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ANIMAL EXPERIMENTATION IN MEDICAL RESEARCH

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ABSTRACT

The use of animals in experimental research parallels the development of medicine, which had its roots in ancient Greece (Aristotle, Hippocrate). The increased interest in and concern about animal welfare issues led to legislative regulations in many countries and the establishment of animal ethics committees. The major areas are drug research, testing of vaccines and other biological and cancer research, whereas about 30% of the animals are used for other purposes such as fundamental research, diagnostics. The increasing demand for high standard animal models together with a critical view on the use of animals led to the development of Laboratory Animal Science in the 1950s with Russell and Burch's three R's of Replacement, Reduction and Refinement as guiding principles, a field that can be defined as a multidisciplinary branch of science, contributing to the quality of animal experiments and to the welfare of laboratory animals. In vitro methods are usually more accurate, easier to perform, and cheaper, such in vitro methods are usually not alternatives in the sense that they substitute for animals; they complement experiments on animals. A legal requirement "that no experiment on a living animal may be performed if the purpose of the experiment can be achieved by alternative means not involving an experiment on a living animal" would be unenforceable. The current trends in 3Rs research are illustrated by the recent successes, however, a real breakthrough in terms of 3Rs depends on the acceptance of a new strategy in the medical research, the consistency approach.

KEYWORDS: Aristotle, Hippocrate, establishment of animal ethics committees.

INTRODUCTION

Today, 75–100 million vertebrates per year are used in research and testing for a wide range of purposes. The major areas are drug research, testing of vaccines and other biological and cancer research, whereas about 30% of the animals are used for other purposes such as fundamental research, diagnostics, etc. Mice and rats are the most frequently used animal species. In many European countries, it is mandatory by national law to grade the level of discomfort for animals in experiments in minor, moderate and severe. On average, 50% of the laboratory animals experience minor discomfort (e.g single blood sampling), 30% moderate (e.g recovery from anaesthesia) and 20% severe (e.g toxicity tests).

Before clinical trials are carried out, the safety and effectiveness of new drugs are usually tested in animal models.[1] Although the use of animals in medical research is controversial, a poll by the Medical Research Council found that most people support their use provided that there are benefits to human health care, no alternative exists, and no unnecessary suffering occurs.[2] The usefulness of animal testing has, however, been questioned.[3–5] Some believe that the results from animal experiments cannot be applied to humans because of the biological differences between the species and because the results of animal experiments often depend on the type of animal model.[3] To date the methods used to assess the value of animal trials include historical analyses, critiques of animal models, surveys of clinicians, and citation analyses. In this paper we compared treatment effects from systematic reviews of clinical trials with those of our own systematic review of the corresponding animal experiments.[6-8] For animal research, a comprehensive set of guidelines on reporting studies has thus far been lacking. This gap has now been filled following the generation of a set of guidelines referred to as ARRIVE (Animals in Research: Reporting In Vivo Experiments)[9] by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), a UK government-sponsored organization. The guidelines recommend the format and content of details relating to animals in a typical scientific report.

Need of the Animal Experiment

The biomedical scientists generally work to unfold the complicated processes of life and to provide new measures for the health and welfare of the society i.e. the humans, the animals and the environment. There is, therefore, need to provide them certain degree of freedom and adequate facilities to use animals wherever necessary. It is evident that certain life processes can not be investigated without involving whole animal system. The in-vitro alternatives can

only provide limited information. These cannot totally replace the animals in experiments. This is why the use of animals continues to be mandatory to meet the statutory regulatory requirements. At the same time, it is an obligation of the scientists t ensure that the experiments conducted on animals are rational and unavoidable, and no unnecessary pain or injury is inflicted on them and they are maintained in best possible environmental conditions. It is, therefore, necessary to have well-defined guidelines which will safeguard the pursuit of knowledge, the interest of society and the welfare of animals.

Guide Line for Animal Experiment

Housing and Environment

Laboratory animals are very sensitive to their living conditions. It is important that they are housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds. The building, cages and environment of animal rooms are the major factors which affect the quality of animals. In planning an animal facility the space should be well divided for various activities. The animal rooms should occupy about 50-60% of the total constructed area and the remaining area should be utilized for services such as stores (8-10%), washing (8-10%), office and staff (8-10%), machine rooms (4-5%) quarantine and corridors (12-15%). The cages should be made of suitable metal (stainless steel, galvanized iron sheet/rods) or synthetic material (polypropylene / polycarbonate). They should be of suitable size for each species of animal and should have adequate arrangement for feeding and watering. They must be free from crevices, corners and sharp edges for easy cleaning and to avoid injury. The bedding should be of right material and sterilized before use. Common bedding materials used in India are paddy husk, saw dust, paper currings, dry grass and crushed corn cobs. The environment of animal room (Macro-environment) and animal cage (Micro-environment) is an important factor on which the production and experimental efficiency of the animal depends. Since animals are very sensitive to environmental changes, sharp fluctuations in temperature, humidity, light, sound and ventilation should be avoided. A constant room temperature is essential, because variation in room temperature causes change in food and water intake. A charge in temperature of 4°C can cause 10-fold alteration in biological responses. The temperature also affects fertility and lactation. Coupled with high humidity the increase in temperature causes ammonia built up. If the ventilation is not proper the high ammonia concentration causes respiratory irritation to both animals and attendants, predisposing them to infection by lowering their resistance. An effective ventilation system with 10-12 air

changes per hour of 100% fresh air must be provided for animal rooms. Light and sound are other important factors. The light intensity, the wave length and the photo cycle affect the health and behaviour of the animals. Sudden and sharp sounds in the animal rooms disturb the health and behaviour of animals and may give rise to ear damage, hypertension, cannibalism, etc.

Nutrition and Feeding

The results of an experiment are likely to be influenced by co-existence of nutritional deficiencies and imbalance. It is, therefore, essential that laboratory animals are maintained on a balanced diet based on nutritional requirements of each species. Special care is needed on nutritional elements, ingredients used in diet, and feeding practices. A balanced diet should contain protein, carbohydrates, fat, minerals, vitamins, roughage and water in required proportions for each species of animal. Only quality ingredients should be used in a diet and they should be free from dust, moulds, fungi and other contaminants. Each animal must get required quantity of feed, based on animal maintenance and production requirements. The feed should be palatable so that it is consumed in adequate quantity by the animals. Any undesirable odor always causes under consumption resulting in nutritional deficiency in the animals. No drug, hormone or antibiotic should be added in the feed as these are likely to disturb the normal metabolism of the animals and produce biased results. The ingredients and the prepared feed must be stored and handled carefully so as to avoid any contamination. The food must be of right consistency and should be presented to animals in proper type of hoppers to avoid wastage. In some cases the feed may be divided in 2-3 meals during the day. Pelleted feeds balanced for different species of animals are now available commercially. These are easy to procure and use without wastage. However one has to be careful on quality of the feed from batch to batch. It should be obligatory on manufacturer to mark each bag with the type of food, date of manufacture, the batch number, the ingredients used and chemical composition. Random chemical analysis must be carried to for major nutrients to monitor the quality of food from time. Clean, chlorinated water should be available to the animals ad lib.

Hygiene and Disease Control

The building for housing the animals should be provided with barriers to control the entry of contamination into the building through men, material and wild animals. Strict barriers should be provided to avoid the entry of wild rodents, birds, insects and pests. Visitors and

service staff should be allowed entry with care and when necessary. On the exit side an efficient monitoring service should be established to monitor the prevalence of any infection in the colony. A regular medical checkup of the staff, postmortem of dead and sacrificed animals and screening of waste material of the rooms are essential.

Personnel and Training

The selection of animal facility staff, particularly the staff working in animal rooms or involved in transportation, is a critical component in the management of an animal facility. The staff must be provided with all required protective clothing (masks, aprons, gloves, gumboots, etc.) while working in animal rooms. Facilities should be provided for change over with lockers, wash basins, toilets and bathrooms to maintain personal hygiene. It is also important that a regular medical check-up is arranged for the workers to ensure that they have not picked up any zoonotic infection and also that they are not acting as a source of transmission of infection to the animals. He should ensure that persons working in animal house don't eat, drink, smoke in animal room and have all required vaccination, particularly against tetanus and other zoonoses. Initial in-house training of staff at all levels is essential. A few weeks must be spent on the training of the newly recruited staff, teaching them the animal handling techniques, cleaning of cages and importance of hygiene, disinfection and sterilization. They should also be made familiar with the activities of normal healthy and sick animals so that they are able to spot the sick animal during their daily routine checkup of the cages. At national level suitable training programmes should be organized by the National Centres to provide training in care, breeding, management, handling of animals for the staff working in animal breeding and holding units. Orientation training programmes should also be initiated for the investigators working in different areas to acquaint themselves with various experimental techniques. Such a course should address the undermentioned topics.

a- biology and husbandry of laboratory animals

b- genetic make-up

c-microbiology and diseases

d-health-hazards in the animal house

e-anesthesia, analysis and experimental procedures

f- alternatives to animal use

g- ethical aspects and legislation

Records and Evaluation

Good quality animals are those which are free from disease. Animal of a specified strain should also have all the characteristics of that strain, i.e. they should be genetically anthesised. The results of regular monitoring of parameters of genetic purity must be scrupulously recorded. Proper record-keeping is extremely important and vital for an animal facility. The forms should be simple but complete and preferably computer compatible. Too exhaustive and unnecessary recording should be avoided as these are not useful. Records of breeding and experimentation and deaths of all experimental animals at various stages are essential. Receipt and issue of food and other stores should be recorded. Log books of various machines such as incinerator, boilers, air-conditioning plant should be maintained. Monthly and annual reports of the activities should be prepared and reviewed for evaluation of work and future planning.

Experimentation and Veterinary Care

The experimental animal units should generally be looked after by qualified investigators. These units must have adequate housing and technical facilities for experiment and post-operative care.

The equipment provided in the experimental unit should be appropriate for the needs of the experiments. No technique should be used which may cause avoidable discomfort to the animals.

The post-operative holding rooms and cages should be comfortable and such animals should remain under the care and supervision of an experienced scientist or a qualified veterinarian. The person actually incharge of animal facility should preferably be a veterinarian or a person qualified in laboratory animal science. In any case an experienced veterinarian must be readily available in an animal holding for health care, monitoring, diagnosis and treatment of diseases and injuries. A veterinarian could also be helpful to investigators in animal anaesthesia and surgery.

Ethical Guidelines for Use of Animals in Scientific Research

1. Animal experiments should be undertaken only after due consideration of their relevance for human or animal health and the advancement of knowledge.

2. The animals selected for an experiment should be of an appropriate species and quality, and minimum number should be used to obtain scientifically and statistically valid results.
3. Investigators and other personnel should treat animals with kindness and should take proper care by avoiding or minimizing discomfort, distress or pain.
4. Investigators should assume that all procedures which would cause pain in human beings may cause pain in other vertebrate species also (although more needs to be known about the perception of pain in animals).
5. Procedures that may cause more than momentary pain or distress should be performed with appropriate sedation, analgesia or anaesthesia in accordance with accepted veterinary practice. Surgical or other painful procedures should not be performed on unanaesthetized animals.
6. At the end of, or when appropriate during an experiment, the animal that would otherwise suffer severe or chronic pain, distress, discomfort, or disablement that cannot be relieved or repaired should be painlessly killed under anaesthesia.
7. The best possible living condition should be provided to animals used for research purpose. Normally the care of animals should be under the supervision of a veterinarian or a person having adequate experience in laboratory animal care.
8. It is the responsibility of the investigator to ensure that personnel conducting experiment on animals possess appropriate qualifications or experience for conducting the required procedures. Adequate opportunities have to be provided by the institution for in service training for scientific and technical staff in this respect.
9. In-vitro systems to replace or reduce the number of animals should be used wherever possible.

The concept of replacement

It was at the Universities Federation for Animal Welfare's 1957 Symposium on Humane Technique in the Laboratory[10] that the concept of the Three Rs (reduction, refinement and replacement) as a means of removing inhumanity from animal experimentation was first discussed in depth at a public meeting, notably by Charles Hume and William Russell. This Symposium, and Russell and Burch's book, The Principles of Humane Experimental Technique[11] resulted from an initiative taken by UFAW in 1954, with the advice of a distinguished committee}which met under the chairmanship of Peter Medawar. Russell and Burch defined a replacement technique as 'any scientific method employing non-sentient

material which may in the history of animal experimentation replace methods which use conscious living vertebrates'. They distinguished between relative replacement}in which animals would still be required}but would not be exposed to any distress in the actual experiment} and absolute replacement} in which animals would not be required at any stage at all.

Alternatives to animal experiments are procedures which can completely replace the need for animal experiments, reduce the numbers of animals required, or diminish the amount of pain or distress suffered by the animals in meeting the essential needs of man and other animals.

The replacement alternatives

If a replacement alternative is defined as a method which does not involve the use of a living protected animal in a regulated procedure, then the range of methods includes the following.[12-13]

1. The improved storage, exchange and use of information about animal experiments already carried out, so that unnecessary epetition of animal procedures can be avoided.
2. The use of physical and chemical techniques, and of predictions based on the physical and chemical properties of molecules.
3. The use of mathematical and computer models, including: a. modelling of quantitative structureactivity relationships (QSAR),Le. taking advantage of correlations between molecular structure and biological activity in the prediction of the potential desired and undesired effects of series of related chemicals; b. molecular modelling and the use of computer graphics, e.g. in actively designing drugs and other chemicals for specific purposes; c. modelling of biochemical, physiological, pharmacological, toxicological and behavioural systems and processes.
4. The use of 'lower' organisms' not protected by legislation controlling animal experiments, including invertebrates, plants and microorganisms, e.g. Limulus in pyrogenicity testing and bacteria in genotoxicity testing.
5. The use of the early developmental stages of vertebrates before they become protected animals. In the case of the British 1986 Act, this is before half-way through gestation (mammalsl or incubation (birds and reptiles), or the stage when independent feeding occurs (amphibians and fish), e.g. early chicken embryos in reproductive toxicity tests.
6. The use of in vitIO methods, including sub-cellular fractions, short-term maintenance of tissue slices, cell suspensions and perfused organs, and tissue culture proper (cell and organotypic culture), including human tissue culture.

7. Human studies, including the use of human volunteers, post-marketing surveillance and epidemiology, e.g. skin patch testing in humans before marketing and monitoring consumer response after marketing, as alternatives to the animal testing of cosmetic products.

Animal use in testing and biomedical research

The use of animals in the basic science of toxicology is, on the whole, similar to animal use in other kinds of fundamental biomedical research. Toxicity testing, however, represents a special case, for two main reasons. Firstly, as practised, the induction of adverse effects, and even of considerable suffering, is often integral to the procedure and is therefore unavoidable. Secondly, the application of such procedures is often required lor at least, perceived to be required) by national and/or international legislation and/or regulatory guidelines. Thus, the application of the Three Rs principles to toxicity testing, as is required by other laws, represents a considerable difficulty for all concerned, be they politicians, regulators, toxicologists, lawyers, or scientists committed to the development of relevant and reliable nonanimal tests.

Toxicity testing is also special in otherways. The maintenance of the status quo is backed by enormous vested interestsin the regulatory authorities, in industry, in academia, and in contract testing establishments. In addition, the weak scientific basis of many current practices in the dominant 'check-list' approach to testing has repeatedly been questioned, not only by animal welfarists, but also by toxicologists themselves, both independently[14-17] and through group discussions such as those of the FRAME Toxicity Committee.[12] That the present unsatisfactory situation is tolerated and progress on scientific grounds, let alone in terms of animal welfare, is so difficult to achieve, testifies to the power and pervasive influence of the toxicity testing industry and its stout defence of the status quo, which is based partly on inertia and partly on self-interest.

Refinement Reduction, and Replacement of Animal Use for Regulatory Testing

Most national laws require that data from animal experiments be produced before a new medical product or device can be permitted for use in humans or animals. This is to protect from possible harmful effects of both new and unknown medicines and surgical devices. Biomedical researchers have therefore taken it upon themselves to develop a code of ethics that achieves their scientific objectives, while at the same time minimizing animal discomfort through humane procedures and animal care reducing the numbers of animals they use, and

in some cases not using animals for their research at all. In a nutshell, this is the philosophy behind the 3Rs of Russell and Burch (the Rs standing for Refinement, Reduction, and Replacement).

There are many hurdles that impede the widespread implementation of the philosophy, including the same national legislations that mandate the use of animals in the first place. Laws and requirements for submission of documents to register new chemical entities (NCEs) are already harmonised and centralised for the European Union through the European Medicines Agency (EMEA), but not in many other countries. In order to register NCEs in multiple countries, it may be necessary to conduct repeat studies that comply with the local laws of that country, thus unnecessarily repeating the same studies. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines may eliminate duplication of tests by mutual acceptance of research data in different countries. ICH guidelines and EMEA can therefore be a major contributor to the reduction of the number of animals used for regulatory compliance.[18, 19]

A critical and positive discussion of animal welfare is an aid to the progress of biomedical research. The issues that affect research animals are best known to scientists, funding agencies, and institutional policy makers. Knowledge of and implementation of the 3Rs can be a proactive response to animal welfare by the primary users of research animals. Therefore, leadership in this area rightly belongs to scientists, animal technicians, veterinarians, and managers of research institutions and less to politicians or militant animal activists. The remainder of this article will attempt to provide a brief overview of the general meaning of the 3Rs with the specific objective of disseminating information on animal welfare and inviting comments and contributions on the subject from readers.

Refinement is not just the improvement of particular experimental techniques. It includes thorough knowledge of adverse experimental effects of research protocols and the safeguards to minimize animal discomfort through the implementation of early endpoints and the availability of experienced veterinarians and animal care staff. Where animals may experience pain, especially after a surgical procedure, a refinement of the procedure will require the administration of adequate anaesthesia and appropriate analgesia as well as humane euthanasia of research animals to avoid pain and suffering.[20, 21] Refinement also includes good experimental design and the proper use of biostatistics.

Several recommendations for implementation of 3Rs methods are provided above. It should be stressed that these recommendations include not only bench-related activities but also, and perhaps even more important, the following activities at the regulatory level: full implementation of good laboratory animal science principles in test guidelines, the harmonization of test guidelines, and a critical analysis of the retesting policy. The investment in time and human resources might be minimal compared with the development and validation of 3Rs methods, and the effect might be many-fold. However, the real breakthrough in terms of animal reduction will be possible only after adoption of the consistency approach. This quality control approach makes us less dependent on the use of animals and therefore paves the way for a complete replacement.

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