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The 3Rs – Past, Present and Future

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Introduction

This paper offers a personal account of how the concepts of replacement, reduction and refinement can be traced through a brief history of the use of animals in science. It considers how they are reflected in current intent and practice, and speculates about how they will influence animal use and progress in the biological sciences in the future.

Russell and Burch's book "The Principles of Humane Experimental Technique" , first published in 1959, is widely recognised as a landmark in thinking about the care and use of animals for experimental and other scientific purposes. In addition to describing what we now refer to as the '3Rs', the authors champion the case that more humane animal care and use promotes better science. The book also draws attention both to the 'direct' welfare costs of the procedures applied to the animals, and the 'contingent' costs relating to factors such as housing and care. 'Suffering', the 'cost' of using animals in science, is equated both to the infliction of that which is unpleasant, and the denial of that which is pleasurable.

These concepts shape the thinking of the current generation of animal care staff, scientists and regulators.

Russell and Burch defined:

- *Replacement* as "the substitution for conscious living higher animals of insentient material". Tissue culture, computer modelling and the use of invertebrate species are classical examples.
- *Reduction* as using the minimum number of animals necessary "to obtain information of given amount and precision". They stressed that the imperative was to use the right number rather than too few or too many.
- *Refinement* as any decrease in the in the nature, severity or incidence of inhumane procedures to those animals which still have to be used.

The Past

We now regard it as self-evident that, like man, many classes of animal are capable of experiencing pain, suffering, distress or lasting harm. That animals are capable of such suffering is a relatively recent concept: indeed there are still some parts of the world where editorial policy acknowledges that animals may 'experience pain and distress', but is loath to acknowledge that they 'suffer' as a result.

The first legislation in the United Kingdom that afforded protection to animals based upon the principle that they were capable of suffering was the Cruelty to Animals Act 1876. All previous legislation in the United Kingdom relating to animals dealt with them only as goods or property.

We now look back at 'classical' observations of gross physiological phenomena made using methods that seem barbaric today – particularly so when the prevailing scientific culture required that individuals base their knowledge on studying the original descriptions and repeating and witnessing the 'classical' observations for themselves.

Much of the pioneering work on the dynamics of the circulation, including that of John Hunter, was performed before the advent of general anaesthesia and used readily available, domesticated, higher species such as dogs and horses.

In 1769 the Reverent Stephen Hales recorded in his journal:

> "In December I caused a mare to be tied down alive on her back. Having laid open the left crural artery about three

> inches from her belly, I inserted into it a brass pipe…..I fixed a glass tube…the blood rose in the tube eight feet three inches perpendicular above the level of the left ventricle…it would rise and fall after each pulse…"

He was repeating an experiment, for his own education, he knew to have been first performed in 1732. Barbaric by our standards, not permissible under current regulatory regimens, but in tune with the culture of scientific curiosity fulfilled by personal observation that prevailed at that time.

Early contributions to science tended to be largely curiosity driven, empirical, essentially descriptive and aimed more at generating knowledge than solving practical problems. They describing natural (or unnatural) events and speculated about the underlying mechanisms.

As time passed, as knowledge accumulated, the scientific method progressed and evolved to seeking to understand the mechanisms that underpin the phenomena observed and described, and to generate insights and knowledge with practical applications.

Deductive science, based upon the ability to formulate and test hypothesis, has come to the fore. The publication and wide availability of experimental data has become the norm. The technologies developed and applied to investigating biological phenomena have multiplied. Powerful insights into the biological sciences, and the scope and limitations of animal models, have been gained.

From the beginning, progress with the 3Rs has always been driven as much or more by a desire to perform better science than by a desire to reduce the welfare cost.

Development of *replacement* alternatives still depends on having a sufficient understanding of the mechanisms of interest to be able to produce non-sentient models reproducing or mimicking key aspects of the systems of interest (often based upon a large body of animal test data) and/or the technology to develop and exploit non-sentient models. In addition to replacing animal use, such advances can also make possible areas of investigation that could not previously be taken forward in classical animal model systems.

Reduction strategies often herald or reflect improvements in experimental design, such as improved statistical methods or the availability of better-defined experimental subjects.

It is a cause for regret that many people still judge progress solely by the numbers of animals used in science. Such judgements fail to give proper regard to the considerable progress and welfare gains that are brought about by refinement.

Refinement takes place continuously on a number of fronts. At times refinement has mirrored progress made in clinical practice: the availability and use of anaesthetics, analgesics, antibiotics and diagnostic imaging are examples. Use of the readily available, higher domesticated species familiar to John Hunter and Claude Bernard has given way to the use of purpose bred rodents of well-defined genetic and microbiological status as the most common laboratory animal. The development of laboratory animal science, and improved conditions of housing and care, particularly in the last 25 years, have been good for animal welfare and for science. The development and application of more sophisticated non-invasive/minimally invasive imaging techniques and telemetric methods of gathering physiological data from unstressed subjects rather than patho-physiological data from stressed or clinically disturbed animals are setting the scene for further refinements by improving the data-stream and identifying humane end-points that precede clinical signs.

The Present

Most societies now regulate the use of animals in science. National regulatory systems vary, but in general they evoke the spirit and principles of the 3Rs and humane research, in addition to implementing various international obligations.

While it is to be hoped that international regulators champion good science and good welfare, it is worth remembering that these are not necessarily their primary considerations.

Throughout the European Union, the welfare of animals used in science is safeguarded by Directive 86/609/EEC "on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of

animals used for experimental and other scientific purposes". Its provisions require that the 3Rs are implemented. The Directive must be enforced by national legislation. However, the primary purpose of the Union and the Directive is to eliminate disparities in national law that may affect the functioning of a common market.

The Council of Europe's role is to promote common cultural and social values, rather than to provide a level economic playing field. Council of Europe Convention ETS123 "on the protection of vertebrate of vertebrate animals used for experimental and other scientific purposes" recognises the moral obligation to ensure animal use is justified and humane. However, at the time of writing, only ten of the 41 member states have signed, ratified and implemented the Convention.

Scientific evidence and advice influence Government policy and underpin important public services. Science is facilitating current moves towards 'evidence based healthcare' and 'clinical governance'. Yet at the same time it is acknowledged that our understanding of many scientific issues is derived from assumptions based upon current but ambiguous or incomplete knowledge. Scientific 'fact' deals with probabilities and opinion rather than facts and certainties – and opinions and advice may need to be revised as more evidence comes to light.

It should be possible to construct a case for contemporary animal based research being of the highest quality and fully implementing the 3Rs. It is required both by national and international regulatory frameworks. It is supported and facilitated by enlightened animal care, scientific and regulatory communities capitalising on the progress already made in science, technology and animal welfare. By regulation, or by other means, some form of ethical analysis or ethical review plays a role in the planning and/or conduct of most animal care and use. Funding agencies should be able to claim that they support and fund only the best, the most important, and the most humane and refined research.

But is this the case?

The Challenges

I would argue that we should not be complacent: there are problems in practice and there is still much to do.

The 3Rs: Integration and Balance

The 3Rs must be applied with the intention of minimising the potential for suffering. Minimising suffering, not just the numbers or animals used, is the key to humane animal use. To do this it is necessary to know what is 'meaningful to the animal'. We will never have the technology or the insights to know what animals actually 'experience'.

Designing and performing the most refined science requires that replacement, reduction and refinement strategies are all considered. Potentially conflicting costs, opportunities and priorities must be acknowledged and resolved. It is a mistake to focus on any one of the 3Rs if the others are unintentionally compromised as a result.

It is important that, as well as independent advances being made on all fronts, that means and resources must be found to identify, prioritise and tackle at an international level specific areas where essential *in vivo* animal-based work can still only be undertaken at a high welfare cost. Vaccine testing would seem to an area ripe for such scrutiny.

Some strategies that focus on reducing the numbers of animal used 'at all costs' have the potential for harm rather than good. It is indefensible to reduce the numbers to be used if the total animal suffering will increase as a result. Examples where this can be the case include the ability to meet objectives by using a small number of a higher species rather than a larger number of lower species, using fewer animals but subjecting them to more aggressive interventions or applying less humane endpoints, or risking using too few animals to draw meaningful conclusions thus requiring that studies be repeated.

Directive 86/609/EEC mandates that the animals used be, amongst other things, of the "lowest neurophysiological sensitivity". This is generally interpreted as meaning the species and stage of development having the least capacity to suffer. Although there might be general agreement about gross inter-species differences (most would acknowledge that non-human primates would be

ranked above fish), there is no established evidence-based rank order of species by capacity to suffer, and there will always be scope to debate the rank-order of animals within classes or species groups.

Measuring Welfare

Because we cannot be certain 'what is meaningful to the animal' it can be difficult at times to be sure that 'refinements' that are intuitively welfare friendly do indeed reduce the suffering experienced by, or otherwise improve the quality of life of, the experimental subjects. Or of animals bred or kept for use in science.

Drawing meaningful conclusions about animal welfare requires much more than consideration of crude morbidity and mortality data. A number of biochemical and behavioural measures, severity scoring systems and disturbance indices have been published and are in use. These allow changes from the normal range to be detected and in some circumstances conclusions to be drawn about the effects of the sum total of numerous and often unrelated factors affecting the animal.

Our current state of knowledge makes it possible to identify broad classes of, and gross changes to, welfare status, but I believe they are not adequate for detecting subtle changes or necessarily what is 'meaningful to the animal'. Without such knowledge there can be dangers in relying on the current measures and 'critical anthropomorphism' when trying to draw conclusions about true welfare costs, and developing or evaluating refinement strategies.

Duplication

Although we might wonder at the desire of earlier scientists to reproduce and observe previously described phenomena, studies are still repeated. Sometimes with good reason – sometimes not.

It is generally expected that significant original findings should be independently reproduced at other centres before they are accepted as scientific fact.

Two widely known, contrasting examples can be cited to illustrate and support this view. An extensively cited publication on the production of transgenic animals following the incubation of sperm with free-DNA was subsequently retracted when the findings could not be reproduced by other laboratories (or, subsequently, by the laboratory that published the original funding). There was some speculation that the reported cloning a mammal by nucleus transfer from an adult cell was due to some form of artefact – until other laboratories independently reproduced the findings.

Work is often repeated for other defensible reasons. For example when beginning for the first time to use an established model system a case can be made for pilot studies to show that the essential phenomena can be reproduced. When work in progress is relocated to new facilities, or if work has been in abeyance for some time, a case can be made for some studies being repeated to confirm that no unrecognised significant variable has been introduced that disrupts the continuity of the data-stream.

However, regulatory toxicology and safety testing can in some circumstances require that more animal test data be generated than might be considered by some to be strictly necessary. Notwithstanding the data-sharing provisions of various European Directives there are still significant obstacles to the sharing of data among and between regulators and manufacturers. The situation is further complicated by regional or national differences in test requirements and obstacles to the mutual recognition of data requiring supplementary testing to satisfy the different regulators. When data sharing is facilitated, test requirements harmonised and mutual recognition of data and regulatory decisions are properly developed, our current practices may appear, in retrospect, to be as logical and defensible as some of the repeated demonstrations performed by earlier scientists.

Validation

Validating 'alternative tests' is fraught with difficulties.

One recurrent difficulty with validation is the inherent variability in, and at times the unreliability of, some of the *in vivo* tests that 'alternatives' are expected to reproduce. An *in vitro* test that has greater specificity or sensitivity

may appear to give results at variance with the established *in vivo* method. This inability to strictly reproduce the results of traditional *in vivo* test methods can delay the acceptance of more refined and more accurate *in vitro* test methods.

The scientific validation and regulatory acceptance of replacement alternative and more refined methods has proved difficult to achieve – but progress is now being made. OECD Guidelines 420, 423 and 425 are more refined than OECD Guideline 401. Unfortunately they are do not seem as yet to have become routinely or universally acceptable to all regulators of countries that are OECD members.

ECVAM and ICCVAM are to be congratulated with progress made in scientifically validating other replacement and refinement alternatives: *in vitro* tests for phototoxic potential and skin corrosion should shortly be added to Annex V clearing they way for their becoming the preferred tests methods expected by European regulators.

The European Union needs to signal clearly to National Competent Authorities and regulators when more refinement test methods have been deemed to be both scientifically validated and appropriate, in truth preferred or required, for regulatory toxicology and safety testing.

Other Factors

Even when the conservatism of those involved with regulatory assessments is not a consideration a number of other factors hamper the introduction of more refined and humane test methods.

Timeliness of 3R Considerations

The intense competition for available funding and resources should ensure that only the best science is funded.

The importance and quality of the science and the likelihood of success of the proposed research strategy influence funding decisions. Some funding agencies may not ask or require that the methodology to be applied is the most refined, and those that do may not take an interest in the detailed protocols and humane endpoints to be applied. The Home Office is working with the main UK funding agencies to ensure that they better ensure that they demonstrably fund the best, and the most refined and humane, science.

Timely advice on the 3Rs can improve both science and welfare. Bids for funding are generally made only after the general research strategy and the model systems to be used have been determined in principle. After funding is secured there can be a reluctance by scientists, regardless of their levels of experience or seniority, to acknowledge that they might either consider reviewing their preferred strategy or benefit from expert advice of specialist colleagues including laboratory animal scientists, animal care staff and veterinary surgeons, information services and those able to advise of various aspects of study design. At present such advice is either not always sought or taken, or the advice is offered too late in the process to determine how the work is actually performed. In the United Kingdom the introduction of local ethical review processes is seen as one means to tackle this problem.

Access to Information

Access to a wealth of information in the literature and IT databases has never been easier. In practice this can make it remarkably difficult to find the best and most up to date information on the 3Rs.

The literature is vast. Much of the relevant material in the general and specialist scientific literature, having its origins in improved methodology and better science, will not be found by searching using 'alternatives' as a keyword. Journals that focus on alternatives may not be known to, easily available to, or regularly referenced by scientists. The situation is complicated by the fact that in some cases the primary objective is to publish the new science, with less priority given to publishing improved, refined methodology.

It can difficult to quality assure much of the information distributed over the world-wide-web.

Custom and Practice

'Custom and practice', can be obstacles to change: if you are successful and productive, why change systems that are already known to work?

There is now only very limited justification for the production of monoclonal antibodies in rodents

using the ascites method. In the United Kingdom exceptional justification must be given on a case by case basis and failure to produce the required product after reasonable *in vitro* attempts have failed is an essential requirement. Nevertheless there is still sufficient demand for *in vivo* produced material for a search of the world-wide-web to reveal many organisations in other countries willing to undertake *in vivo* production on contract without having exhausted *in vitro* methods.

In addition to signifying reluctance by some to embrace more refined methods, this example also exemplifies how relying on regulation to impose the 3Rs may simply displace less refined animal use to other countries rather than resulting in the more refined alternatives being adopted.

Analgesia and Anaesthesia

Although it is to be expected that best practice will always be followed with respect to the use of analgesics and anaesthetics, this should not be taken for granted.

The view that animals are not capable of suffering significant post-operative pain and discomfort is not tenable. Nevertheless there can still be some reluctance by some to routinely using post-operative analgesics lest by altering the mechanism of interest, or the animal's response, data may be compromised. Often no *a priori* justification for this belief is forthcoming – and concern is that the earlier data derived from animals not provided with proper analgesic cover may be suspect also has to be addressed.

It was disappointing to note that a recent monograph on retro-orbital bleeding of rodents included a paper describing the use of ether (a known irritant and stressor) as the general anaesthetic of choice.

Resource Implications

Reluctance to change to reasonably and practically available refined methods may at times be based upon resource implications.

Some more refined technologies may require changes to laboratories, the procurement of new equipment, and staff recruitment or retraining.

It is disappointing when discussing or negotiating proposals for new facilities to find the establishment's focus is often on accommodating existing (and sometimes historical) needs rather than planning for the future.

Accommodation and Care

Tremendous advances have been made with respect animal accommodation and care over the last 25 years and animal welfare and science have benefited as the result.

Science has benefited from standardisation of experimental subjects resulting from better control of genotype, environmental conditions and microbiological status.

However it needs to be remembered that current statutory requirements and guidelines for animal care and accommodation generally set only minimum, empirically derived standards. Although the Council of Europe is working towards producing revised standards it is unlikely that these will set out evidence-based optimum requirements.

Matching Production and Demand

'Progress' can throw up other problems.

Welfare-friendly systems for group-housing rabbits have increased the demand for female rabbits. This poses the question of whether demand for male animals will match production, and whether male animals can be kept in harmonious groups without resorting to chemical sedation or castration.

There may also be the potential for discrepancies between production and demand when there is a strong user preference of animals of one sex, or of very precise age or weight requirements. The maintenance breeding of little used colonies and multiple lines of genetically modified animals may also result in the production of a biological surplus.

These issues have still to be fully addressed.

Public and Political Support and Confidence

Public and political confidence and support are vital to building and sustaining a successful science base.

It is becoming clear that these can only be fostered by systems and practices that explain and justify why animal use is important and currently indispensable, when high standards of conduct and care are guaranteed, and the user community has a culture of care that encourages and supports the continuous development and implementation of more refined methods. Scientists must justify and explain what they want to do, why they want to do it, and how they are going to do it.

Public concerns about the rate of change in science, and the ethical issues raised by scientific advances (such as the 'disbenefit' that might be seen from the application of new knowledge or technologies: genetically modified food, cloning and xenotransplantation are topical examples), must also be acknowledged and addressed. Debate and resolution of some of these concerns are beyond the competence of any single government department, or any single national government.

The Future

I would like to think that when the progress made in the years to come is judged that we will be given credit for having tackled and resolved some of these challenges and problems.

Specifically I would hope the groundwork is already being done to reduce and minimise the welfare cost of animal-based research by:

- The scientific community, not its critics, remaining committed and being clearly seen to be at the forefront of developing and implementing 3R strategies.
- The 3Rs pervading and supporting the scientific culture from the 'concept' stage through the design and conduct of work to the review of completed work, influencing funding decisions, and being required, supported and facilitated by funding agencies and the establishments at which research is undertaken. Continuous improvement must be the objective.
- Developing and implementing true measures of welfare and disturbance that allow better informed conclusions to be drawn about what is meaningful to the animal, and underpinning better science and better welfare.
- Devising and implementing optimum, evidence based standards of animal accommodation and care.
- Rapid validation, acceptance and use of alternative methods.
- Effective data sharing, harmonised regulatory test requirements and mutual acceptance of data and assessments.
- Public confidence being maintained in the scientific community's commitment to well justified, refined, studies.

Summary

This paper sets offers a personal account of how the concepts of replacement, reduction and refinement can be traced through a brief history of the use of animals in science. It considers how the 3Rs are reflected in current intent and practice, and speculates about how they will influence animal use and progress in the biological sciences in the future.

The scientific community, not its critics, must always be, and be seen to be, at the forefront of developing and implementing 3R strategies. Good welfare is a pre-requisite for good science, and 'alternative methods' are generally driven by a need to improve the model systems used.

All of those involved with *in-vivo* animal research have moral and legal obligations to ensure that animal models are only used when

- they are scientifically valid,
- the benefits likely to accrue are judged to exceed the likely animal welfare cost,
- there are no available replacement alternatives, and
- the animal suffering likely to be caused has been minimised.

Public opinion and the political climate should not be hostile to the use of animals in science providing these principles are demonstrably applied and the scientific community offers sufficient justification for the use of animal models.

In practice a number of considerations have to be balanced to define the best 3R strategy. Research strategies and methods should be regularly

challenged, reviewed and revised as continuous progress is made with respect to replacement, reduction and refinement opportunities.

Whilst legislation can set the scene for refined science, real progress requires that funding bodies, the regulatory authorities, and the user community demonstrate their commitment to participating in the best and most humane science underpinned by an appropriate culture of care.

Commitment to the 3Rs is essential to a successful science-base:

- To maintain public and political confidence and support.
- To continuously raise welfare standards.
- To support and facilitate better science.

Our improving knowledge of animal welfare and laboratory animal science sets the scene for further progress. This knowledge also reinforces the general principle that good welfare is a prerequisite for good science.

Science tends to evolve from the empirical and descriptive to the mechanistic and problem solving. In the biological sciences this facilitates reduction, refinement and replacement strategies. Progress with 'alternative methods' should be science and welfare driven.

There are obstacles to the general and rapid introduction of alternative methods: they must be validated, accepted by the scientific community, and reasonably and practically available. Publishing details of more refined methodology is still seen by some as less important than publishing the new science.

Despite the progress that has been made with replacements, reduction and refinement there is still much to do. We should not be complacent.

Hopefully the stage is already set to identify and remove some of the barriers that still exist to the development, promulgation and implementation replacement, and reduction and refinement alternatives.

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