Animals/transgenic animals as experimental models for human:microbe interactions

Why do scientists still use animals to study diseases of people?

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Source Wikipedia: Mus musculus has been the most frequently used living mammalian model in biomedical research for the past 100 years.

Storyline

Scientists use animals to understand health and disease in both humans and animals. Researchers believe that it would be wrong to experiment with humans, meaning deliberately exposing people to health risks in order to observe the course of a disease.

Animals are also used to study the effectiveness and the safety of new treatments, because animals are biologically similar to humans. Some treatments involve processes that can only be studied in a living organism. Studies in a living animal also allow researchers to determine whether potential new treatments are effective and whether they have harmful side-effects on other parts of the body (American Physiological society, www.physiology.org).

Opponents of the use of animals in research state that it is morally wrong to use animals for human benefit/purpose. Most of those people also object to eating meat (vegetarians, vegans). Indeed, the motivations for a meat-reduced or meat-free diet are similar to ethical concerns about animal welfare. However, it should be acknowledged that Western society is moving away from anthropocentrism (a philosophy supporting that humans are superior to animals) to biocentrism (the view or belief that the rights and needs of humans are no more important than those of other living things). "Moderate biocentrism" is a moral theory which is widely acceptable these days. Moderate biocentrism ascribes a moral status to all forms of life, although the value of this status increases with the species' position on the evolutionary ladder (hierarchy of the organisms). This means, for example, that there would be more reason to protect the life of a dog than that of a hamster or a worm (Max Planck Society, https://www.mpg.de/798717/dfg_S_27-29_engl.pdf).

It is true that in the past, animal experiments have been performed that would not be accepted nowadays by medical ethics committees. Fortunately, in the last century researchers and governments have started to develop regulations to ensure/guarantee that animal rights ethics are respected. The '3R principles of animal experimentation' were formulated by Russell & Burch (1959), and advise to reduce, refine and replace. Reduction includes methods that minimise the number of animals used per experiment. Refinement refers to methods that minimise animal suffering and improve welfare. Replacement corresponds to methods that avoid or replace the use of animals. European legislation is available. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines issued in 2010 were intended to maximise the publication of information and minimise unnecessary animal studies (https://www.nc3rs.org.uk/arrive-guidelines). That year, a team of researchers evaluated the application of the ARRIVE guidelines in a medical journal and compared the compliance of reporting with ARRIVE before and after 2014. There is still room for improvement. Paying better attention to reporting will lead to improvement in reproducibility, replicability, and quality of animal research (Reynolds and Garvan, 2020)

The Microbiology and Societal Context Animal experimentation has historically played a central role in microbiology. It has been essential for studying the pathogenesis of bacteria, viruses, fungi, and parasites; developing vaccines (e.g., for tuberculosis, influenza, COVID-19), testing antimicrobial drugs and investigating resistance mechanisms; and modelling host–pathogen interactions and immune responses. However, many of these advances have relied on animal models such as mice, zebrafish, non-human primates, raising significant ethical concerns about animal welfare.

In response, microbiology is undergoing a broader shift toward more ethical and responsible practices. This includes recognizing that scientific progress must align with ethical standards, ensuring experiments are reproducible and not unnecessarily duplicated and addressing growing societal concerns about animal rights and research integrity. Within this context, the principles of the 3Rs - Reduce, Refine, Replace - are especially relevant guiding efforts to minimize animal use and improve experimental design.

In today's context, microbiology is increasingly shaped by the development and adoption of alternatives to animal models, including advanced cell cultures, organ-on-chip systems, and microbiome simulations. Additionally, improved reporting standards, such as the ARRIVE guidelines, are enhancing the reproducibility of infection models while reducing unnecessary animal use caused by poor experimental design. Global regulatory frameworks, including EU directives further support the harmonization of ethical standards across countries

This shift offers societal benefits (SDG1, SDG3). It Increases public trust in microbiological research, promotes efficient use of resources by reducing redundant experiments and supports ethical innovation, especially in emerging areas such as pandemic preparedness and antimicrobial resistance

Microbiology-relevant Issues

Nowadays, scientists use animals for microbiological studies when there is no alternative. This will be explained below. The goals of animal models for microbiology and microbial diseases are shown in Table 1.

Table 1 Goals of animal experimentation

To study the human and animal microbiome in health and disease
To understand the process (pathophysiology/pathogenesis) of infections caused by microbes
To understand why infected people are affected to different extents
To study the virulence of microbes
To weigh the impact of biofilms in colonisation and infection
To study the effect of antibiotic resistance of microbes
To discover and test new medicines and treatments
To test new diagnostic and surgical procedures for animals and humans

Thoughtful selection of animal models is essential.

Because of complex biological interactions, the choice of animal model must be a clearly defined process in order to provide relevant and translatable scientific data, and to ensure the most beneficial use of the animals. A well-designed animal model requires a thorough understanding of similarities and differences in the responses between humans and animals and incorporates that knowledge into the goals of the study. If similarities and differences between the animal model and human are not accurately defined, that can lead to false extrapolations and conclusions) (Swearengen, 2018). According to the 3Rs principle, the smallest, "lowest" animals should be used if these animals are suitable to obtain relevant data to reach the goals described in Table 1.

The mouse as animal model champion.

The house mouse, *Mus musculus*, has been the most frequently used living mammalian model in biomedical research for the past 100 years._Mice and humans share over 90 percent of the same genes._However, there are significant physiological and genetic differences. Nevertheless, mice have certain advantages that explain their use as a model. They are small, easy to handle and transport in the laboratory setting. Because of fast breeding, large numbers of animals are obtained quickly at a relatively low cost. Their life cycle is short and disease processes can be studied over the course of only ~2 years, versus decades in humans (Masopust & Sivula, 2017). For example, this allows researchers to study the effect of age on the severity of a disease, as shown in COVID-19 experiments (Sun et al., 2020) or the response to COVID-19 vaccines (see below). To solve the problem of the differences in immune system between mice and humans, so-called '*humanized mice*' have been developed, in line with the above-mentioned Refinement principle. *Humanized* mice, defined as mice engrafted with functional human cells or tissues, are suitable for the study of immune responses to various pathogenic microbes and for the development of treatments.

Transgenic mice are genetically modified animals that have had their genes changed in some way (https://www.understandinganimalresearch.org.uk/animals/areas-research/breeding-and-gm-mice/) K*nock-out* mice carry a gene that has been inactivated, which creates less expression and loss of function; *knock-in* mice are produced by inserting a transgene into an exact location where it is overexpressed. Since 2012, a powerful genome-editing tool named CRISPR-Cas, has been developed for this purpose. The CRISPR technique can change the DNA of a cell with high precision (Sun et al., 2020).

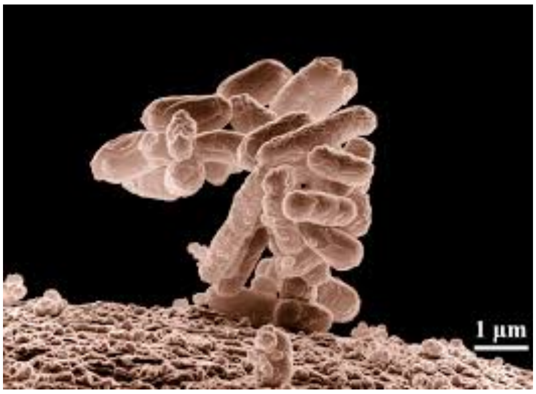
Today, genetically modified mice are considered vital for medical research. As an example, an *Acinetobacter baumannii* transgenic mouse pneumonia model has been used to identify factors involved in virulence (the side of the bacterium) and pathogenesis (the side of the host) of this deadly disease in humans (McConnell et al., 2013).

Smaller experimental organisms

Recently, much smaller Refined experimental organisms such as the worm *Caenorhabditis elegans*, *Danio rerio* (zebrafish) or *Galleria mellonella*, the larval stage of the greater wax moth, infected with human pathogenic microbes have shown their usefulness for the study of host immunity and pathogen virulence as well as for investigating the efficacy of antibacterial agents in vivo at an early stage. It takes zebrafish about 90–100 days to reach adulthood (from egg to larva and pre-reproductive juvenile). They produce up to 200 eggs per week, and have a reproductive lifespan of up to 3.5 years. Different kinds of larvae and all life stages of *C. elegans* have the important advantage that they are transparent, enabling ready visualization of fluorescently labelled microorganisms in the gut (Douglas, 2019).

Another application of these smaller animals is microbiome research. Recent research on lower vertebrate and invertebrate species shows how microbiome composition is shaped by host control (immune factors, nutrition), transmission patterns (e.g., the host's food or habitat preference) and microorganism interactions. Hydra, squid and *Apis mellifera* (honeybee) are valuable alternative models to address specific questions. Research on microbiome-dependent host traits has identified how specific metabolites and proteins released from microorganisms can influence behavioural traits, such as the transition from hive bee (which processes and distributes food) to forager bee (which collects food) (Douglas, 2019). Microbiome research has also been performed on *Danio rerio* (zebrafish) and *Drosophila melanogaster* (fruit fly).

"Simple animal models"



Source: Wikipedia

However, some questions cannot be answered by studying these small invertebrates.

Larger animals

Even now, larger animal models are sometimes used in human medicine when there is a medical urgency. An example is the West African Ebola outbreak from 2013-2016. Ebola virus of the family Filoviridae causes severe, often fatal disease with fever and bleeding. Non-human primates (NHP) such as rhesus macaques (monkeys) are favoured for Ebola virus disease research due to their similarities with humans regarding the pathogenesis, clinical presentation, laboratory findings, and causes of fatality. To comply with the regulatory requirements for the approval of countermeasures against high-consequence pathogens, the Food and Drug Administration instituted the Animal Rule, which permits efficacy studies in animal models instead of human clinical data when such studies are not feasible or ethical (St Clare et al., 2017). The ongoing

COVID-19 pandemic is another example of a medical emergency. COVID-19 is caused by the novel coronavirus SARS-CoV2.

Concerning regular medicines research, including antibiotics, the FDA has the following information on their website: https://www.fda.gov/about-fda/fda-basics/why-are-animals-used-testing-medical-products

Animals are used in the testing of new medicines (for example antibiotics) and vaccines, mainly to determine the safety of the product. For medicines, the focus of animal testing is on the nature, chemistry, and effects (pharmacology) and on its potential damage to the body (toxicology). Animal testing is used to measure

- how much of a medicine is absorbed into the blood
- how a medicine is broken down chemically in the body
- the toxicity of the product and its breakdown components (metabolites)
- how quickly the product and its metabolites are eliminated from the body

There are still many areas where animal testing is necessary and non-animal testing is not yet a scientifically valid and available option. However, the FDA has supported efforts to reduce animal testing. In addition, the FDA has research and development efforts underway to reduce the need for animal testing and to work toward the replacement of animal testing.

In vaccine development, larger animal models can optimize vaccine formulation (antigen, adjuvant), route of administration (e.g., intramuscular vs. mucosal) and dosage. The induced immune response can be assessed to find immune correlates of protection. This preclinical research maximizes the chances that a vaccine candidate will be protective and safe before it is applied to humans. It is beyond doubt that a good vaccine represents the most effective way to prevent diseases caused by pathogens.

Finally, it is important to realize that not all animal research poses a risk or inconvenience to animals. Dogs have superior olfactory systems compared to humans. They smell volatile organic compounds (VOCs), and can be trained to detect these. Sniffing by dogs has been used for many years to detect diseases such as cancer and diabetes. Bacterial diseases can also be detected by dogs as was demonstrated during a hospital outbreak of *Clostridium difficile* (Bomers et al., 2012).

Other examples of helpful sniffing dogs are their contributions to diagnosing of malaria, and recently of COVID-19.

Earlier work has shown that people infected with malaria parasites produce a body odour that is detected by mosquitoes, which results in malaria mosquitoes preferentially feeding on symptomatic, malaria-infected individuals. Dogs, with their highly advanced sense of smell, could be trained to detect people carrying malaria parasites. In the future, application of malaria-detection dogs could be used to detect individuals infected with malaria at ports of entry in countries or regions that are malaria free or approaching malaria elimination (https://www.wired.com/story/a-malaria-breathalyzer-its-closer-than-you-think).

In September 2020, four COVID-19 sniffer dogs have started working at Helsinki airport in a state-funded pilot scheme that Finnish researchers hope will provide a cheap, fast and effective alternative method of testing people for the SARS-CoV2 virus. "A dog is capable of detecting the presence of the coronavirus within 10 seconds and the entire process takes less than a minute to complete," said a researcher of the University of Helsinki, who is overseeing the trial. "If it works, it could prove a good screening method in other places such as hospitals, care homes and at sporting and cultural events."

https://www.theguardian.com/world/2020/sep/24/close-to-100-accuracy-airport-enlists-sniffer-dogs-to-test-for-covid-19

Potential Implications for Decisions

Future policies should acknowledge earlier discoveries in medicine that were made possible by conducting animal experiments. The importance for humans of animal research was highlighted on 5 October 2020 when the Nobel Prize for medicine was awarded for the discovery of the causative pathogen of non-A non-B transfusion hepatitis that could have severe consequences such as liver cancer when left untreated. Worldwide, more than 70 million people are infected with hepatitis C, mostly in Africa and Asia. One of these Nobel Prize awardees, Dr. HJ Alder, showed in 1978 that after injecting five chimpanzees with serum of patients with hepatitis the animals developed similar but mild disease. The virus was subsequently identified as hepatitis C virus by the two other Nobel Prize awardees.

Glossary

Anthropocentrism. The belief that human beings are the most important entity in the universe, often placing human needs and values above those of other organisms.

Antibiotic resistance. The ability of bacteria and other microorganisms to resist the effects of an antibiotic that once could successfully treat them.

Biofilm. A structured community of microorganisms that are attached to a surface and embedded in a self-produced matrix.
Biocentrism. An ethical perspective that extends inherent value to all living organisms, not just humans.
Diagnosis. The identification of the nature of an illness or other problem by examination of the symptoms.
Diagnostic procedure. A test used to identify a disease or condition.
Ethical. Relating to moral principles that govern a person's behavior or the conduct of an activity.
Microbiome. The collection of microorganisms (such as bacteria, fungi, and viruses) that live in a particular environment, including the human body.
Olfactory. Relating to the sense of smell.
Pathophysiology. The disordered physiological processes associated with disease or injury.
Surgical procedure. An operation performed to treat a medical condition.
Virulence. The degree of pathogenicity or ability of a microorganism to cause disease.
VOCs (Volatile Organic Compounds). Organic chemicals that have a high vapor pressure at ordinary room temperature.

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