

# CHAPTER 1

# SCIENTIFIC CONSIDERATIONS AND CHOICE OF SPECIES

# CHAPTER 1.1

## 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, cardiopulmonary 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 20<sup>th</sup> 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 20<sup>th</sup> 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 21<sup>st</sup> 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.

# EXPERIMENTAL ANIMAL MODELS IN BIOMEDICAL RESEARCH

Robert Ng

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The use of animals in *in vivo* experiments is an essential feature of any Experimental Surgery Laboratory. In the choice of species, the most important considerations are the goals of the study, the constraints imposed by the animal study design, the familiarity of the laboratory with a particular species, cost and relevance of the obtained data for clinical use.

## Mice and Rats

Small animals such as rats and mice are preferred for many experimental studies as they have short life cycles, are inexpensive to purchase and easy to maintain in limited space. They are extensively used in veterinary and biomedical research for the discovery and manufacture of vaccines, antibodies and hormones and in the testing of drug potency and toxicology. In pharmaceutical industries, rodents are widely used to study the metabolism of drugs and food additives, since extrapolation of their results to humans is a necessary prerequisite to clinical trials. In addition, when a large number of graded doses of an investigational therapeutic agent are being examined and when larger number of animals is needed for studies with survival as an endpoint, economic feasibility takes priority as a consideration. The following information is mostly specific to studies conducted at the Department of Experimental Surgery, Singapore General Hospital.

### A. Rats

Rats have been used in experimental neurosurgery and in cardiac transplantation and research on abdominal heart allografting is a well established model in the rat. Also,

the rat body size is suitable for microCT imaging. Some of the rat models used include:

- **Outbred Wistar rat**

This outbred rat has become a standard feature in most experimental laboratories today and has its origin in the Wistar Institute in Philadelphia, and was named after Professor Casper Wistar (1761–1818). Good breeders with long lifespan (30 months) and are excellent parents. However, they have the tendency to become very fat with age.

- **Outbred Sprague-Dawley rat**

This strain was developed in 1925 by Robert W. Dawley, who named the strain after himself and his first wife, whose maiden name was Sprague. A commercial firm, Sprague-Dawley Inc., was subsequently set up in Wisconsin and was dedicated to the establishment and sale of this rat strain. Descendants of the Sprague-Dawley strain today are random bred and extremely popular in experimental laboratories worldwide. These rats are highly intelligent and are thus popular models for psychology tests.

- **Inbred Buffalo and Fisher 344 rats**

At the Columbia University in 1921, five inbred lines were initiated using rats from four local vendors namely August, Fisher, Marshall and Zimmerman and one vendor from Copenhagen. From this first litter of pedigree, rats after the 344<sup>th</sup> mating was subsequently derived as the well-known Fisher 344 strain. The Buffalo and Fisher 344 were two of the seven inbred strains that were established in 1953 at the National Institute of Health and these inbred were used as models for oncogenesis. Cell line Morris 7777 is specific for the development of hepatocarcinoma in Buffalo rats (Fig 1.2.1).



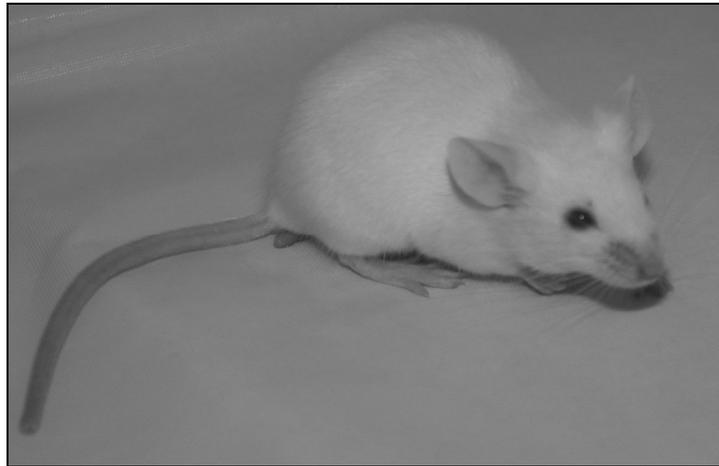
**Fig 1.2.1: Inbred Buffalo rat.**

- **Inbred Brown Norway rat**  
This rat has moderate incidence of urinary bladder and ureteral carcinoma (35%) in old males. There is also moderate incidence (14% male, 26% female) of pituitary adenomas in older animals. This rat is a good immunologic model as the strain is unique in its expression of high IgE-responder phenotype. They are resistant to experimental allergic encephalomyelitis and to induction of autologous immune complex glomerulonephritis. Poor to moderate breeders with moderate lifespan of approximately 25 months for male and 28 months for female.
- **Inbred Dark Agouti rat**  
Its uses include studies on transplantable salivary gland adenocarcinomas, transplantation immunology and autoimmune disease, for example thyroiditis.
- **Inbred Lewis rat**  
The rat is susceptible to induction of experimental allergic encephalomyelitis, adjuvant induced arthritis, induced autoimmune myocarditis and autologous immune complex glomerulonephritis. It is also a host for a number of induced neoplasms (lymphoma 8, renal sarcoma and fibrosarcoma).
- **Mutant Athymic Nude rat**  
These are homozygotes having little or no hair and lack of thymus gland (referred to as athymic). They have similar dysgenesis to the nude mutation in mice. Cell mediated immunity is greatly reduced or absent with marked reduction of T-lymphocyte function. They are thus suitable for tumour xenograft studies. They are extremely susceptible to infection with *Clostridium piliformi*.

#### B. Mice

Mice are mainly used for experimental oncology research and their small sizes are most suitable for bioimaging procedures like microCT and microPET scanning as these tests involve use of expensive drugs and chemicals. The most commonly used mice include the following:

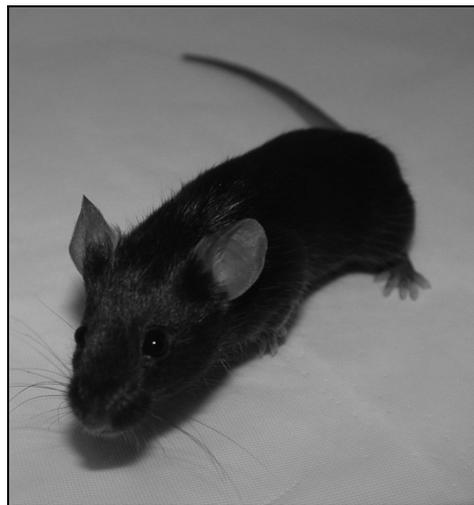
- **Outbred Swiss mice**  
This is a general-purpose mouse recommended for dissection and any work not requiring the special qualities of inbred strains.
- **Inbred BALB/c mice**  
This mouse is susceptible to chronic pneumonia and extremely sensitive to radiation. BALB/C (Fig 1.2.2) has a high level of alpha-fetoprotein. Commonly used for ascitis fluid production. Injection of mineral oil intraperitoneally induces a high incidence of transplantable plasmacytomas. They have low mortality after neonatal thymectomy.



**Fig 1.2.2: Balb/c mouse.**

- **Inbred C57BL mice**

This is an intense black mouse with very low incidence of mammary tumours. Used most commonly as the background for various mutant genes (for example, nude, beige, knockouts etc). These mice (Fig 1.2.3) are used in alcohol tolerance studies and are susceptible to the development of atheromatous lesions after 20 weeks on high fat diet. When fed high fat, high simple carbohydrate diet, they develop noninsulin dependent diabetes mellitus and hypertension. They are notorious for developing a progressive, ulcerative dermatitis, which may be responsive to an altered fatty acid diet.



**Fig 1.2.3: C57BL mouse.**

- **Inbred CBA/CaH mice**

This mouse line is known to be missing the lower third molar in about 18% of the offspring. They do not develop antinuclear antibodies or LE cells with ageing. They are resistance to *S. typhimurine* and to *Leishmania* infection, but are highly susceptible to the Edmonton strains of measles virus.

- **Mutant BALB/c Athymic nu/nu mice**

Nude is an autosomal recessive mutation located on Chromosome 11. The two major defects are failure of hair growth and dysgenesis of thymic epithelium. Nude mice (Fig 1.2.4) respond poorly to thymus-dependent antigens because of a defect in helper T-cell activity. They have often been used for tumour xenotransplantation studies.



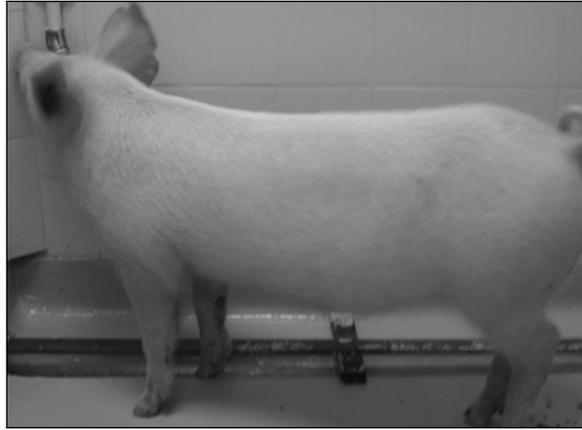
**Fig 1.2.4: Mutant Balb/c Athymic nu/nu mouse.**

- **Mutant SCID mice**

The severe combined immunodeficient (SCID) mouse arose as spontaneous autosomal mutation in C.B-IgH-I (CB17) congenic strain. Homozygotes have little or no immunoglobulin in serum. Lymphoid organs consist of vascular connective tissue and macrophages and are devoid of lymphocytes. Although B and T-cells are absent early B and T-cells are present. They are a useful model for studying the relationship between immunity and disease, studies on engraftment of xenogenic cells and tissues and studying human severe combined immunodeficiency.

## Pig

Apart from primates, the pig (Fig 1.2.5) is the laboratory animal species nearest to humans in terms of anatomy and physiology. Being relatively inexpensive to obtain and maintain, the pig is quite popular for physiological and pharmacological studies. However, the rapid growth rate of the domestic pig makes it unsuitable for chronic experiments. For experiments where there is need for post operative maintenance of six months to 2 years, the mini- or micro-pig (Gottingen, Yucatan, PWG or Bama) should be used, Micro-pig's weight at two years is less than 50 to 60 kg as opposed to the normal domestic pigs like Yorkshire or Landrace where weight can go beyond 200 kg for the same period. In spite of this limitation, the pig is still the animal of choice for colorectal, angioplasty and transplantation surgeries or other acute procedures as its size and anatomy mimic the human structure. It is also suitable for experiments on trauma management as in dermal burns, resuscitative shock or traumatic brain injury.



**Fig 1.2.5: Pig.**

## Sheep

Domestic sheep are placid animals of manageable size and are choice animals in several areas of biomedical research. They tolerate implanted electrodes and indwelling catheters in blood vessels and lymphatics better than most other species and make little effort to remove them. Hence, they are often used for chronic experimental preparation for studies of endocrine function and for immunological or isotope research involving chronic collection of lymph draining from different anatomical areas. They also recover well from foetal instrumentation surgeries. Sheep are an appropriate animal for cardi thoracic surgeries such as heart valve implantation where they have shown high resilience against vascular insult during such surgeries, a lower tendency toward thrombosis formation than many species.

## Rabbit

New Zealand White is a popular non-inbred strain for various research projects especially for polyclonal antibody production. It is a commonly used animal model for research studies involving orthopaedic surgery and ophthalmology. Rabbit (Fig 1.2.6) is also widely used for paediatric intensive care courses as it offers good simulation of human infant patient for chest tube placement.



**Fig 1.2.6: Rabbit.**

## Golden or Syrian Hamster

This animal is agouti or golden yellow in colour with many coat mutations. An excess vitamin in the diet results in fatty liver and teratogenesis. It is used for a variety of experimental purposes including immunology, parasitology, reproductive physiology and cancer research.

## Guinea Pig

The white strain, Hartley is the most commonly used outbred stock for routine and experimental procedures such as skin patch test for efficacy test on antiseptics and antibiotics efficacy test. This animal has a tendency to be more prone to deafness than non-white stock.

## Nonhuman Primates

*Macaca fascicularis* (Fig 1.2.7) is the common nonhuman primate used for experimental research in Southeast Asia. Although costly to work with, they remain invaluable for selected studies by virtue of their similarity to humans. However, nonhuman primates are generally reserved for preclinical experiments when only small sample size is needed.



**Fig 1.2.7: *Macaca fascicularis* (longtail or cynomolgus macaque).**

# NONHUMAN PRIMATES AS MODELS IN BIOMEDICAL RESEARCH

Jason Villano and Bryan Ogden

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Nonhuman primates (NHP) belong to the order Primates, which contains two suborders:

1. **Strepsorrhini**, the ‘wet-nosed primates’, which include the lemur and the loris; and
2. **Harplorrhini**, the ‘dry-nosed primates’ which are the true primates divided into infraorders, Tarsiiformes (tarsiers) and Simiiformes. Simiiformes is further divided into parvorders:
  - a. Platyrrhine or New World monkeys (NWM) – found in Central and South America. Examples include marmoset, tamarins, squirrel monkeys and capuchins.
  - b. Catarrhine or Old World monkeys (OWM) and hominids, including man, chimpanzees, and gibbons belong.

The Old World monkeys will be the focus of this discussion.

## Old World Monkeys (OWM)

The Old World monkeys (OWM) of Africa and Asia belong to the family Cercopithecidae. Their common characteristics include having a specialized digestive mechanism for processing a folivorous diet, narrow noses with comma-shaped nostrils separated by a narrow nasal septum, ischial callosities, opposable thumbs, marked sexual dimorphism, and cheek pouches.

To this group belong the most commonly used NHPs in biomedical research: the *Macaca fascicularis*, also known as cynomolgus or crab-eating or longtail macaque and the *Macaca mulatta*, the rhesus macaque.

- *Macaca fascicularis* (Fig 1.3.1) — found mainly throughout Southeast Asia, including Singapore. Its tail is longer than its head and body, which is brown with grey or black tones with crown hair, directed either backward and outward or in a crest. Adult females generally weigh from 2 to 6 kg and adult males from 4 to 8 kg. But, there are ten sub-species of *M. fascicularis* each with distinctive fur, morphometric and genetic differences.
- *Macaca mulatta* — found in the northern half of the Indian continent, Northern Burma and Indochina, and much of China. It has a medium length tail and is brown with a reddish tone on its hind parts, including hindlegs. Adult females weigh 4 to 9 kg and adult males weigh 6 to 11 kg, though weights exceeding this range are not uncommon. As with *M. fascicularis*, there are regional differences within the species, most notably immunologic differences between Indian- and Chinese-rhesus as noted by AIDS researchers.

In Singapore's booming biomedical research industry, the cynomolgus macaque is the most widely used NHP since it can be found locally and on some nearby islands like Bintan, Indonesia.

## Anatomy and Physiology

The phylogenetic proximity between NHPs and humans is reflected by many similarities between them. From an anatomical perspective, NHPs are the closest model to humans among many laboratory animals. Similarities include, but are not limited to, skeletal structure, growth and development, organ structure and dentition. Physiological similarities include immune function, neuroendocrine function and processes such as aging of the brain and other body systems. These and other similarities make them a preferred model for biomedical research in such areas as pharmacology (especially with biologics), immunology, pathobiology, neurobiology, behavioural neuroscience and aging processes.

## Current Uses in Biomedical Research

The use of nonhuman primates in research has led to significant discoveries during the past 100 years. Studies on relapsing fever, typhoid and yellow fever benefited from NHP use. The discovery of Rh factor depended on work in rhesus macaques. The role of NHPs has expanded to cover a multitude of research areas, including pharmacology, aging, metabolic disorders, gene therapy (Fig 1.3.2), virology and many others. A summary of a few of these areas follows:

### 1. *Pharmacological research*

Animals have been essential in pharmacokinetics and toxicological studies. Besides establishing the efficacy of a certain compound to produce a desired effect and the specifics of its ADME (absorption, distribution, metabolism, and excretion), the safety of a compound must be established in a rodent and a non-rodent species (usually dogs, but increasingly NHPs). While research involving nonhuman primates provides a meaningful translation towards understanding human disease and the development of treatments, their similarities to humans also becomes the drawback to their use as significant bioethical issues must be addressed. Many of these issues are discussed elsewhere in this text. Justifications for using NHPs for pharmacological and toxicological research often depend on the characteristic of the test compounds. For example, large molecules or biologics need to be tested on species with the most immunologic similarity to humans. Also, the receptors targeted by some molecules are unique to human and nonhuman primates.

### 2. *Human aging and metabolic disorders research*

There has been substantial interest regarding primate aging. Study areas involving NHPs included the neurobiology of aging as well as reproductive senescence. Metabolic disorders such as obesity and diabetes, spontaneously-occurring and experimentally-induced, have been frequently studied in NHPs. Macaques will consume carefully formulated high fat, high cholesterol diets. This and their similarity to humans make them susceptible to many of the other chronic diseases, such as atherosclerosis, that affects human populations.

Streptozotocin is a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. While it is used as medicine for treating certain cancers of the islets of Langerhans, it is also used in biomedical research to produce Type II diabetes in NHPs and other laboratory animals.

### 3. *Neurological research*

Nonhuman primates offer a valuable choice of animal model in studies of neurological disease and cognition. With their cerebral organization approximating that of humans, they can be used to study cognition, functional connectivity, and neurotransmitter pathways. It is important to note that certain disorders in humans occur spontaneously as well in NHPs. For instance, epilepsy, cerebral amyloidosis and cognitive changes due to aging can be found in these animals. Meanwhile, experimental inductions of Parkinson's disease, focal and generalized epilepsy, stroke and multiple sclerosis have also been performed.

### 4. *Gene therapy research*

Gene therapy involves the delivery of specific genes to a patient as a treatment for disease. A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vectors used are viruses that have been genetically altered to carry normal human DNA. Research involving gene therapy on nonhuman primates often focuses on diseases caused by

single-gene defects. These include cystic fibrosis, haemophilia, muscular dystrophy and sickle cell anaemia.

#### 5. *Virology research*

Nonhuman primates are often the only species that can be used to study certain human viruses due to the relative homology and conservation of critical molecules used by viruses in their life cycle. Also, some of the natural viruses in these animals have their human counterpart. An example is the human immunodeficiency virus (HIV) and the simian immunodeficiency virus (SIV). The immune system of NHPs also closely resembles that of humans, in respect to development, genetics, function and anatomy. Recombinant HIV/SIV viruses (SHIVs) and hepatitis (B and C) have been widely studied in NHPs.

#### 6. *Others*

There are numerous other research uses for nonhuman primates.

In 1996, a tuberculosis model using Philippine cynomolgus macaque was reported. Animals inoculated intratracheally with various doses of *Mycobacterium tuberculosis* developed either a rapidly progressive, fatal lobar pneumonia or a chronic, progressive localized form of pulmonary tuberculosis, similar to the disease in humans.

Cynomolgus macaques have also been used extensively to study cocaine and alcohol abuse, including social factors associated with addiction. One study demonstrated that subordinate animals found cocaine to be more enforcing than did their dominant counterparts.

Osteoporosis poses a major health problem for women, especially those at the post-menopausal stage. In macaques, the peak bone mass is at about 9 years of age. Procedures for measuring bone mass and density in women can be similarly applied to animals. Also, oestrogen deficiency (e.g., through surgical menopause) in cynos causes rapid bone loss that progresses for at least 18 months. This can be completely prevented with oestrogen treatment.

The menstrual cycle and reproductive hormone profile of macaques are similar to women. Hence, they offer a good model for reproductive biology studies. Rhesus macaques, are seasonal breeders, but cynos are not allowing for reproductive function to be studied year-round. Macaques have also been used to investigate breast and uterine cancer, particularly in relations to oestrogen exposure.

## **Future Advances in Primate Models**

The use of NHPs in biomedical research is likely to increase as emphasis is placed on developing translational models for drug development that will be more predictive of efficacy in humans. Newer imaging modalities, such as PET-CT and functional MRI will be increasingly applied.

With the SARS epidemic in the recent past and the current threat of an avian influenza (bird flu) pandemic, more attention is being paid to emerging infectious disease research. Nonhuman primates and other animal models are needed to study disease prevention, disease progression and effectiveness of possible treatment strategies.

While the research and medical community has long been interested in the possibility of transplanting organs from animals to humans (xenotransplantation), success has been limited. Many studies have used monkeys as the model recipient of pig organs in order to develop xenotransplantation strategies. With proper consideration of critical issues, such as rejection, zoonoses and ethics, the exploration of xenotransplantation will continue and NHPs will play a major role.



**Fig 1.3.1: An anesthetized cynomolgus macaque maintained on isoflurane, an inhalant anaesthesia.**



**Fig 1.3.2: Ophthalmology surgery performed on NHP.**