Journal of Anatomical Sciences

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J Anat Sci 4 (1)

ANIMAL EXPERIMENTATION: CONTEMPORARY ETHICAL CONSIDERATIONS

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ABSTRACT
Throughout history, researchers have been solving medical and other challenging problems, developing new techniques and treatments, and curing diseases – largely by conducting animal experimentation. Animal testing gives researchers the opportunity to control *in vivo* the genetic and environmental factors that may influence the development of disease and establishment of its complications, and thus gain new information about its handling and treatment in humans. Most experiments are carried out on rodents, though other species with human-like biological characteristics are also used. In this review, an overview of ethical considerations in the use of animals in research, which have become highly topical and contentious, especially in recent times, are presented. The usefulness and contributions of animals in biomedical research are equally highlighted with some historical perspectives.

Keywords: Animal models, Animal testing, Animal experiment, Ethics, Endocrinology, Diabetes, Thyroid, Quinine.

INTRODUCTION

Animal testing is the use of non-human animals for the purpose of scientific experimentation. There are many different words used to describe the practice of animal testing. Some of these words are ***in vivo* testing, animal experimentation and animal research.**

Accurate global figures for animal testing are very difficult to obtain. Global estimates are extremely difficult to compile because recording criteria differ greatly from country to country, and only very few countries have even partial national figures. About 80*% of countries do not appear to publish the number of animals used in their laboratories*[1]. Estimates of worldwide annual laboratory animal use are consistently imprecise and unsubstantiated. Estimates are that between 50 and 100 million vertebrate animals are experimented on around the world every year. Many invertebrates— including flies and worms— are also used in experiments but their numbers are mostly not enumerated and unaccounted for.

The types of animals used in medical research vary depending on laboratories and locations. The vast majority of the animals used are rodents, which constitute over 80% of animals used in research. Others are fish, amphibians, reptiles and birds (including many fertilized hen's eggs) (about 10%), sheep, cows, pigs and other large mammals (about 1.5%), rabbits and ferrets (about 0.7%), dogs (about 0.2%), cats (about 0.05%), non-human primates marmosets and macaques (about 0.1%). Restrictions have been placed on the use of chimpanzees, orangutans and gorillas in some facilities.

Vivisection is historically defined as the dissection (or operation on) of living animals for experimental purposes. It is derived from two Latin words, *vivus*, meaning "alive", and *sectio*, meaning "cutting", and can therefore be defined as surgery conducted for experimental purposes on living organisms, typically animals having a central nervous system, with a view of studying the living internal structure[2,3]. The term is now often used by organizations opposed to animal experimentation.

An animal model is a living, non-human animal used during the research and investigation of human diseases, conditions and processes (physiological and anatomical) for the purpose of achieving a better understanding of such diseases, conditions and processes. Animal model can also be referred to as a living organism with an inherited, naturally acquired, or induced pathological process that in one or more respects closely resembles the same phenomenon occurring in human[3]. A model must provide a new insight, have relevance to a particular problem and respond predictably[4]. An animal model for biomedical research is a living organism, a non-human animal, organ, tissue or cell in which normative biology or behaviour can be studied, or in which a spontaneous or

induced pathological process can be studied, and in which the phenomenon in at least one respect resembles the same phenomenon in humans or other species of animals[5-7]. According to the National Research Council (U.S.) Committee on Animal Models for Research on Aging (1981)[6], animal models used in biomedical research can be classified into five groups: (a) spontaneous or natural models in which diseases or conditions occur spontaneously in animals as they do in humans, (b) experimentally modified models, whereby diseases or conditions are induced chemically or surgically (c) genetically engineered models in which diseases or conditions are induced by genetic manipulation; (d) negative models, including animals resistant to a particular condition or disease and (e) orphan models, including animal models with disease unknown to human counterparts.

Animal models can be further be distinguished by the phylogenetic distance of the model species from humans. Animal models can also be classified based on fidelity— how well the model resembles humans— as well as based on validity— how well what you think you are measuring corresponds to what you really are measuring. In addition, animal models can be considered based on reliability— the precision and accuracy of the measurement[8].

HISTORICAL PERSPECTIVES

Vivisection

The use of animals in scientific research has a surprisingly long history, dating back to the 5th century B.C. The scientific dissection and vivisection of animals may have begun with the work of Alcmaeon (ca. 500 B.C.) of Crotona in Italy and Empedocles (490 - 430 B.C.) in Sicily (largest island in the Mediterranean Sea in Southern Italy). Alcmaeon published a treatise titled “On Nature”. In preparation for this book, he dissected many animals and described his findings in detail. Alcmaeon experimented with live animals, severing the optic nerve to study vision in them[9].

Aristotle (384 - 322 B.C.), a Greek philosopher and polymath, a student of Plato and teacher of Alexander the Great, is one of the first known to have performed experiments on living animals.

One of the most well known and controversial, early vivisectionists was Galen of Pergamon (129 - ca. 200 A.D.). He used animals to investigate anatomical questions in humans. Galen, physician to Roman Emperor Marcus Aurelius dissected goats, monkeys, pigs (and other animals), a practice which later earned him the title “the father of vivisection.” He is noted for his pioneering use of vivisection of animals to understand health and disease in the human body.

Early Animal Research

The first observations of significance to modern science were made in the 1600s, when William Harvey, a pupil of Hieronymus Fabricius, used animals to observe and describe the blood circulatory system. William Harvey (1 April, 1578 - 3 June, 1657), an English physician, was the first to describe completely and in detail the systemic circulation and properties of blood being pumped to the body by the heart, though earlier writers had provided precursors of the theory. Harvey's experiments involved both direct dissection and physiological experiments on animals.

In 1714, Stephen Hales, an English clergyman, opened an artery of a horse, inserted a brass tube, and measured the pressure of the blood. This was a careful, scientific experiment demonstrating that the heart exerts pressure in order to pump blood. Natural philosopher and inventor, Stephen Hales (1677 - 1761), undertook these lengthy series of experiments on animals described in “Haemastaticks” (1733) which led to the first direct measurement of blood pressure[10].

Antoine Laurent Lavoisier (26 August, 1743 - 8 May, 1794), the "father of modern chemistry, a French nobleman, prominent in the histories of chemistry and biology. Antoine Lavoisier used a calorimeter and a guinea pig to demonstrate that respiration was a type of combustion (1780).

Ivan Petrovich Pavlov (14 September, 1849 - 27 February, 1936), a famous Russian physiologist, used salivating dogs to show classical conditioning. Pavlov trained dogs to salivate at the sound of a bell, by teaching them to associate the sound with food. Pavlov's research into the physiology of digestion led him logically to create a science of conditioned reflexes. In his study of the reflex regulation of the activity of the digestive glands, Pavlov paid special attention to the phenomenon of psychic secretion, which is caused by food stimuli at a distance from the animal. By employing the method (developed by his colleague D.D. Glinskii in 1895) of establishing fistulas in the ducts of the salivary glands, Pavlov was able to carry out experiments on the nature of these glands. The Nobel Prize in Physiology or Medicine 1904 was awarded to Ivan Pavlov "in recognition of his work on the physiology of digestion, through which knowledge on vital aspects of the subject has been transformed and enlarged".

In 1922, insulin was extracted from dogs to treat diabetes mellitus, following the work of Frederick Banting, Charles Best, John Macleod and Bertram Collip. Hailed as one of the most dramatic events in the history of the treatment of disease, the discovery of insulin extended the life-span of patients with diabetes and brought succor to many homes.

The Russians used a dog, Laika (the first living being in space), to test the effects of space on mammals in 1957 by rocketing her into orbit. At that time some scientists believed humans would be unable to survive the launch or the conditions of outer space, so engineers viewed flights by animals as a necessary precursor to human missions. Laika, a stray dog, originally named Kudryavk, underwent training with two other dogs - Albina and Mushka, and was eventually chosen as the occupant of the Soviet spacecraft Sputnik 2 that was launched into outer space on the 3rd of November, 1957. The physiological variables that were monitored and telemetered back to Earth included electrocardiogram (chest lead), blood pressure, respiration rate, and motor

activity[11]. Food was automatically delivered twice a day. The heart rate was 103 beats/min before launch and increased to 240 beats/min during the early acceleration. However, after 3 hours of weightlessness, it was back to 102 beats/min[12]. On the 14th of April, 1958, the spacecraft carrying the body of Laika fell out of orbit and burned up during re-entry into the Earth's atmosphere. Laika's death sparked debates on animal rights across the world.

Dolly, a Finn Dorset sheep (5 July, 1996 - 14 February, 2003) was cloned from an adult cell (taken from a healthy six-year-old sheep), proving the possibilities of cloning technique in 1996. Dolly was a female domestic sheep, and the first mammal to be cloned from an adult somatic cell, using the process of nuclear transfer[13,14]. She was cloned at the Roslin Institute in Midlothian, Edinburgh, Scotland, and lived there until her death when she was about 6.5 years old. Dolly was euthanized after contracting an incurable and debilitating viral disease.

The Discovery of Insulin

The history of insulin discovery is marked by a long row of conflicts and controversies. The name, insulin has itself quite a disputed story. Prior to the discovery of insulin, diabetes mellitus was a dreaded disease that most certainly led to death. Doctors and other caregivers knew that sugar worsened the condition of patients with diabetes and that an effective treatment was to put the patients on very strict diets where sugar intake was kept to a minimum. At best, this treatment could procure patients a few extra years, but it never saved them. In some cases, the harsh diets even caused patients to die of starvation.

During the 19th century, observations of patients who died of diabetes mellitus often showed that the pancreas was damaged. In 1869, a German medical student, Paul Langerhans (25 July, 1847 - 20 July, 1888), found that within the pancreatic tissue that produces digestive juices there were clusters of cells whose functions were unknown. Some of these cells were eventually shown to be the insulin-producing beta cells. Later, in honour of the person who discovered them, the cell clusters were named the islets of Langerhans.

In 1889 in Germany, physiologist Oskar Minkowski (13 January, 1858 - 18 July, 1931) and physician Joseph von Mering (28 February, 1849, in Cologne - 5 January, 1908, at Halle, Germany), showed that if the pancreas was removed from a dog, the animal got diabetes. But if the duct through which the pancreatic juices flow to the intestine was ligated, the dog developed minor digestive problems but no diabetes. Their experiments, conducted in the University of Strasbourg, showed that the pancreas must have at least two functions: to produce digestive juices and at least a regulator of blood sugar.

During the first two decades of the 20th century, several investigators prepared extracts of pancreas that were often successful in lowering blood sugar and reducing glycosuria in test animals. However, they were unable to remove impurities, and toxic reactions prevented its use in humans with diabetes[15].

In 1920, Dr. Frederick Grant Banting (14 November, 1891 - 21 February, 1941), a young Ontario orthopaedic surgeon, wanted to make a pancreatic extract, which he hoped would have anti-diabetic qualities. Banting had the idea that the pancreatic digestive juices could be harmful to the secretion of the pancreas produced by the islets of Langerhans. Banting's hypothesis was that ligation of the pancreatic ducts before extraction of the pancreas, destroys the enzyme-secreting parts, whereas the islets of Langerhans, which were believed to produce an internal secretion regulating sugar metabolism, remained intact. Banting's conception was not completely new or altogether correct, because the digestive enzymes of the pancreas must be activated in the intestine before they can exercise their destructive action.

Early in 1921, Banting took his idea to **John James Rickard Macleod** (6 September, 1876 - 16 March, 1935), a Scottish biochemist and physiologist, professor of physiology and head of Department of Physiology at the University of Toronto and a leading authority on carbohydrate metabolism. Banting managed to convince him that his idea was worth trying. Macleod gave Banting a laboratory with a minimum of equipment and ten dogs. Banting also got an assistant, an American-Canadian medical student and scientist, named Charles **Herbert** Best (27 February, 1899 - 31 March, 1978). The experiment started on 17th of May, 1921. After several weeks of ligation of pancreatic ducts, the pancreas, unable to secrete fluid into the duodenum, would gradually atrophy and would be removed and processed to extract the internal secretion. The extract would then be administered to other dogs made diabetic by removal of the pancreas[15].

Banting and Best managed to test this extract on dogs that had diabetes. They managed to keep a dog, which had pancreatectomy, alive throughout the whole summer by administering it the extract. The extract regulated the dog's blood sugar levels. Banting and Best showed their result to Macleod, who was impressed, but he wanted more tests to prove that their pancreatic extract really worked, and so requested for a re-run of the whole trial. After doing so he decided to get his whole research team to work on the production and purification of the extract.

For the increased testing, Banting and Best realized that they required a larger supply of organs than their dogs could provide. They had to start using pancreases from cattle, and this new source enabled them to produce enough extract to keep several diabetic dogs alive.

In late 1921, a third person, James Bertram Collip (20 November, 1892 - 19 June, 1965), a biochemist, joined the team, which now consisted of Banting, Best, Collip and MecLeod. Collip was given the task of purifying the extract so that it would be good enough for testing on humans. The team managed to produce enough extract, in a form, that was pure enough to be tested on patients. The team was eager to start testing on humans. There is an account that speculated that Banting and Best, began

by injecting themselves with the extract before the proper clinical trial. On 11th of January, 1922, the extract was tested on Leonard Thompson, a 14-year-old patient with diabetes in Toronto General Hospital[17]. At first Thompson suffered a severe allergic reaction and further injections were cancelled. The scientists worked hard on improving the extract and then a second dose of injections were administered on Thompson. Following the positive and remarkable response of Thompson, the extract was tried on other patients. Success with purification was largely the work of J.B. Collip. Yield and standardization were improved by cooperation with Eli Lilly and Company.

In 1923 Banting and Macleod were awarded the Nobel Prize in Physiology or Medicine. As of September 2011, Banting, who received the Nobel Prize at age 32, remains the youngest Nobel laureate in the area of Physiology/Medicine. In 2004, Frederick Banting was voted 4th place on The Greatest Canadian. When the Nobel Prize was awarded to Banting and Macleod for the discovery of insulin, it aggravated the contentious relationship that had developed between them during the course of the investigation. Banting was outraged that Macleod and not Best had been selected, and he briefly threatened to refuse the award. He immediately announced that he was giving one-half of his shares of the prize money to Best and publicly acknowledged Best's contribution to the discovery of insulin. Macleod followed suit and gave one-half of his money award to Collip[16].

It is very pertinent to briefly trace the origin of the word "insulin" at this stage. In 1905, the English physician and physiologist, Ernest Henry Starling (17 April, 1866 - 2 May, 1927) coined the term "hormone" (Greek: *hormaein*, to set in motion) to designate the chemical messengers of the body's endocrine glands. The duo of Starling and the English physiologist, Sir William Maddock Bayliss (2 May, 1860 - 27 August, 1924) discovered secretin (the first hormone to be discovered) in 1902. Three years later the two brothers-in-law introduced the hormone concept with recognition of chemical regulation[18] (Henriksen and de Muckadell, 2000). Sir Edward Albert Sharpey-Schafer (2 June, 1850, Hornsey, Middlesex - 29 March, 1935, North Berwick, East Lothian, Scotland), an English physiologist, regarded as a founder of endocrinology, coined the term "endocrine" for the secretions of the ductless glands[20]. In 1894, Sharpey-Schafer suggested that on morphologic grounds the islet tissue might be responsible for the internal secretion by which the pancreas produced its effect on the blood sugar concentration. In 1913, in lectures at Stanford University, he suggested the name "insuline" for the still hypothetical substance in the islets[19]. He later acknowledged that he was unaware that the term had been introduced by the Belgian physician, Jean de Meyer (1878 - 1934) in 1909. "Insulin" (Latin: *insula*, island) was independently adopted by the Toronto workers in 1922.

According to Macleod, the word insulin was his invention, which he made public on 3rd of May, 1922, during the Washington meeting of The Association of American Physicians. He then explained that the word came from the Latin, *insula*, meaning island in English. From that moment, insulin was the official name for the pancreatic hormone[17].

The controversies on the discovery of insulin involve yet another researcher, Nicolae Constantin Paulescu (30 October, 1869 - 17 July, 1931), a Romanian physiologist and professor of Medicine. Since 1916, Paulescu had developed a pancreatic extract used successfully in normalizing blood glucose levels in diabetic dogs. Unfortunately, with the outbreak of World War I, Paulescu was forced to abandon his research until 1921, when he wrote an article on the role of the pancreas in food assimilation, published in August 1921, in "Archives Internationales de Physiologie." Paulescu's discovery (method of manufacturing insulin under the name "pancreine") was patented on 10th of April, 1922 by the Ministry of Industry and Commerce of Romania. He had called the pancreatic extract pancreatine ("pancreine"). The "pancreine" was a crude extract of bovine pancreas in salted water, after which some impurities were removed with hydrochloric acid and sodium hydroxide.

The Nobel Committee received furious letters of protest. Nicolas Paulescu in Bucharest was outraged. He claimed his work was stolen by the Toronto team, and he demanded justice from the Nobel Committee. While Paulescu had patented his technique in Romania, no clinical use resulted from his work. The work published by Banting, Best, Collip and McLeod represented the injection of purified insulin extract into diabetic individual ameliorating symptoms of the disease. Not surprisingly, Banting and Macleod received the 1923 Nobel Prize in Physiology or Medicine for the discovery of insulin. While it seems fair to say that Paulescu deserved to share in the prize, so did Collip and Best, who were left out. International recognition for Paulescu's contribution to the discovery of insulin came only years later.

THE IMPORTANCE AND BENEFITS OF ANIMALS IN BIOMEDICAL RESEARCH

Scientists have developed many valuable non-animal models (*i.e.*, cell culture, computer) that are useful for medical research, but these models cannot exactly imitate the complicated processes that occur in a living system.

Animals are similar to humans and can be good models when humans cannot be used. Organs and body systems of animals are similar to humans. Animal life is based on a similar genetic, biochemical and physiological principles as human life.

Animals have short life span that allows them to be studied throughout their entire life. Several generations of animals can thus be studied by a single research team. The environment of laboratory animals and other factors are easily controlled to keep experimental variables to a minimum, and when needed, the environment and these other indices can easily be manipulated for the purpose of study. For example, rats

are amenable to study the interactions of genetics and environment that may be critical for disease expression in humans.

In addition, genetic linkage studies in humans are always hampered by the need to collect a large number of families, reliance on whatever pattern of mating that has occurred within the population, genetic heterogeneity and concerns regarding paternity. Animal studies circumvent all these problems as it is possible to study large numbers of offspring from whichever mating researcher chooses to set up. The issue of doubtful paternity does not arise when animals are well bred.

Moreover, animals are susceptible to quite a number of diseases that affect humans, and so serve as models to study these diseases. Some of the many health problems affecting both human and animals include: allergies, arthritis, birth defects (including spina bifida), cancer, tuberculosis, asthma, epilepsy, heart diseases, kidney disease, lyme disease, peptic ulcers, cataracts (and other visual impairments), measles, influenza, hypertension, glaucoma, diabetes mellitus, bronchitis, leukaemia, deafness, bleeding disorders such as haemophilia, rabies, brucellosis, trichinosis, giardiasis, emphysema, hyperthyroidism, hypothyroidism, atherosclerosis, dermatophytosis and toxoplasmosis,

Animal models enable researchers to explore potential therapies in ways which would be impossible in humans. Studying disease mechanisms in animal models leads directly to the development of new technologies and medicines, including vaccines that benefit both humans and animals. Advances in genetic technology have allowed the development of transgenic animals, which have new genes inserted into their DNA, allowing them to develop human diseases which do not naturally affect them. In particular this has allowed rats and mice to model many human diseases which were previously difficult to study, and which do not spontaneously occur in animals. Animal models help researchers to understand the features observed following damage to body structure or disruption of body physiology, and thus help in the development of new or better therapies.

Once researchers learn more about a particular disease, animals are used to develop and test these potential therapies as part of the applied research process. Models are an essential part of applying biological research to real medical problems, allowing new targets for disease intervention to be identified. Data from animal studies are essential before new therapeutic techniques and surgical procedures can be tested on human patients.

New medicines require testing because researchers must measure both the beneficial and the harmful effects of a compound on a whole organism. A medicine is initially tested *in vitro* using tissues and isolated organs, but legally and ethically it must also be tested in a suitable animal model before clinical trials in humans can take place. For example, apart from the use of animals in studying the pathogenesis of diabetes and its complications, all new treatments for diabetes, including islet cell transplantation and preventative strategies, are initially investigated in animals[20].

In order to circumvent the ethical and logistical constraints inherent in studying type 1 diabetes in outbred populations of humans exposed at random to chemical and microbiological agents, investigators continue to rely on animal models that can be readily tested, biopsied, and autopsied. It is possible to breed these models to study and manipulate inheritance and to test for their response to environmental agents[21].

The safety and effectiveness of newly developed diagnostic tools such as scanners, and implants such as heart pacemakers or artificial hips, are tested on animals before using them on humans. Many surgical techniques and life saving procedures, such as open heart surgery and heart transplants, rely on methods and equipment that were developed using animals.

Animal tests provide data on efficacy and safety. They not only identify potential safety concerns, but also give guidance in the determination of the doses which are given to volunteers and patients during the first human trials. Testing on animals also serves to protect consumers, workers and the environment from the harmful effects of chemicals. All chemicals for commercial or personal use must be tested so that their effect on the people and animals exposed to them is well understood. The chemicals that are used day-to-day can accumulate in the water, ground or air, and their potential impact on the environment must be adequately assessed.

WHEN TO USE ANIMALS IN RESEARCH

Animals are used in scientific research to further science in many arenas. They are used most often in disease treatment, prevention, treatment of injuries, basic medical testing and medical diagnosis. Animal testing should, however, be undertaken only: following adequate literature searches and comparison of data to previous research; after computer model and simulations have been considered and undertaken in some cases; after cell and/or tissue culture alternative have been explored; following the approval of protocols by an Institutional Animal Care and Use Committee (IACUC); after extensive training and education on the handling, care, and use of animals; and before human clinical testing.

RESEARCH CONDITIONS FOR ANIMALS

Human Responsibility

The use of animals in research is a privilege granted only to those investigators and programs that commit to meeting the highest ethical and regulatory standards. Ethical treatment of animals implies that animals used in experiments should be treated well. The use of these animals must be monitored by Institutional Animal Care and Use Committees (IACUCs). Ethical treatment of animals includes: providing a comfortable living environment, minimizing discomfort from testing and humanely euthanizing animals.

Housing
Animals are housed in cages designed to provide a comfortable environment that contributes to their well being. Approximately 4 cm of clean wood shavings are used on the floor of the cages. Soiled bedding is removed as often as necessary to keep the animals clean and dry. Environment must minimize variables that can modify an animal's response during experimentation. Environmental factors such as temperature/humidity ranges, room air exchange rates, lighting, noise levels, and even odours are considered in housing the various species. Noise from human activity in the area should be kept to a minimum in order to minimize stress and discomfort to the animals. Radios and other sound producing equipment should not be used in the animal area unless part of an approved research protocol. Periodic dusting with boric acid compound is used to control cockroaches. The dust is applied to the entrances to the food storages area, research rooms, animal rooms and the cage washing area.

Behavioural Management
Young rats should be housed in small social groups 2-4 unless the research protocol requires isolation. Adults are maintained in same sex pairs unless breeding or research protocols dictate otherwise. If adults are not compatible, then isolation may be necessary. Some small items (such as tubes and blocks) may be added to cages for activity based enrichment.

Death of an Animal in the Colony
In the event of a death of an animal in the colony, all pertinent information should be recorded, including information from cage tag and any recent use of the animals. The carcass should be placed in a plastic bag for necropsy. If the latter cannot be undertaken within two hours, the bagged carcass should be placed in a dedicated refrigerator for later examination. After necropsy, carcasses should be stored in the freezer compartment until removed for disposal.

ANIMAL ANAESTHESIA AND SURGICAL PROCEDURE
Euthanasia
Vertebrate animals are sentient. They have the ability to feel, perceive, or be conscious, or to have subjective experiences. The minimization of distress, pain and suffering is a moral imperative. Animals are normally euthanized at the end of a study for the purpose of sample collection or post-mortem examination. Animals may be euthanized because they are experiencing pain or distress. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in humans may cause pain or distress in other sentient animals. Justification must include documentation of alternatives considered. A description of the methods and sources used to determine that alternatives to the use of physical sacrifice alone were not acceptable or available must be clearly stated and substantiated. Some of the agents that can be used include pentobarbital, halothane and CO_2. Death must be verified after euthanasia and prior to disposal. Unintended recovery must be obviated by the use of appropriate CO_2 concentrations and exposure times or by other means. Researchers may only use euthanasia methods that are approved in their IACUC Animal Care and Use Protocol.
Volatile agents used to euthanize animals should not be stored or used in animal rooms because of improper ventilation, toxicity to laboratory animals, and possible effects on experimental results.
Chloroform is not acceptable for either anaesthesia or euthanasia as it is very toxic to many species of mice. Additionally, this compound has been shown to be carcinogenic[21]. Ether is irritating, flammable and explosive, and should not be used in animal rooms. In addition, animals euthanized with ether must be left in a fume hood for several hours so that the carcasses are not explosive when disposed[23,24].

Anaesthesia
Anaesthesia of laboratory animals is an art as well as a science. It is also a serious responsibility. Significant animal pain and distress can result from poorly administered or inadequately monitored or inappropriate anaesthesia. Only trained personnel should undertake animal anaesthesia.

Analgesia
The relief or prevention of pain in conscious animals is extremely important. It should be generally assumed that any procedure that causes pain in humans would cause pain in laboratory animals, and arrangements must be made to minimize pain. Unrelieved pain or distress is not acceptable except in circumstances where they have been scientifically justified and approved by the IACUC. Even after analgesics have been administered, animals must be monitored closely for adequacy and persistence of analgesia. Protocols should describe plan for monitoring animals and should indicate who on the research team will be responsible for monitoring the animals.

Surgery
Careful attention should be paid to sterility, thorough understanding of appropriate methods of anaesthesia and an understanding of concerns specific to care unique to individual animals during and after surgery are imperative to the success of these techniques. It is also critical that the surgeons have experience in the use of surgical instruments and basic surgical technique. Practice with suturing techniques and anatomical dissections should be performed on inanimate objects and postmortem animals respectively prior to beginning experimentation on live animals.

Any proposed surgical procedures, both survival and nonsurvival, must be fully described in the approved protocol. Nonsurvival surgery is defined as a surgical procedure from which the animal does not awaken. Nonsurvival surgery must meet applicable standards (*e.g.*, clean instruments, surgeon appropriately garbed,

in a suitable environment). Survival surgery, where the animal recovers from anaesthesia, must follow strict standards for aseptic technique.

ANIMAL RIGHTS AND ETHICAL CONSIDERATIONS

Institutional Animal Care and Use Committee (IACUC) and the Three "Rs"

The IACUC reviews all facilities and programs twice a year; identifies and ensures correction of any deficiencies; reviews the researcher's proposal for animal use; monitors animal use; and can suspend any animal activity that does not meet the required standards.

Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of the society.

The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Alternative methods should always be considered before settling for the use of animals. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain consistent with sound scientific practices, is imperative. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anaesthesia. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Veterinary care must be provided routinely. Investigators and other personnel must be appropriately qualified and experienced for conducting procedures on living animals.

The protocol review of IACUC frequently include the following queries: Does the research address an important question? Does the research require the use of animals? Is the research necessary, in that it does not needlessly repeat previous work? Is the experience of each animal in the study adequately described and justified?

The use of animals in research has guided several experiments to medical and other scientific advances. As a result, both human and animals are now able to enjoy longer and healthier lives than they could have in previous generations. The conduct of animal research is not a right but a privilege. The IACUC strongly encourages researchers to incorporate and implement "the Three R's" (the principles articulated by Rusell and Burch (1959)[25] for the concept of humane animal experimentation) in their studies. The "principles of humane experimental technique", published in 1959, proposed that all research using animals should be evaluated to see if the Three Rs (**Reduction**, **Replacement** and **Refinement**) could be applied. It was recognized that while the replacement of animals as research subjects was a desirable goal, considerable gains could be made in humane science through reducing the numbers of animals used and by refining the techniques that were applied to animals. In the last 50 years, the Three Rs have become widely accepted ethical principles to be embedded in the conduct of animal-based science.

The Sundowner Principles

In May of 1998, the American College of Laboratory Animal Medicine (ACLAM) published a monograph of a symposium that had taken place in October of 1996 at the Sundowner Inn in California. The catalyst for the meeting was public concern over the use of primates in space research by the National Aeronautics and Space Administration (NASA). One of the papers in this monograph referred to a discussion about ethical guidelines, called the "Sundowner Principles." The use of animals in research involves responsibility - not only for the stewardship of the animals but to the scientific community and society as well. Stewardship is a universal responsibility that goes beyond the immediate research needs to include acquisition, care and disposition of the animals, while responsibility to the scientific community and society requires an appropriate understanding of and sensitivity to scientific needs and community attitudes toward the use of animals. The basic principles particularly relevant to the ethics of research involving animals have been well articulated in the Sundowner Principles, and these are becoming the "standards": Respect to life, societal benefits and Nonmaleficence.

Respect for Life: Living creatures deserve respect. This principle requires that animals used in research should be of an appropriate species and health status and should involve the minimum number required to obtain valid scientific results. It also recognizes that the use of different species may raise different ethical concerns. Selection of appropriate species should consider cognitive capacity and other morally relevant factors. In addition, methods such as mathematical models, computer simulation, and *in vitro* systems should be considered and used whenever possible.

Societal Benefit: The advancement of biological knowledge and improvements in the protection of the health and well being of both humans and other animals provide strong justification for biomedical and behavioral research. This principle entails that where animals are used, the assessment of the overall ethical value of such use should include consideration of the full range of potential societal goods, the populations affected, and the burdens that are expected to be borne by the subjects of the research.

Nonmaleficence: Vertebrate animals are sentient. They possess the ability to feel, perceive, or be conscious, or to have subjective experiences. Nonmaleficence entails that the minimization of distress, pain and suffering is a moral imperative. Unless the contrary can be appropriately established and supported, researchers

should consider that procedures that cause pain or distress in humans may cause pain or distress in other sentient animals.

ALTERNATIVES TO ANIMAL EXPERIMENTATION

Background

The University of London Animal Welfare Society (ULAWS) was founded in 1926 of by Major Charles Hume. The name of the organization was changed to the Universities Federation for Animal Welfare (UFAW) in 1938, reflecting the increasingly wide range of people and institutions involved. In 1954, the UFAW with its founder and director at the time, Major Charles Hume established a committee to undertake a scientific study of humane technique in laboratory animal experiments. The committee was chaired by Sir Peter Medawar, the Nobel prize-winning and well respected immunologist, while Hume served as the committee's secretary. William Lane-Petter, secretary of the Research Defense Society, an organization established to defend animal research, was also a member of this committee. Christine Stevens, founder of the Animal Welfare Institute (AWI) in the U.S., provided financial support for the project. The committee appointed William Russell (a zoologist) and Rex Burch (a microbiologist) to carry out the project[26]. The couple published the work "The Principles of Humane Experimental Technique" in London in 1959. In the book animal testing alternatives were defined as the Three Rs: Replacement, Reduction and Refinement.

Definition

"Alternatives" or "substitutes" are defined as anything from absolute to partial replacement of live animals in biomedical research and testing[26]

The Four "Rs"

Russell and Burch (1959)[25] encapsulated their definition of "alternatives" in "the Three Rs – Reduction, Replacement and Refinement".

Reduction: Reduction means a decrease in the number of animals used previously with no loss of useful information. This may be achieved by reducing the number of variables through good experimental design, by using genetically homogeneous animals/phylogenetic reduction or by ensuring that the conditions of the experiment are rigorously controlled. Reduction can also be implemented by improved statistical design and use of better quality animals such as animals with implanted catheters and flow probes which are used to study physiological functions in major organ system toxicology and telemetry systems[27,28].In the case of teaching protocols, it may entail students working in groups rather than individuals working with an animal.

Replacement: Replacement often means the use of non-animal living systems (*e.g.*, cell and tissue cultures) or use of non-living systems as alternatives (*e.g.*, a computer model or program, a video, a mannequin). It can also mean the replacement of sentient animals (usually vertebrates) with less sentient animals (usually invertebrates such as worms, bacteria, *etc*).

Refinement: Refinement means a change in some aspect of the experiment that result in a reduction or replacement of animals or in a reduction of any pain, stress or distress that animals may experience. Refinement is also implemented by generally decreasing invasiveness, improving instrumentation and improving techniques used for animal research[28,29]. The establishment of early endpoints for intervention in a study that has the potential to cause pain or distress is an example of refinement.

Responsibility: The 4th "R", was added by Ronald Banks[30,31]. It has grown into a new era of performance-based outcomes, which reflects integrity, honesty, and scientific correctness in appropriate and reasonable use of laboratory animals. This ensures that animal life is required and necessary for biomedical advancements[32].

The Alternatives

These alternatives can be physico-chemical techniques, microbiological systems, tissue/organ culture preparation, computer or mathematical analysis (*in silico* testing), epidemiological surveys, and plant analysis (*e.g.*, toxicity assays in plants). Research methods superior to using animals to learn about human disease or predict the safety of new drugs are stem cells, microdosing, DNA chips, microfluidics chips, human tissue, new imaging technologies, and post-marketing drug surveillance[33]. Other alternatives include the use of humans for skin irritancy tests, donated human blood for pyrogenicity studies, Modular immune *in vitro* construct and use of a fungal model for mammalian drug metabolism. The arguments against *in vitro* cell culture techniques and *in silico* computer simulation are that these two cannot be true alternatives since simulations use data from prior animal experiments, while cultured cells often require animal derived products, such as serum. It has also been argued that they cannot replace animals completely as they are unlikely to ever provide enough information about the complex interactions of living systems[34].

CHOICE AND NUMBER OF ANIMALS FOR STUDY

In the determination of the choice and number of animals to use in an experiment, ethical consideration is of utmost importance. The Four "Rs" of reduction, replacement, refinement and responsibility, earlier discussed, offer useful guide.

The significance and validity with respect to the usefulness of results generated in an animal model will largely depend on the selection of a suitable model. A good knowledge of comparative anatomy and physiology is a definite advantage when developing or employing an animal model.

The number of animals used in a particular study should be the minimum number that provides adequate data for statistically valid results. This number varies with the type of study, but should generally conform to the norms of the particular discipline. In making decisions about the numbers of animals, adequate consultation with faculty scientists, statisticians, and other researchers is highly encouraged.

Considerations will include diseases or body systems being studied, animals that are most similar to humans and animals that have been used in past research on the topic in question. For a given disease or body system, some species are more appropriate than others. However, for economic reasons, most initial testing is done on rodents especially rats and mice, while the follow-up studies may be done in other animals. Moreover, certain infectious diseases only affect primates, and thus monkeys or chimpanzees have to be the animal studied in such cases.

There is a natural tendency to equate the value of an animal as an experimental model with its *fidelity*, or how closely it resembles the biological structure of the target organism (*e.g.*, human). In some areas of research, close homology (structural similarity) may be important. For example, if one were developing a new surgical procedure or instrument for humans, similarity of anatomical structure in the animal chosen might well be essential. However, this factor should not be overstretched. In fact, a lot of caution must be exercised in this regard. The idea that overall anatomic or genetic (evolutionary) similarity is, in general, necessary, or even desirable, may be erroneous. Russell and Burch (1959)[25] discussed the concept of the "hi-fi (high-fidelity) fallacy." This view emphasizes the importance of not placing inordinate weight on the fidelity of an animal model. The fallacy arises when one assumes, for purposes of human disease research, that mammalian models are inherently better and, by extrapolation, that the use of nonhuman primates is inherently best.

Another very important concept is that of *discrimination*, or the ability of an animal model to reproduce a particular property desired, or to provide good predictive ability for a condition (or normal biology) of the target species. Discrimination is related to the concept of biological reductionism. Reductionism is a method of study that seeks to break a system down into its component parts, study each part individually, and then reach a conclusion about the system as a whole or at least the role of the individual parts[35]. This should always be treated with great caution as the summation or the reconstruction of the individual steps or processes may not necessarily generate the highly complex entity or system.

A major issue in deciding on the animal model to use for an experiment should be based on the model that best answers the research question(s). The answer may be that nonhuman primates are necessary, *e.g.*, in many types of infectious disease research where other animals simply do not become infected; one classic example is Hepatitis B virus infection.

Another example is the study of HIV (human immunodeficiency virus) and AIDS (*acquired immune deficiency syndrome*). Although the chimpanzee is the only animal species that becomes infected with the human immunodeficiency virus, the virus does not seem to exhibit similar behavioral pattern as observed in humans. Nonhuman primates have their own immunodeficiency viruses, and study of these has provided valuable clues in our understanding of HIV. It is, however, to mention that a great deal of information has also been derived from studies of feline immunodeficiency virus (FIV), a natural disease of cats that resembles HIV/AIDS in many respects.

In many other cases the greater simplicity and ability to manipulate the system in lower species may be scientifically quite powerful and become a major consideration. A good example is the tremendous amount of basic genetic information that has been accumulated over the years from studies of the fruit fly (***Drosophila melanogaster***).

Other considerations in choosing an animal model include: good literature base; historical usage (*i.e.*, accepted animal model); ready availability to other researchers; sufficient size for obtaining necessary samples (blood, urine, biopsies, *etc.*) and substance administration; accommodation in existing animal facilities (caging, environmental controls, exercise and environmental enrichments); experience of animal care staff; experience of research staff; good reproductive performance; length of survival to allow functional and meaning deductions; minimal indigenous disease; microbiological and genetic characterization and public relations implications.

Many researchers are of the opinion that the use of nonhuman primates carries a greater ethical cost than other "lower" species, and similar views held by the public implies that the use of these animals carries a greater risk of media attention and public criticism. In addition, nonhuman primates, especially larger species such as macaques or baboons, are by no means easy to work with, and are dangerous not only because of their strength and sharp teeth, but because they can carry diseases transmissible to humans. Finally, nonhuman primates are expensive to purchase, and maintenance costs are considerably high because of the need for large, sturdy cages, along with programs to provide for their psychological well-being (*e.g.*, group housing and appropriate environmental enrichments).

EXTRAPOLATION FROM ANIMAL EXPERIMENTATION

Olson *et al.*, (2000)[36] examined the strengths and weaknesses of animal studies in predicting human toxicity. Their results showed the true positive human toxicity concordance rate of 71% for rodent and nonrodent species, with nonrodents alone being predictive for 63% of human toxicities and rodents alone for 43%. The highest incidence of overall concordance was seen in haematological, gastrointestinal and cardiovascular human toxicities, and the least was seen in cutaneous human toxicity.

Data generated in the rat have correctly predicted the

outcome of several human diabetes prevention trials, notably the failure of nicotinamide and low dose parenteral and oral insulin therapies[35].

In toxicology, determination of human equivalent of pharmacologically active dose is complicated and depends upon many factors such as pharmacokinetics and differs markedly among pharmacological classes of drugs and clinical indications[37].

Diabetic animals may be regarded as models of the disease in man. However, a lot of caution must be exercised because such animals display a wide diversity of pathophysiology[38]. In fact, no animal syndrome corresponds precisely to any type of diabetes in human subjects. The assumption that homo sapiens are identical to other animals in his/her bodily functions has led to a number of errors in the history of medicine. The anatomical research of Galen (Greek physician and philosopher) was based almost entirely based on studies of apes and pigs. Unhesitatingly, he extrapolated his discoveries directly to humans, thus initiating many errors. The combination of Galen's immense authority and the dogmatic prohibition by the Church of postmortem dissections of the human body conserved these errors well into the late 16th century. A closer look reveals that his mistake was to draw wrong conclusions from the results of these first scientific "animal models" because of uncritical interspecies extrapolation. As a result of a variety of caveats, the safety and effectiveness of interventions in human subjects can only be speculated from animal studies[39].

Prior to any discovery in humans, there is a critical stage involving animals to determine if the new treatment or procedure is safe and effective. Biomedical research follows a rigorous path of science and discovery leading to highly regulated testing that proves or disproves the value of a new idea that may lead to new treatments and cures. One portion of that research involves animals. Animal models are therefore essential tools to mankind. In fact, animal experimentation has led to the development of procedures and medicines that have improved and saved the lives of both humans and animals all over the world, including treatments for heart disease, cancer, mental illness, and a wide range of animal ailments. Nevertheless, caution must be greatly exercised in extrapolating findings from animal research to humans because "All flesh is not the same: Men have one kind of flesh, animals have another, birds another and fish another"[40].

Conclusion

There is no iota of doubt that animal testing, has contributed to (and will most certainly continue to) the well being of both humans and animals. Notwithstanding, researchers in all countries need to do more, especially in the humane treatment of animals. Investigators should further incorporate the ethical standards of the Four Rs (reduction, replacement, refinement and responsibility) and the **Sundowner Principles (respect for life, s**ocietal benefit **and n**onmaleficence**) in the conduct of animal experimentation.** Relatively few countries collate and publish animal use statistics, yet this is an essential step toward public accountability and an informed debate on the relevance of animal testing, as well as being important for effective policy-making and regulation. One can comfortably predict that animal rights groups will become more vibrant and stronger in the coming decades even in developing countries. Researchers need to conduct animal experiments with greater attention to ethical issues. The implementation of the Four Rs, without regular, accurate statistics, cannot be monitored. It is imperative therefore, that adequate policies and regulations are enacted where not available and where already in place, more vigorous mode of enforcement should be engineered. Finally, careful and robust synthesis of data from multiple animal studies is needed to assess the concordance of interspecies extrapolation.

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