. These committees are tasked with reviewing proposed experiments, ensuring compliance with laws and regulations, and advocating for the humane treatment of animals in research environments.

In summary, the evolution of animal welfare movements throughout the 20th century has had a profound impact on the scientific use of animals, particularly in the field of pharmacology. The introduction of ethical principles like the 3 R's and the enactment of key legislation have significantly improved the standards for animal welfare, ensuring that scientific progress is achieved without unnecessary animal suffering. Today, animal research is conducted within a framework of ethical oversight and responsibility, reflecting society's growing concern for the welfare of all living beings.

6. The 3 R's Principle in Modern Preclinical Pharmacology

The 3 R's principle—Replacement, Reduction, and Refinement—has become a guiding framework for ethical animal use in modern preclinical pharmacology. This principle helps balance scientific progress with the moral responsibility of minimizing animal suffering. Each of the 3 R's targets a specific aspect of research design: replacing animal models with alternative methods where possible, reducing the number of animals required for meaningful results, and refining experimental techniques to minimize pain and distress. In today's research environment, the 3 R's are not only ethical imperatives but also contribute to the improvement of scientific outcomes, ensuring that animal models are used as responsibly and effectively as possible. **Figure 2.** Illustrates the modern relations

between human observation and manipulation of animals for acquisition of new knowledge supporting new applications for benefit of clinical and veterinary medicine and species survival (Huang et al., 2012).

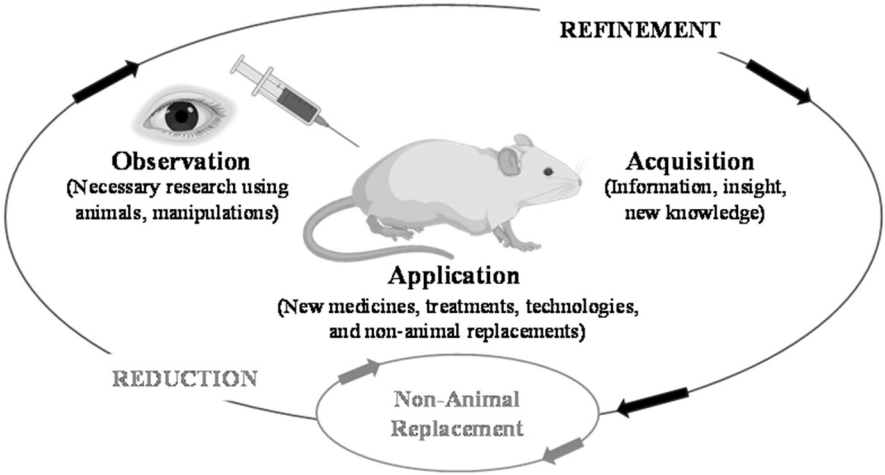

Figure 2. Depicts the modern relations between human observation and manipulation of animals for acquisition of new knowledge supporting new applications for benefit of clinical and veterinary medicine and species survival.

6.1. Replacement Strategies: Alternatives to Animal Use

The first of the 3 R's, Replacement, focuses on the development and application of methods that allow researchers to avoid the use of animals altogether. This can be achieved by substituting animals with alternative technologies such as in vitro (test tube) systems, computer simulations, or advanced cell culture techniques. For example, organoids miniaturized and simplified versions of organs grown in the lab can replicate key physiological responses, reducing the need for animal models in certain drug discovery processes. Similarly, microfluidic systems that mimic human tissues and organs, often referred to as "organ-on-a-chip" technology, offer promising avenues for studying disease progression and drug interactions without animal involvement (Hendriksen, 2009).

Additionally, computational models and *in silico* approaches, which use computer simulations to predict how a drug will behave in the body, have advanced significantly. These technologies allow researchers to simulate the interactions between molecules, cells, and tissues in a virtual

environment, reducing the reliance on live animal experiments. Although not every research question can be addressed with non-animal methods, advances in biotechnology, computational biology, and molecular modeling have provided substantial progress in this area (Knudsen and Ritskes-Hoitinga, 2021).

6.2. Reduction Techniques: Minimizing Animal Numbers in Research

The second aspect of the 3 R's principle, Reduction, focuses on minimizing the number of animals used in experiments without compromising the integrity or validity of the research. This is achieved through a variety of strategies, including better experimental design, statistical techniques, and collaboration between research teams to share data and resources (Festing, 2011).

One of the key methods to reduce animal numbers is through the use of power analysis, a statistical tool that helps researchers determine the minimum number of animals needed to achieve reliable results. By ensuring that experiments are neither over- nor under-powered, researchers can avoid using excessive numbers of animals while still obtaining statistically significant results. Additionally, advanced imaging technologies and biomarkers allow for longitudinal studies where fewer animals are needed because they can be studied multiple times over the course of an experiment, reducing the overall number required (Stallard and Whitehead, 1995).

Collaboration between different research groups and data sharing can also play a crucial role in reduction. By encouraging open access to existing data and results, researchers can avoid unnecessary repetition of experiments, thus further reducing the number of animals used across the scientific community.

6.3. Refinement Methods: Enhancing Animal Welfare

Refinement, the third component of the 3 R's, seeks to improve experimental procedures and living conditions to minimize pain, suffering, and distress in animals. Refinement techniques focus on improving both the humane treatment of animals and the quality of the scientific data obtained from experiments, recognizing that stress and suffering can negatively impact research outcomes (Buchanan-Smith et al., 2005).

One key area of refinement is the use of anesthesia and analgesia in experiments that involve potentially painful procedures. By ensuring that animals experience minimal pain, researchers can enhance animal welfare and also obtain more reliable data, as stress-related variables are minimized. Similarly, the use of humane end points the predetermined point at which an experiment is stopped to prevent unnecessary suffering ensures that animals are not subjected to prolonged distress (Lloyd et al., 2008).

Refinement also includes improvements in the housing and care of laboratory animals. Enhancing the animals' environment, such as providing enrichment (toys, nesting materials, or social housing for social species), can significantly improve their well-being. These measures help to reduce stress, which in turn can lead to more reliable experimental results. Refinement techniques are continually evolving, as advances in veterinary science, behavioral research, and animal husbandry contribute to better ways of caring for animals in research settings (Olsson et al., 2008).

In summary, the implementation of the 3 R's principle—Replacement, Reduction, and Refinement—has become essential in modern preclinical pharmacology. These strategies not only promote ethical responsibility toward animal subjects but also enhance the quality and relevance of scientific research. With continued advances in alternative methods and a growing commitment to animal welfare, the future of pharmacological research will likely see an increased reliance on innovative non-animal models, improved experimental design, and higher standards for animal care and treatment. **Table 3** outlines how the 3 R's—Replacement, Reduction, and Refinement are applied to modern preclinical pharmacology to make research more ethical and scientifically efficient.

Table 3. The 3 R's Principle in Modern Preclinical Pharmacology: Replacement, Reduction, and Refinement

Principle	Definition	Application in Preclinical Pharmacology	Examples
Replacement	Substituting animal models with non-animal alternatives	Use of *in vitro* methods, computer simulations, and cell cultures instead of live animals	Human cell cultures for drug testing; organ-on-a-chip technology; *in silico* models for drug screening