Replacement, Reduction and Refinement*

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Summary
In 1959, William Russell and Rex Burch published "The Principles of Humane Experimental Technique". They proposed that if animals were to be used in experiments, every effort should be made to Replace them with non-sentient alternatives, to Reduce to a minimum the number of animals used, and to Refine experiments which used animals so that they caused the minimum pain and distress. These guiding principles, the "3Rs" of animal research, were initially given little attention. Gradually, however, they have become established as essential considerations when animals are used in research. They have influenced new legislation aimed at controlling the use of experimental animals, and in the United Kingdom they have become formally incorporated into the Animal (Scientific) Procedures Act.
The three principles, of Replacement, Reduction and Refinement, have also proven to be an area of common ground for research workers who use animals, and those who oppose their use. Scientists, who accept the need to use animals in some experiments, would also agree that it would be preferable not to use animals. If animals were to be used, as few as possible should be used and they should experience a minimum of pain or distress. Many of those who oppose animal experimentation, would also agree that until animal experimentation is stopped,

Zusammenfassung: Replace/Ersetzen, Reduce/Reduzieren, Refine/Verminderung der Belastung
1959 veröffentlichten William Russell und Rex Burch das Buch "The Principles of Humane Experimental Technique". Sie schlugen vor, beim Einsatz von Tieren in Experimenten jede Möglichkeit zu nutzen, sie durch nicht-schmerzfähige Alternativen zu ersetzen, die Tierzahlen auf ein Minimum zu reduzieren und die Experimente in der Weise zu verfeinern, dass Schmerzen und Leiden auf ein Minimum reduziert werden. Diesen Leitlinien, dem 3R-Prinzip in der tierexperimentellen Forschung, wurde anfänglich wenig Aufmerksamkeit zuteil. Nach und nach wurden sie jedoch als wichtige Betrachtungsweise etabliert, wo immer Tiere in der Forschung verwendet werden. Sie haben die neuere Gesetzgebung zur Kontrolle von Tierversuchen beeinflusst, im Vereinigten Königreich wurde dieses Prinzip sogar formal in die Animal (Scientific) Procedures Act übernommen.
Das Prinzip Ersetzen, Reduzieren, Vermindern der Belastung, hat sich als gemeinsame Gesprächsbasis erwiesen für Wissenschaftler, die mit Tieren experimentieren, und Tierschützer, die dagegen sind. Wissenschaftler, die damit einverstanden sind, dass in einigen Experimenten Tiere nötig sind, stimmen gleichzeitig zu, dass es vorzuziehen wäre, wenn man keine bräuchte. Wenn Tiere verwendet werden, sollten es so

Russell and Burch's 3Rs provide a means to improve animal welfare. It has also been recognised that adoption of the 3Rs can improve the quality of science. Appropriately designed experiments that minimise variation, provide standardised optimum conditions of animal care and minimise unnecessary stress or pain, often yield better, more reliable data.

Despite the progress made as a result of attention to these principles, several major problems have been identified. When Replacing animals with alternative methods, it has often proven difficult to formally validate the alternative. This has proven a particular problem in regulatory toxicology, especially when combined with the labyrinthine processes of the various regulatory authorities.

The principle of Reduction would appear less contentious, but its application has highlighted the difficulties of providing appropriate expert statistical advice, especially in academic research facilities. In some instances, concern to implement Reduction strategies can result in the use of too few animals, which leads to inconclusive results, and wasteful experiments.

It is in the area of Refinement, however, that major problems have arisen. Much of our judgement of what represents Refinement is based on little more than common sense. We make assumptions about animals and their feelings that often have little scientific basis. In many instances we may be correct, but these assumptions may become incorporated into institutional or national policies, without any attempt to verify them. To give an example – it is reasonable to assume that animals will experience pain after a surgical procedure, so pain-relieving drugs should be given to prevent this. We have some idea of the appropriate dose of analgesics for most animals, but effective pain relief requires that the dose given is adjusted to meet the requirements of the individual animal. Requiring every animal to have the same dose of the same drug after any surgical procedure is not the best way of dealing with post-operative pain.

Discussion of these problems should not detract from the very significant progress that has been made in the 40 or so years since Russell and Burch set out their guiding principles. What is needed now is greater academic focus on this area, not only to work on new methods of implementing the 3Rs, but also to disseminate current "Best Practice", and to revise this advice as further progress is made.

wenige wie möglich sein, und sie sollten ein Minimum an Schmerzen und Leiden erfahren müssen. Auch viele Tierversuchsgegner stimmen dem Ansatz zu, dass bis zu einem Ende aller Tierversuche das Prinzip von Russell und Burch eine Möglichkeit bietet, Tierschutz zu praktizieren. Es wurde ebenfalls erkannt, dass das 3R-Prinzip die Qualität der Forschung steigern kann. Aus einem sorgfältigen Experimentaldesign, das die Variabilität minimiert, für optimal standardisierte Bedingungen bei der Tierpflege bürgt sowie unnötige Schmerzen und Leiden minimiert, entstehen oft zuverlässigere Versuchsergebnisse.

Trotz der Fortschritte, die es dank der Berücksichtigung des 3R-Prinzips gibt, müssen einige Probleme bedacht werden. Beim Ersatz von Tierversuchen durch Alternativmethoden hat es sich häufig als schwierig herausgestellt, die Alternativen formal zu validieren. Dies erwies sich als spezielles Problem bei den gesetzlich vorgeschriebenen Toxizitätsprüfungen, vor allem wenn man in das Dickicht der Regelungen verschiedener Behörden gerät.

Das Prinzip des Reduzierens ist dagegen unbestritten, doch hat seine Anwendung die Schwierigkeiten aufgezeigt, die es vor allem in der akademischen Forschung bei der Akzeptanz der richtigen statistischen Beratung gibt. In einigen Fällen kann das Bestreben, die Tierzahlen zu reduzieren, zum Einsatz von zu wenigen Tieren führen, was nicht bewertbaren und damit überflüssigen Tierversuchen gleichkommt.

Die grössten Probleme haben sich jedoch auf dem Gebiet des Refinement ergeben. Vieles von unserem Beurteilungsvermögen, was Refinement eigentlich bedeutet, beruht mehr oder weniger auf gesundem Menschenverstand. Wir stellen Vermutungen über Tiere und deren Gefühle an, die oft auf einer sehr geringen wissenschaftlichen Basis stehen. In manchen Fällen mögen wir richtig liegen, aber diese Annahmen werden dann in lokale oder nationale Regelungen aufgenommen, ohne dass auch nur der Versuch gemacht würde, sie zu verifizieren. Um ein Beispiel zu nennen: Es ist berechtigt anzunehmen, dass Tiere nach einem operativen Eingriff Schmerzen empfinden, also sollen schmerzstillende Medikamente gegeben werden, um dies zu verhindern. Wir haben zwar bei den meisten Tieren eine ungefähre Vorstellung von der nötigen Dosierung der Analgetika, aber eine wirksame Schmerzbekämpfung erfordert, dass die verabreichte Dosis individuell den Bedürfnissen eines jeden einzelnen Tieres angepasst wird. Zu fordern, dass jedes Tier die gleiche Dosis des gleichen Mittels nach jedem beliebigen chirurgischen Eingriff erhalten soll, ist nicht der beste Weg, mit postoperativen Schmerzen umzugehen.

Die Diskussion dieser Probleme soll den beträchtlichen Fortschritt nicht schmälern, der in den etwa 40 Jahren gemacht wurde, seit Russell und Burch ihre Leitlinien veröffentlichten. Wir brauchen nun mehr akademischen Fleiss auf diesem Gebiet. Es sollen nicht nur neue Methoden zur Verwirklichung des 3R-Prinzips erarbeitet werden, es müssen auch die zur Zeit besten Methoden eingeführt, aber auch wieder revidiert werden, wenn es weiteren Fortschritt zu verzeichnen gibt.

Keywords: principles of humane experimental techniques, replace, reduce, refine, animal welfare, statistical advice, analgesics

1 Introduction

The use of animals in biomedical research continues to be a cause of considerable public concern. Until recently, the scientific community has failed to treat this concern seriously and the result, in many countries, was the development of groups that were prepared to use extreme measures to try to prevent the use of animals in research. Results of public opinion polls indicate that the majority of the population would support some use of animal experimentation, but their approval falls when asked to support research that causes significant pain or distress. This concern is reflected in legislation currently in force in the European Union and in individual member states. It should also be an issue that is addressed by all scientists who use laboratory animals.

The use of animals in biomedical research began very early in the development of the biological sciences. The significance of some observations in animals was however not always recognised. Around 1540, Valerius Cordus described the synthesis of ether, and the Swiss alchemist, Paracelsus, noted "Even chickens will eat it, whereupon they sleep for a moderately long time and re-awake without being injured" (Leake, 1925). It was, however, another 300 years before ether was used as an anaesthetic in man and animals ref. Other use of animals did lead to significant advances, but animal use was very limited. During the 19th century, however, animal experimentation began to increase, as the new discipline of physiology developed. Initially, this involved surgical procedures in non-anaesthetised animals – since anaesthesia had not been developed at the time. Demonstrations of some of these experiments in the UK, which at the time was a common means of teaching the subject, led to a public outcry and the establishment of a Royal Commission to investigate animal experiments. In 1876, this led to the passing of the Cruelty to Animals Act, the first law that sought to control animal experimentation (Orlans, 1993).

It is easy to condemn the practices of investigators who were prepared to carry out surgical procedures without anaesthesia. It is important to note, however, that many still accepted the views of Descartes, who considered animals incapable of experiencing pain. Other philosophers, such as Bentham, disagreed with this, providing several generations of animal welfare organisations with a memorable quotation "The question is not, can they reason? Nor, can they talk? But, can they suffer?" (Bentham, 1789). Bentham's utilitarian philosophy sought to minimise harm, and included harm to animals in that concern. Subsequently numerous other views on this issue have been advanced, for example Singer's "speciesism" and the concepts of animal rights, and these are discussed elsewhere (Brom, 2002). Many scientists and other members of society have views that do not consciously follow from any rigorous philosophical analysis of the issues. For the majority, there seems to be a recognition that the use of animals in research, or any other use of animals by society needs to be justified. Although a small minority would take up extreme positions – either accepting no use of animals for any purpose, or allowing any use at all, these extreme views are uncommon. Between the extremes lies a very wide spread of positions on animal use by society. What many people share however, is a common ground that accepts that animals are worthy of our concern and that if we are to use them in research, for example, we should minimise the harms that we cause them. It also follows that we should use them only when absolutely necessary and use as few animals as possible. This widely held view of animals and their importance perhaps accounts for the current popularity of Russell and Burch's concept of Replacement, Reduction and Refinement (Russell and Burch, 1959).

2 The 3Rs concept

Like many new ideas, the initial publication, in 1959, of "The Principles of Humane Experimental Technique" attracted little attention. William Russell and Rex Burch proposed that if animals were to be used in experiments, every effort should be made to Replace them with non-sentient alternatives, to Reduce to a minimum the number of animals used, and to Refine experiments which used animals so that they caused the minimum pain and distress. Gradually, these principles have become established as essential considerations when animals are used in research. They have influenced new legislation aimed at controlling the use of experimental animals, and in the United Kingdom they have become formally incorporated into the Animal (Scientific) Procedures Act, 1986.

The three principles, of Replacement, Reduction and Refinement, have also proven to be an area of common ground for research workers who use animals and those who oppose their use. Scientists, who accept the need to use animals in some experiments, would also agree that it would be preferable not to use animals. If animals were to be used, as few as possible should be used and they should experience a minimum of pain or distress. Many of those who oppose animal experimentation would also agree that until animal experimentation is stopped, Russell and Burch's 3Rs provide a means to improve animal welfare. It has also been recognised that adoption of the 3Rs can improve the quality of science. Appropriately designed experiments that minimise variation, provide standardised optimum conditions of animal care and minimise unnecessary stress or pain, often yield better more reliable data. This concept of "Alternatives" was restated by Smyth (1978) as "All procedures which can completely replace the need for animal experiments, reduce the number of animals required, or diminish the amount of pain or distress suffered by animals in meeting the essential needs of man and other animals."

Despite the progress made as a result of attention to these principles, several major problems have been identified. When replacing animals with alternative methods, it has often proven difficult to formally validate the alternative. This has proven a particular problem in regulatory toxicology, especially when combined with the labyrinthine processes of the various regulatory authorities. Progress has been made, however, and there are

now realistic prospects for a further clear replacement of animals with *in vitro* alternatives in this area.

The principle of Reduction would appear less contentious, but its application has highlighted the difficulties of providing appropriate expert statistical advice, especially in academic research facilities. Analyses of publications in peer-reviewed scientific journals have repeatedly demonstrated the poor design of some of these studies (Festing, 1994). In some papers, too large a group size was selected, resulting in excessive animal use, where in others, *too few* animals were used, leading to inconclusive results, and wasteful experiments. Current opinion on this issue emphasises the need to select the *appropriate* number of animals for each specific study (Festing et al., 2002).

3 Refinement

It is the area of Refinement, however, that is perhaps causing the greatest controversy. When considering how to Refine experiments, so that they cause a minimum of pain and distress to animals, Russell and Burch identified two distinct areas of concern. They referred to "Contingent inhumanity" – pain and distress caused simply as a result of housing animals in a research laboratory, and "Direct inhumanity" – pain and distress caused as a direct result of research procedures. The concept of "contingent inhumanity" is useful since it emphasises the need to consider the entire life-span of a laboratory animal: how it is bred, transported, housed, cared for and handled, its general health and freedom from disease, and the method of euthanasia employed at the end of a study. Very considerable changes have been made in these areas – for example clinical disease problems have been virtually eliminated in many research animal facilities. We house animals in a carefully controlled environment, in well-constructed caging, with standardised bedding and standardised diets. This has virtually eliminated problems such as pododermatitis, ringtail and dietary deficiencies such as hypovitaminosis C in guinea pigs (Laber-Laird et al., 1996). The move to standardisation has also reduced the variability in research data obtained from our laboratory animals, leading to smaller group sizes being required to demonstrate biologically significant differences between experimental groups. The methods used to achieve this standardisation have been questioned however, specifically whether the standards result in improvements in animal welfare. One area of particular concern is the apparent barren environment of many laboratory animal cages. This has led to numerous attempts to introduce methods of environmental enrichment for a wide range of species (Baumans, 2000; Stauffacher, 2000; Fraser et al., 2000). It has now been recognised that enrichment strategies may change the biological characteristics of laboratory animals, and may also increase the variability of some parameters. Superficially, it would seem that we must decide whether to use more, but "happier" animals, or fewer that experience adverse effects from a barren environment.

4 Assessment of pain and distress in animals

This is just one example of the numerous controversies that arise in relation to refinement. Similar issues occur when dealing with "Direct inhumanity". Very considerable progress is being made in the introduction of more humane endpoints for experiments (Hendriksen and Morton, 1999). Use of analgesics to control pain is becoming more widespread. However, in all of these areas, our judgement of what represents Refinement is based on little more than common sense. We make assumptions about animals and their feelings that often have little scientific basis. This principle of analogy – assuming that animals will have similar experiences to humans, unless evidence to the contrary is available – is widely used (van Zutphen, 2002). It is often expressed as "When uncertain about whether a procedure could cause pain or distress, give the animal the benefit of the doubt". In many instances we may be correct and this approach encourages a concern for animal welfare. The problem is that these assumptions concerning an animal's likely experience of pain and distress may become incorporated into institutional or national policies, without any attempt to verify them. To give an example – it is reasonable to assume that animals will experience pain after a surgical procedure, so pain-relieving drugs should be given to prevent this (Flecknell, 2000). We have some idea of the appropriate dose of analgesics for most animals, but effective pain relief requires that the dose given is adjusted to meet the requirements of the individual animal. Requiring every animal to have the same dose of the same drug after any surgical procedure is not the best way of dealing with post-operative pain (Flecknell, 2000; Roughan and Flecknell, 2002). Effective management of post-operative pain requires a means of measuring pain, which will then enable the efficacy of analgesic treatment to be assessed. Until recently no validated means of measuring pain has been available. Work in farm animals (Wood et al., 1991), companion animals (Firth and Haldane, 1999) and laboratory rats (Roughan and Flecknell, 2001, 2002) suggests that methods can be devised that could be used routinely for pain assessment. For example, studies in the author's laboratory have shown that laboratory rats exhibit a series of distinctive behaviours following abdominal surgery (Roughan and Flecknell, 2001). These behaviours were initially identified following extensive analysis of video-taped material, filmed in undisturbed rats over a prolonged period. Further work has shown that several key behaviours occur sufficiently frequently to form the basis of a practically-useful means of pain assessment (Fig 1). The behaviours are also exhibited when animals are briefly removed from their home cage for a short (10 minute) period of observation, in normal lighting conditions. The behaviours are sufficiently distinctive that inexperienced investigators can easily be taught to recognise them. We have also been able to demonstrate that if these criteria are not used, and investigators simply rely on their prior experience to assess pain, the results are very poor. At present, this scoring system has been applied only to

rats following abdominal surgery, but it is hoped to develop similar systems for other procedures and for other species. In other areas, we have made even less progress and considerable work is needed to evaluate the signi cance of a wide range of apparent re nements. In the interim, we can only fall back on our principle of analogy, but should recognise its limitations and seek to progress beyond this stage.

5 Conclusions

Discussion of these problems should not detract from the very signi cant progress that has been made in the 40 or so years since Russell and Burch set out their guiding principles. What is needed now is greater academic focus on this area, not only to work on new methods of implementing the 3Rs, but also to disseminate current Best Practice , and to revise this advice as further progress is made.

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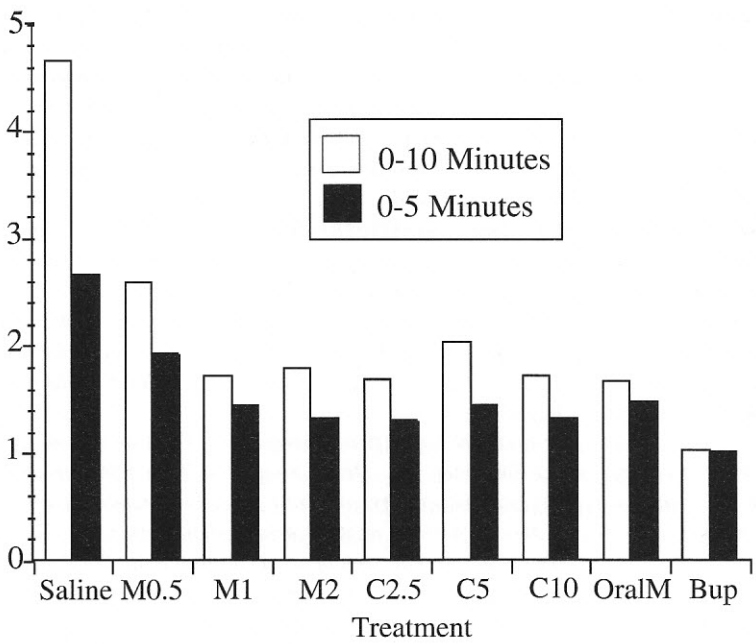

Fig. 1: Pain score comprising the frequency of several pain-related behaviours in rats following laparotomy with and without the use of analgesics. Significant differences occur between all analgesic treated groups and those animals receiving either saline, or a low dose of meloxicam (M0.5, 0.5mg/kg s/c). This difference was detectable over both a 5 and 10 minute observation period. An intervention analgesic protocol was followed so that control animals (receiving saline) could be withdrawn from the study and given pain relief (Buprenorphine 0.05mg/kg s/c). Analgesic treatments were meloxicam s/c at 0.5, 1 and 2mg/kg (M0.5, M1, M2), carprofen s/c at 2.5, 5 and 10mg/kg (C2.5, C5 and C10), oral meloxicam, 2mg/kg (OralM) and buprenorphine, 0.05mg/kg s/c. All drugs were administered 1 hour pre-operatively.

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