Of Mice and Ethics

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ABSTRACT Using animals in research has always been a subject of debate amongst scientific communities worldwide. The decision to perform certain experiments on live animal subjects is always taken after a series of experiments were already performed, leaving this as a final requirement before live human testing. It should always be performed when it is determined that it will provide a significant advancement in the field and will later result in an improvement to the human, social or environmental well-being. Historically, animals have been used in scientific experiments since ancient times. Their usage has varied greatly, and so did the moral implications. As medicine advanced through the centuries, using live or dead animals was of crucial importance for physiology or anatomy experiments. In modern society, animal experiments are a right attributed to competent researchers who can ensure humane care and use of the animals in controlled environments, with as little discomfort as possible to the living animal. Mice were used as prime subjects for medical experiments, especially in pharmacokinetics studies and treatment.

KEY WORDS Mice; Laboratory animals; Ethics; Animal experiments

Introduction – the mouse as an animal model

Text Murine experiments are some of the most commonly used small animal models for testing various types of treatments and effects, primarily because of their convenience in acquiring, breeding, housing and transportation. Mice genomics are well established and a high number of different strains exist in order to accommodate almost all experimental needs. They have a relatively high reproductive performance, new generations being produced in a short period of time. They are easily fed, their daily cycle can be regulated and their housing needs can be met with relative low costs of operations in a small space. However, their small size poses problems with certain procedures and the quantity of working tissue obtained for some types of experiments is relatively low. Their high numbers do however overcome the latter limitation, as several animals may be included in the same experimental lot, thus providing a larger volume of data in a certain direction.

As with all experimental animal models, many scientific advancements were obtained from their usage, therefore validating their use in controlled experiments. The morality of the usage of such animals in experiments that require physical damage or result in animal death has always been a question of debate, either philosophical or in the realm of humane activism [1].

Short history of animal usage in scientific experiments

The first live animal experiments date back to 500 BC, when in Antic Greece the first observations regarding the structure and function of organs in live animals were recorded. Pythagoras proclaimed that the soul passes through animal and human forms, even embodying plants as well; Hippocrates, considered by many as the father of medicine, related the first aspects of diseased organs in both humans and animals. During the first century of our era, in the Roman Empire, Galenus uses vivisection in some of the first recorded physiological studies, thus establishing experimental medicine as we know it today. His research was however preceded by a number of both Greek and Roman anatomists that used vivisection to characterize the appearance of organs and hypothesize on their function.

Vesalius (1514-1564) recreated some of Galenus' experiments and almost a century later, W. Harvey published the first scientific experiment involving the use of animals, in 1638, stating his theory of reproduction "*Exercitatio anatomica de motu cordis et sanguinis in Animalibus*".

During the 1800's, animal experiments were the only way to study physiological processes naturally occurring in a living organism. Louis Pasteur tested his anti-rabies vaccine on dogs in 1885, while the diphtheria anti-toxin was synthetized by injecting guinea pigs with the toxin.

The rules for experimenting on live animals were set as early as in the 19th century, as Jeremy Bentham (1748-1832) proclaimed utilitarism, the ethic principle that tried to correlate the benefit obtained with the level of the action required to

obtain it. He also introduced the notion of morality when using animals for experimentation, due to their capacity to feel pain. In 1824, in England, the *Society for the Preservation of Cruelty to Animals* was established, being the first organized body that militated for animal rights, in particular for that period referring to carriage horses. The modern embodiment of this society has broadened its spectrum, and its branches worldwide militate for the rights of all animals, including the ones involved in scientific experiments [2]. Claude Bernard, considered by many as the father of modern physiology, extensively used animals in his demonstrations; his wife created the first association dedicated to protecting the rights of laboratory animals, in 1860. The "British Cruelty to Animal Act" was signed in 1876 in England, effectively being the first law to regulate the usage of animals in laboratory settings; later on, in 1909, its counterpart was adopted in the United States of America [3]. Efforts intensified in both Europe and the USA towards limiting the use of animals in laboratory studies, this leading to the foundation of the National Anti-Vivisection Society (NAVS) in 1875, and the British Union for the Abolition of Vivisection (BUAV) in 1898, both by active militant for animals rights, Frances Power Cobbe. The English writer Henry Stephens Salt was the first to write a piece on animal rights, in his book "Animals' Rights: Considered in Relation to Social Progress" published in 1894. He however defended the use of animals in justified medical experiments.

The principle of the "3 Rs"

In the 2nd half of the 20th century, zoologist Wiliam M.S. Russel and microbiologist Rex I. Burch defined the principle of the three Rs: Refinement, Reduction and Replacement, which should govern any scientific procedure and complex set of experiments. This practical strategy was cited by any major act governing the ethics of animal research, ever since it was published in 1959. They thus became the international approach to decision-making when designing experiments and the use of animals in a humane fashion [3–5].

Replacement refers to finding alternative methods in experimental models which circumvent the use of animals altogether, or the usage of species lower on the phylogenetic scale (for instance, replacing vertebrates with invertebrates).

Refinement refers to altering experimental procedures in order to minimize or eliminate discomfort or pain for the animals. This requirement is however hard to accomplish in certain studies which require by design experiments that should inflict a certain degree of distress upon the animal. However, even in these settings, all efforts should be made towards establishing the cost-benefit ratio for performing this part of the experiment.

Reduction refers to performing the experiments in certain ways designed to minimize the number of animals, discomfort and pain inflicted, while maintaining the level and significance of information obtained. Animal reuse is not a valid reduction strategy as their well-being may be severely compromised when they were already subjected to medical experiments.

Applying any of these principles should always be performed under the supervision of a trained veterinary specialized in identifying animal discomfort, who can intervene when pain or distress levels surpass those intended by the study protocol. Studies resulting in chronic pain or severe alteration of physiology of the animal should include humane end-points for animal termination.

European and international regulations regarding animal rights

UNESCO adopted the Universal Declaration of Animal Rights in Brussels, 1978, which proclaims the equality between animals' rights for existence and the respect humans should show towards them [3,4]. Through this declaration, it was stipulated that laboratory animals have the right to protection and justified use in experiments. This declaration also stipulated that medical experiments conceived to inflict physical or psychic suffering on an animal should not be performed, while developing methods to replace animals from such experiments.

On March 18th 1986, in Strasbourg, the European Council ratified the convention for the protection of vertebrate animals utilized in experiments and other scientific purposes. Its stipulated goals were the reduction of the number of animals used in research, while using them only for diagnostic, prophylactic and treatment purposes, under general anesthesia or any other methods employed to reduce pain and discomfort. All European countries adopted this convention in the next two decades, Romania publishing it as an official act in 2006.

The United Nations also regulated the use of animals in laboratory settings through the 86/609/EEC directive, given on the 24th of October 1986. It re-stated the use of the "3 Rs" as an active governing principle of medical research, further enforcing that no experiment should

involve animals if a safe alternative exists, yielding similar results, and, whenever possible, alternative techniques should be employed towards reducing the number of specimens used and decreasing the pain derived from procedures.

In following years, several bodies emerged with a common goal of restricting the use of animals in medical experiments to the required minimum while maintaining the quality of the scientific results. Their methods involved alternative financing and resourcing options, cooperation with research centers towards validating results and creating open fora for alternative methods. Such bodies were the European Centre for Validation of Alternative Methods (ECVAM), founded in 1991 or the German Centre for the Documentation and Evaluation of Alternative Methods (ZEBET), a renowned database for alternative methods to animal experiments.

In 1990, the International Conference on Harmonization (ICH) attempted to unify guidelines for animal testing, thus greatly reducing the numbers used in research; in present, its results are the main accepted rules for reporting test results.

The 86/609/EEC directive was replaced on the 22nd of September 2010 by the Directive 2010/63/EU which regulated "the protection of animals used for experimental and other scientific purposes". This new directive took the issue one step further, taking it in line with recent advancements and updating the legislation accordingly. It also set minimal standards for housing, laboratory techniques and methods and especially the training of personnel involved in animal handling.

Editorial policies regarding reporting animal experiments

The accepted method of disseminating scientific knowledge is through publication in peer-reviewed journals. Therefore, all results of scientific experiments pass the filter set by these publications, and their ethical standards should in turn influence the methods used during the research period, by actively enforcing the internationally adopted directives.

For the purpose of standardization, the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines were developed by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). This came as a necessity after the installment of the high standards of quality required for reporting trials involving human subjects [6–8]. Another example of regulations is the Gold Standard Publication Checklist (GSPC), effectively a list of items designed to increase the level of standardization in animal experimentation [9, 10].

A number of studies were concerned with the quality of the editorial policies of scientific publications in regards to animal usage [10–15]. One study found that, out of a sample of 138 in-print journals, 85 contained on-line instructions towards the authors clearly stating the need for written adhesion to ethical requirements for animal studies [12]. Most studies concluded that no relationship between how long a journal has existed, its open-access policy or board composition influences the ethical standards required for publication of animal studies. Biomedical journals are however more prone to include clear ethical requirements for animal studies, of all journals in the general field of biology [12–14]. Standardized regulations such as ARRIVE and GSPC are standard requirements to be fulfilled for submission to most journals. However, one recent study has shown that ethical issues related to how accurate procedures are related and even suspicion of altering protocols to "fit" within standardized guidelines, still plagues a rather high number of studies [10]. The rejection rate of many journals on ethical grounds is relatively low, proving that most studies do conform to ethical regulations stipulated. Whether this is an attempt to fraudulently bend protocols in order to comply to editorial policies, or it represents a real effort to ensure the optimum conditions for humane approaches when experimenting on animals, remains to be determined.

Conclusions

Mice are one of the most commonly used species in animal model, making for almost 90% of all species involved in medical experiments. The usage of animals for scientific research contribute towards scientific progress in all areas. It is however imperative to establish standardized welfare conditions in order to ensure the humane treatment of laboratory animals. For this, fundamental principles were established and the whole academic and scientific communities have taken important steps towards providing a framework for experiments. An important help should come from scientific journals, which should refine their editorial policies to ensure that all studies conform to basic ethical principles.

"Hyperthermic magnetic nanoparticle ablation of liver and pancreatic tumors".

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