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Refinement in Laboratory Animal Science: Is it a Cinderella subject, and is there conflict and imbalance within the 3 Rs?

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Introduction

In Europe, and particularly within the UK, more and more people question the use of animals for food, safety testing and hunting. Indeed, some even consider the use of animals in biomedical research to be an abuse of power. Most people, however, have a more balanced view regarding the use of animals to increase scientific knowledge and to counter important diseases.

Russel & Burch's (1) concept of the 3 Rs (Replacement, Reduction and Refinement) has had a profound impact upon the manner in which the use of laboratory animals has developed. The extent to which it has been assimilated into subsequent legislation on the subject clearly demonstrates the significance of this visionary publication.

Although it concentrates on Refinement, this paper also discusses the complex interrelationships between the 3 Rs. It will not consider issues such as the refinement of experimental design to use fewer animals, or the complete replacement of animals with non-sentient material.

Replacement is an easy concept to deal with. Although it will never be possible to completely avoid the use of animals in biomedical research, replacement of animals in many assays, tests and other contexts has been succesfully accomplished.

Reduction of the number of laboratory animals used for experimental procedures might be seen as a logical consequence of Replacement. However, great care must be exercised if Reduction is seen as the overriding principle. For instance, it is vital that experimental group sizes are kept at a level which guarantees statistical confidence in the final results. All that Reduction below such levels achieves is to render worthless any compromise that might have occurred to the test animals' welfare.

We now come to an area of potential conflict between Reduction and Refinement. The main thrust of recent European legislation is the emphasis on lowering "the pain, suffering, distress and lasting harm" experienced by individual animals. This is very much the case with the UK's Animals (Scientific Procedures) Act 1986, and is a very clear statement of the principles of Refinement. However, the inevitable and inexorable political pressure is on reduction in the annual statistics of animals used in biomedical research. One only has to witness the outcry that is generated if a particular year's statistical return fails to show a significant reduction in animal useage, to realise which part of the 3 Rs that those outside the field consider to be paramount. Considering that the public's primary access to information on this subject is through the annual returns, this emphasis is only to be expected. Whilst Reduction and Refinement are not mutually exclusive, some care has to be exercised if they are to be mutually inclusive. A fundamental question is whether it is more acceptable to put 100 animals through a mild procedure, or to put 10 animals through a substantial procedure, in order to obtain the same data. The authors' "Refinementist" answer to this dilemma runs, in terms of the numbers of animals used, quite contrary to the current poli-

tical imperative. It also requires some clarification and debate, if it is to be fully explained to the general public. We, thus, find it to be of critical importance that Reduction does not compromise the standards of welfare of those animals which continue to be used. A reduction in animal numbers will be consistent with our aims to increase laboratory animal welfare, only if experimental procedures are constantly reviewed and refined.

Adequate knowledge of laboratory animal science and welfare amongst all those who work with laboratory animals is therefore a prerequisite in order to ensure the humane and ethical use of animals. A recent editorial in the Lancet advocated a more compassionate approach to animals in biomedical research, and stated that "an important aspect of the way ahead is to educate not only medical researchers but also anti-vivisectionists concerning the needs, particularly the social needs, of (experimental) animals" (2).

The remainder of this paper will consider the current status of various aspects of laboratory animal science which make direct contributions to Refinement.

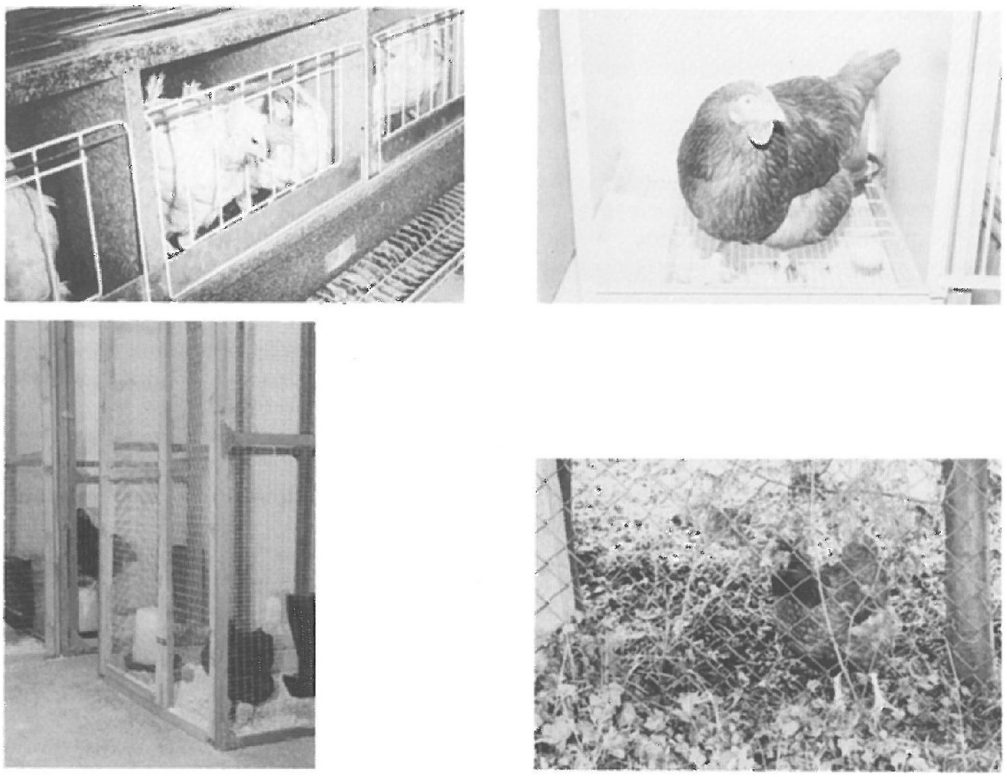
Environmental Enrichment as Refinement

In recent years considerable attention has been paid to the care and husbandry of laboratory animals, in order to ensure that they are treated as humanely as possible. Environmental enrichment may very well be regarded as Refinement. It is now generally accepted that the provision of an environment which allows the animal to exhibit a broad repertoire of characteristic behavioural activities, increases that animal's welfare (3, 4). The word "humanely" should be a keyword in the manner in which we treat experimental animals. It implies that the aim is to treat animals under our care, as considerately as we would treat a fellow human being who is subjected to an identical degree of discomfort and pain. However, the danger of this approach is that it can lead to anthropomorphism which should always be avoided. It seems widely accepted that the objective assessment of animal welfare cannot be decided by a single approach (5). Physiological measures, as well as behavioural studies, must be made within a defined environmental (6). Both approaches, however, rely on a detailed knowledge of the biology and behaviour of the individual species in question.

Due to the limited knowledge of how animals perceive their own situation within a given environment, it is extremely difficult to determine whether one procedure or husbandry system is the most appropriate with regard to animal welfare. This is particularly true when comparing different housing systems for laboratory animals, as these systems often limit the exhibition of obvious signs of stress and deprivation. This contrasts with the high mortality and high incidence of fractured bones, which can be seen in farm animal husbandry systems such as battery cages and sow tethers. The current debate on the optimal housing system for laboratory rabbits is a good example of continuing efforts to improve the welfare of animals which are often housed singly in cages, over a long period. Since it is difficult to assess stress in animals housed in cages, and since it is generally agreed that environmental enrichment is beneficial to animals, it has become common to compare husbandry systems for animals in captivity with the way in which animals choose to live in the wild.

Husbandry and Experimental Procedures

The maintenance of good animal health is probably the most important single component of welfare and increasing resources are being allocated to monitoring the health status of laboratory animals. It is very important to strike the right balance between environmental enrichment and maintaining good standards of hygiene whilst, at the same time, achieving rational working routines for the animal technicians. It is also important to remember that environmental stresses may not be recognised by the animal care personnel. A good example of this is ultrasound, which can be emitted from a wide variety of



Chickens are widely used as laboratory animals. The housing of chickens has been the subject of intensive debate, and this is an area where environmental enrichment is a welcome refinement. The figure shows battery chickens as they can be found in industrial egg production in many countries, a chicken housed singly in a rabbit cage, an enriched pen system for singly housed chickens and a chicken housed out-doors in semi-natural conditions.

sources including water taps, computers and plant machinery (7).

Refinement is also relevant in relation to the handling of animals. Physical restraint of conscious animals should always be performed as calmly and gently as possible. Time and effort should be given to gentling all animals that are to be used in experiments involving handling and restraint, and to train animals in order to obtain willing co-operation in place of the need for physical restraint. This is now becoming widely recognised, not least in commercial facilities. For instance, in a large Swedish pharmaceutical company the animal technicians now spend much of their working day socialising, training and conditioning the animals, which are predominantly dogs.

Refinement can, and should, also be applied to routine experimental procedures such as injections and blood sampling, ensuring that animal fear, anxiety and discomfort is minimised. Again, a major problem (and future research area) is the assessment and comparison of the stress associated with, say, restraint and blood sampling, using different collection techniques. Initiatives have been made to assess the severity of different procedures

(8, 9), but much work remains to be done. *Caroline Manser's* excellent book (10), compiling and reviewing the literature on assessment of stress in laboratory animals, is highly recommended and should be read by all those active in this field.

Imaging and non-invasive monitoring of parameters such as blood pressure and foetal development is rapidly replacing the traditional methods, particularly in larger species being used in long-term experiments.

One of the products of the project licensing system that exists under the UK's Animals (Scientific Procedures) Act 1986 is the establishment of a series of humane endpoints and severity limits for each experimental procedure, prior to the start of the research. These allow rational and objective decisions to be made regarding animal welfare, once the work is in progress. Wherever possible, long-lasting experiments should be replaced by acute experiments or a series of acute experiments, and studies should be terminated as soon as the humane endpoints are reached. This may very well result in the need for greater numbers of animals in a given project. So, once again, there is the paradox that Refinement may lead to an increase in the number of animals used in biomedical research. We would argue that this is justifiable since the net result is an improvement in the welfare of individual animals.

Selection of Species

Darwin (11) has had a marked influence on present day society's view of living creatures. Ascending the evolutionary tree, we ascribe greater value to the individuals of different species, culminating in man himself. Apart from the economic value of the individual animal, we are far more concerned with the well-being of a diary cow than we are with the well-being of a honey bee. This view is reflected in the legislation which protects animals, with most of that concerning laboratory animals predominately covering the vertebrate species.

It is, therefore, logical that the choice of which species to use in an experiment is part of the process of Refinement. If, for instance, an ecotoxicological test can be conducted using *Daphnia* instead of a fish species there can be no doubt that this would achieve Refinement, as well as Reduction and Replacement. It becomes more difficult when the choice of alternative species is limited to mammals. The decision as to whether a rat is of a higher ranking to a cat is inevitably influenced by emotional pre-conceptions regarding biological characteristics and the traditional role that a particular species has played in human society. Companion animal species, such as dogs, cats and horses, are used more reluctantly (and are usually afforded greater legislative protection) than farm animal species, such as sheep, goats and pigs (although it will be interesting to see if the public's attitude towards the last of these species changes, given the current vogue for keeping mini-pigs as pets). Equally rodents, which are still being killed in huge numbers as vermin, are more readily used than, say, ferrets and rabbits.

Life expectancy and fecundity can also be considered as relevant characteristics when selecting species. An animal species with a short life-expectancy, which is invariably associated with high fecundity (e. g. the housefly) is described as having an "r" selection strategy and is said to be of lower biological significance than a larger, less fecund (the so-called "K" selection) species (e.g. the elephant). "K" selection species are characterised by slow development from young to adult, greater competitive ability, delayed reproduction and larger body size (see *Krebs*, 1972 (12) for further details). In general mammals are considered to be "K" selection species. However, within the Class it can be seen that small rodents represent "r" selection, whereas primates and dogs represent "K" selection.

Anaesthesia, Analgesia, Surgery and Euthanasia

One of the most rapidly developing areas in veterinary science, in recent years, has been the field of anaesthesiology. There are now a wide range of agents available which can produce surgical anasthesia in laboratory species, with minimal side-effects. The concept of balanced anaesthesia, where different combinations of drugs are used to produce the desired effect, represents real progress in Refinement, especially when compared to classical agents such as ether and pentobarbitone. The advent of reversible anaesthetics has been yet another significant improvement, particularly when the reversing agent is a partial agonist (such as buprenorphine) and has an analgesic action of its own, in addition to reversing the original anaesthetic. One challenge that, perhaps, remains is to overcome the innate conservatism of some research workers by educating them about these excellent new techniques, and encouraging their use.

As with anaesthetics, a number of powerful new analgesics have become available recently, particularly in the non-steroidal anti-inflammatory group. Even more significantly, there has been considerable (and belated) recognition of the importance of post-operative analgesia, and its use has, quite properly, become more widespread. The discovery of the phenomenon of central sensitisation to pain has had an important impact on the timing of administration of analgesics, with the best results being seen when analgesics are administered either as part of the premedicant, or prior to recovery from surgical anaesthesia. It would be fair to say that laboratory animal science has provided the lead to conventional veterinary practice in this area.

The move towards a much higher standard of aseptic surgical technique, even in the rodent species, is clearly a Refinement which is to be welcomed. It is, however, an area of some controversy, and some research workers require active education and encouragement in the maintenance of standards.

There has been increased awareness of the importance of the use of euthanasia techniques which are humane, and which cause minimal stress of animals.

Toxicology and Safety Testing

Toxicity testing of substances accounts for approximately 20 % of animal experiments within the UK and is an area which usually attracts severe criticism from anti-vivisectionists. Progress in Refinement of the methods employed in safety testing tends to be considerably slower than in other disciplines. This is primarily because the test protocols are statutory, legislative requirements and approval has to be granted by the regulatory bodies in many commercially important countries, before they can be amended. An example of the progress that has been made is the "fixed dose procedure" which has been developed as an alternative to the LD50 test and which does not have death as the endpoint (13). It has been developed, through international collaboration, by the British Toxicology Society and the EC and OECD subsidised its international evaluation.

Another excellent example is to be found in the ECETOC monograph (14) on the OECD guidelines regarding the use of a low volume eye irritation assay, and the use of a single anaesthetised animal if the test substance is suspected of having severely irritant properties. Both of these procedures are meant to replace the controversial Draize eye test. Other tests are also being modified to use earlier endpoints than death, which are based on clinical and physiological criteria. In the separate area of studies into learning, aversive stimuli such as electric shocks should be avoided and, wherever possible, food reward systems introduced in their place.

Production of Antisera

Both the production of mono- and polyclonal antibodies use techniques which result in

some degree of suffering for the animals involved. A particular problem with polyclonal antibody production is the continuing use of both the aggressive Freund's Complete Adjuvant, and inappropriate injection sites (15). Welfare problems are seen in the production of monoclonal antibodies in association with the use of the carcinogenic primer pristane, with the production of large volumes of ascites fluid and with multiple tappings from the same animal. One approach to the Refinement of these techniques has been the creation of very strict guidelines on issues such as numbers and sites of injection and the frequency with which individual animals are used in the procedure. There has also been the very encouraging development of an *in vitro* production method for monoclonal antibodies. Once again though, Refinement, such as the use of milder adjuvants, may very well result in an increase in the number of animals that have to be used in order to produce the same quantity of antibody.

Refinement of Animal Models

The development of induced and spontaneous animal models of disease is a vast area and is one where particular efforts are being made to apply the 3 Rs. Space does not allow for further discussion of this general observation. However, the exponential growth in the field of transgenics does have distinct implications for animal welfare. Given the fact that the expression of foreign genes is, by its very nature, highly unpredictable, there must be a high probability that some transgenic strains will suffer some inherent compromise to their welfare. This only serves to illustrate the need both for objective assessment methods for pain, stress and compromised welfare, and for particular vigilance amongst all animal care staff when looking after transgenic animals.

Education and Welfare Monitoring

Another area that has received increasing attention and prominence recently, is that of training. One very effective means of achieving Refinement is to ensure that all those who work with laboratory animals, be they animal technicians, veterinarians or research workers, possess the knowledge and skills which are required to maintain the highest possible standards of animal welfare.

Having done this, there is a definite requirement for some systems for monitoring animal welfare to ensure that the necessary standards continue to be maintained. Everyone accepts the existence of systems to monitor environmental conditions within the animal house. Unfortunately, welfare monitoring is less amenable to automation and computerisation. However, management systems must be put in place if we are to be seen to be doing the best for the animals that are under our care. It is a cynical fact of life that, in these increasingly hostile times, providing the very best standards of care is not enough on its own. We must be able to demonstrate, to a sceptical public, exactly what steps we take to ensure the welfare of our laboratory animals.

Conclusions

Considerable emphasis has been placed on both Reduction and Replacement. It could, perhaps, be argued that these have received greater publicity, particularly in the public arena, than the efforts that have been made towards Refinement.

Although very important, both Reduction and Replacement can be viewed as medium- to long-term strategies. It is quite unlikely that they will ever be so successful that they will completely eliminate the requirement for the use of animals in biomedical research. Therefore, we strongly believe that, for all those who work in laboratory animal science, the daily imperative lies with Refinement as a focus of our efforts towards the 3 Rs. It is the emphasis on this area which offers the best prospect of immediate improvements in the welfare of the laboratory animals in our care.

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