

THE RATIONALE FOR THE USE OF ANIMAL MODELS IN BIOMEDICAL RESEARCH

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The use of animal models in science, and in particular, biomedical research, is accepted by the majority of lay people and scientists alike as being necessary to the advancement of useful knowledge that brings about relief from suffering. Few outside of the biomedical scientific community, however, have a clear understanding of why these animal models are important. This is unfortunate. Animals and man are symbiotic in many real ways and not just on an ideological level. Arguments regarding whether biomedical science can advance without the use of animals are frequently mooted and makes as much sense as questioning if clinical trials are necessary before new medical therapies are allowed to be widely used in the general population.

Addressing these questions has, however, become increasingly urgent with the spectre of both bio-terrorism and the increasing use of therapies derived from biological systems. While the use of animals has apparently declined in the last two decades, advances in genetic research and the demands of research to counter bio-terrorism are expected to reverse this trend and lead to an increase in animal use. At the heart of it all is the health and safety of human populations.

The rationale for using animal models in biomedical research is scientific and animal models are likely to remain necessary until science develops alternative models and systems that are equally sound and robust. This chapter discusses the nature of experimental research, the role of models in science and the relevance of these in biomedical research.

The Place of Experimental Studies in Biomedical Research

Scientific research implies the systematic and empirical investigation of hypotheses. In the biomedical sciences such systemic investigations may be classified as observational or experimental.

Observational studies are frequently (and most usefully) carried out when the variables influencing the outcomes of the phenomena under study either cannot be controlled directly or cannot be easily manipulated. These variables are, thus, carefully observed (occasionally over long periods of time) and an attempt is made to explain or determine the correlations between them. Examples include observing animals in their natural habitat and understanding how recent ecological changes impact on their survival.

Such observational studies also abound in clinical medicine and include *descriptive case series*, *retrospective (case control) studies*, *prospective (cohort) studies* and *cross-sectional studies* or surveys. These studies are particularly important where the conditions are rare and where it is important to understand the natural history of a particular condition (including the outcomes of currently accepted therapy). Investigations of these nature are also common in the basic biomedical sciences e.g. in molecular epidemiology and in comparative anatomy.

Experimental studies require intervention and attempts are made to directly control selected variables and to measure the effects of these variables on outcome. Such studies are necessary to establish cause and effect relationships in an unequivocal and rigorous manner. The results of experimental studies tend to be more robust compared to observational studies (although not necessarily more important) and many breakthroughs in the biomedical sciences are made possible only through experimental studies. Data arising from interventional studies also lend themselves easily to statistical analysis.

The definitive experimental study in clinical medicine is the *randomized controlled trial* some of which can run into large numbers of patients. Clinical trials could of course also be carried out in non-randomized manners such as in *sequential self controlled* or *cross over trial* protocols. There are legislative requirements for most if not all new therapies (such as pharmaceutical and device related) to have undergone rigorous clinical trials before being accepted in mainstream medical practice and be considered standards of care.

Experimental studies are similarly important in pre-clinical biomedical research. These studies may be carried out on ***in vitro biological systems*** such as isolated cells, cell culture systems, tissue slice preparations or isolated perfused organs. Experiments using *in vitro* systems are particularly useful in the early phases of studies where the screening of large number of potential therapeutic candidates may be necessary.

In vitro systems are, however, by definition, nonphysiological and have important limitations. Living creatures are biologically complex and this especially true in higher order animals including man. While data from experiments carried out in *in vitro* systems can establish mechanisms and define toxicities, ***in vivo biological systems*** using live animals (whole organisms) are necessary to study how such mechanisms behave under clinical or pathophysiological conditions.

Intact (whole) animal systems are, thus, extremely important for “proof of principle” research. It is frequently possible to have a clearer understanding of the efficacy, pathophysiological interactions and potential toxicities of novel therapies only with whole *in vivo* biological systems. Many *in vivo* interactions are complex and cannot be predicted from *in vitro* data. Such information is especially important when assessing the safety and efficacy

of biologics. Biologics are therapeutics (drugs, vaccines, antibodies etc.) synthesized from living organisms. Biologics have made great advances in the last decade through advances in genetics and molecular biology especially recombinant DNA technology. Such therapies are increasingly developed and have contributed significantly to better outcomes in diseases e.g. in cancer therapy.

Models in Biomedical Research

A model is “a representation of a real or actual object” (Oxford English Dictionary). Models are, thus, meant to mimic and it is not expected that a model be necessarily identical to the subject under study. Models are widely used in all branches of physical, biological and social sciences. In biomedical research, models allow the investigator to understand and investigate pathophysiological processes and the impact of intervention. As described above, these models can be *in vitro* or *in vivo*.

Biomedical research models can also be either analogues or homologues. **Analogous models** relate one structure or process to another and are not unique to biomedical research. Such models are also common in physics, engineering and mathematics. A scaled-down model of an aeroplane is not an aeroplane but allows appreciation of how the various parts of the structure relate to one another and how improvements may be usefully made. Similarly, large animal models like the pig allow the development of new minimally invasive surgical techniques and instruments.

Homologous models reflect counterpart genetic sequences and are only used in biomedical research. Many animal models are both analogues and true homologues.

The ideal model for a human is another human, which is why randomized controlled clinical trials will always be important in the evaluation of new therapies. Famous historical examples using a human subject as a model will of course include Edward Jenner’s classical “proof-of-principle” experiment of the efficacy of inoculation against smallpox using a hapless farm boy as a subject, presumably without informed consent (and without the approval of an Institutional Review Board!).

Research using human subjects is only justified and should only be allowed if there is sufficient understanding of the underlying mechanisms of action and of the bio-safety parameters involved in the research. Robust preclinical data of this nature are most accurately derived from the use of animal models and must pass the scrutiny of institutional review boards and health authorities. This is especially important with “first-in-man” studies of novel therapies. The use of higher order animal models with close genetic homology to man, such as nonhuman primates, is particularly important in studies involving therapeutics derived from biological systems i.e. biologics.

Animal Models in Biomedical Research

In biomedical research, an animal model is defined as “a living organism with an inherited, naturally acquired or induced pathological process that in one way or another closely resembles the same phenomenon in man” (Wessler 1976). The ultimate goal of experimental research using animal models is to solve problems in clinical practice and to

develop new methods and approaches to the cure and alleviation of disease and disability (Isselhard, Kushe 1986).

Both invertebrate and vertebrate animals are used as models in biomedical research. ***Invertebrate models*** are very useful in the fields of neurobiology, genetics and development and notable examples of invertebrates use for such purposes include the *C. elegans* and *Drosophila*.

Vertebrate models are responsible for many advances in biology and medicine and are extremely important in translational research. This includes the use of both small animal models (e.g. mice, rats, rabbits) and large animal models (e.g. dogs, pigs, monkeys).

Broad areas of how vertebrate animal model are used in biomedical research include:

1. *Pharmaceutical research including the development of biologics*
2. *Toxicology testing*
3. *Development and testing of new medical devices*
4. *Surgical research*
 - a. the development of new surgical techniques e.g. techniques of gastrectomy, open heart surgery, coronary artery surgery, microsurgery, endoscopy and the use of arterial ligation in treating aneurysms (by the pioneer surgical scientist John Hunter).
 - b. the development of new therapies e.g. organ and tissue transplantations, cardio-pulmonary resuscitation.
5. *Pathophysiological research*

Animal models were crucial to the understanding of basic and important pathophysiology processes such as shock and the body's response to trauma, regeneration and malignancy. In particular the development of the concept of the "milieur interieur" in physiology (by the pioneer physiologist Claude Bernard) and the concept renal dialysis all depended on the use of animal models.

The above is not exhaustive. The vast majority of animals used in biomedical research are in the fields of pharmaceutical research and toxicology testing.

When animal models are used for therapeutic testing, an established principle is to use the minimum number of animals necessary to arrive at scientifically robust data and to ensure the humane and proper care of animals so that the scientific data is reliable. Generally, two or more species (one rodent, one non-rodent) are tested because a drug may affect one species differently from another. Besides treatment efficacy, animal models are also used to determine how much of a drug is absorbed into the blood, how it is broken down chemically in the body, the toxicity of the drug and its breakdown products (metabolites), and how quickly the drug and its metabolites are excreted from the body.

What Makes a Good Animal Model?

Not all animal species are useful for the purposes of biomedical research and the limitations of the models selected as well as the methodology involved must always be kept in mind. Biomedical research is a very vast field and there are both general and specific uses for animal models. In the early years of biomedical research, animal models were mainly used for general research purposes i.e. to uncover broad pathophysiological phenomena and principles. The recent development and widespread use of transgenic animal models in biomedical research have made many animal models very specific to the nature of individual research projects.

While there are always exceptions, a good and useful animal model suitable for *general use* in a research facility should have the following characteristics (adapted from Isselhard, Kushe 1986):

1. The animal model should closely reproduce the disease or condition under study.
2. The animal model should be easily available to many researchers, that is, not a rare or exclusive animal. This allows validation and stimulates further investigations.
3. The animal model, in the case of a vertebrate model should be large enough for multiple biological sampling (tissue, blood etc).
4. The animal model should fit into available animal facilities of the average institution.
5. The animal model should be easily handled by most investigators.
6. The animal model should be available in multiple sub-species.
7. The animal model should survive long enough for results to be meaningful.
8. The animal model should be sufficiently robust for the purpose of the study.

Transgenic animal models, spontaneous animal models (see below) and highly specialized animal models such as non-human primates do not fit these traditional guidelines. Such *special animal models* are, however, increasingly used in biomedical research

Consideration in the Selection of an Appropriate Animal Model

The researcher should consider using established models where possible or available (Table 1.1.1). The model must, however, be relevant to the aims of the study. The following serves as examples:

1. *Relevance of species*

For example, animals are suitable for studies on muscle contraction but data obtained from the whole body has little relevance to humans. In gastrointestinal tract and liver

studies, herbivores have highly specialized gastrointestinal parts (e.g. for cellulose digestion) and associated metabolism, which has no counterparts in humans. Omnivores are, thus, most suitable e.g. pigs.

2. Numbers required

In studies where the outcomes between the control and study groups differ only in degree, large numbers of animals are required to achieve statistical significance. Mice and other small mammals are ideal.

3. Transplant and other immunological studies

Inbred or naturally immunosuppressed species may be required.

The animal model that is required to address the specific research question may, however, not have been previously developed or validated in some instances. The research effort must then begin by developing and validating a suitable model rather than using an established one. The development of a suitable model in this case becomes critical because it is essential that the model be reliable, reproducible and valid. The model must also be a reasonable representation of the actual situation and the limitations of the model must be identified. The validity of the results in experimental research depends on the qualities of the experimental model.

Table 1.1.1: Examples of established general animal models

Models	Species
Haemorrhagic Shock	Rat, rabbit and pig
Stress Ulcers	Rat restraint model
Hypercholesterolaemia	Minipig
Sepsis Model	Rat, dog and pig
Primary Liver Cancer	Rat
Liver Regeneration	Rat and pig
Acute Pancreatitis	Dog and rat
Inflammatory Bowel Disease	Rabbit
Myocardial Infarction	Baboons
Vascular Grafts	Dog, pig and sheep
Bone Fracture	Rabbit

Specific Animal Models

Occasionally, researchers may seek to use animal models that specifically mimic conditions of interest as opposed to using or developing general models. Such animal models may either spontaneously mimic these conditions or be induced to simulate those conditions.

Spontaneous animal models are those models that have arisen through spontaneous mutations to mimic specific conditions. Notable examples of these are the Gunn rat (for hereditary hyperbilirubinemia) and the BB Wistar rats (for type I diabetes).

Induced animal models can be created through surgical manipulations, chemical manipulation and genetic manipulations (including negative models).

The surgically induced model is in many ways the classical biomedical research model and was used to understand brain plasticity (nonhuman primates), develop organ transplantation (dogs and pigs), discover the role of insulin in diabetes (dogs) and to develop card-pulmonary resuscitation (dogs).

Examples of chemically induced models include the chemical ablation of beta cells to create diabetes (rats, rabbits, pigs, monkeys) and the use of carbon tetrachloride to create cirrhosis (rats).

Transgenic animal models are important induced animal models. A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. An example of a transgenic animal model is mice with type I diabetes ($Cd38^{tm1Lnd}$). Homozygous mutant mice show impairment in glucose-induced increases in ADP-ribosylcyclase/cyclic ADP-ribose (cADPR), intracellular calcium concentrations and insulin secretion.

Some Special Roles of Animal Models

In the development of drugs against bio-terror agents, controlled studies of clinical effectiveness in humans are unethical. Since generally few people would have been previously exposed to these agents/diseases and have been treated, observational studies may not provide sufficient data.

Under these circumstances the role of animal models becomes especially important. Instead of depending on human studies, the FDA allows approval of drugs shown to be effective in two animal models, without clinical trials for effectiveness. Examples of such circumstances are treatment against anthrax, botulism, plague, smallpox, tularaemia and viral haemorrhagic fevers.

A Short History of the Use of Animal Models in Biomedical Science

In the western scientific tradition, the initial use of animal models was in experimental surgery, which pre-dated all other scientific uses by more than a millennium. In antiquity, the earliest records of physiology research were carried out by Erasistratus of Alexandria (302 – 258 BC) on the functions of the heart and respiratory systems in pigs. The first textbooks on anatomy by Galen (129 – 200) were based on dissections not on human cadavers (which was forbidden by religious and legal authorities) but on pigs and apes. Although these observations and their interpretations were frequently erroneous, they established the discipline of comparative anatomy. While animal models remain central to the development of new surgical techniques and the invention of novel medical devices, the number of animals used in experimental surgery today is only a small fraction of the total number of animals used in biomedical research.

In 1628, William Harvey published his great work on circulation based on studies in animals. The “father” of modern physiology, Claude Bernard (1813 – 1895) established the basis of the discipline based on animal experimentation and Louis Pasteur (1822 – 1895) used animals in the validation of the experimental method in microbiology. In the 20th century, cardio-pulmonary resuscitation, the discipline of immunology and translational research on organ transplantation were all primarily developed through the use of animal

models. Koch's postulate for the carcinogenesis of the *Helicobacter* bacteria was fulfilled in gerbils in the 1990s.

The explosion in molecular biology in the second half of the 20th century increased the importance of *in vivo* models. In the 1980s, the pathology of Hepatitis C was established through infecting chimpanzees with the virus. Examples of other diseases where the use of animal models were crucial to the recent elucidation of pathogenesis include cystic fibrosis, rheumatoid arthritis and spongiform encephalopathies. The use of naturally immunosuppressed animals such as SCID and nude mice to harbour cancer cells were similarly crucial to the development of experimental oncology and new therapies in cancer. Increasingly, animal models are now being produced to exhibit specific symptoms and pathology of diseases through selective breeding and genetic modification.

The development of *in vivo molecular imaging* modalities such as the micro-PET and MRI and their application to animal models in the 21st century has brought about a degree of accuracy and sophistication on biomedical research not previously possible. Such *in vivo* imaging and documentation of cellular processes in animal models confers increased scientific vigour to experimental design and leads to fewer animals being required in each experimental protocol. The robustness of such data increasingly contributes to the ease of translation of biomedical breakthroughs from preclinical studies to clinical applications.

The Limitations of Animal Models

All models have their limitations concerning transferability and predictability and this is true in every branch of science. The extent of the validity of extrapolating data derived from specific experiments using animal models to the general human clinical conditions depends on the degree to which the animal model is an appropriate reflection of the condition under investigation, the design of the experiment and the technical experience of the researchers.

These limitations are, however, an intrinsic part of all modelling approaches that use surrogates and do not render the scientific method invalid. They are also similarly found in clinical and *in vitro* studies. This explains why unexpected adverse reactions can sometimes still occur when medicines are brought into the market even after extensive clinical trials.

The question of the scientific validity of data derived from animal models is often confused with questions pertaining to complex ethical issues. The separation of science and ethics is important in such discussion. Each scientific study has to be judged on its own merits after careful evaluation of the methodological and statistical rigor. Scientific experience and balance are important attributes in such judgement.

A recent review by the Nuffield Council on Bioethics concluded that "animal research has been, and can potentially be, scientifically valid, in that it is possible to extrapolate from animal models to humans (or other animals)...." (Nuffield 2005). The Council further cautioned that data on the validity of animal experiments have been interpreted and used in different ways by both opponents and proponents of the scientific validity of using animal models.

The public health perspective on the use of animal models in scientific research is, however, unequivocal. It is unlikely that any health authority will allow novel therapies in medicine be approved for use in the general population without scientifically rigorous supporting animal and clinical data. Likewise, responsible Institutional Review Boards are

unlikely to allow clinical trials on novel therapies to be carried out within that institution without supporting animal data.

Improvement in the technology used in animal research (such as *in vivo* molecular imaging) continually refines the interpretation of data derived from animal models today. Together with improvement in methodology (e.g. the use of orthotopic models and tumour explants in experimental oncology), there is an expectation that the extrapolation of data derived from animal models to the human condition will be even more valid in time to come.