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# The Three Rs: past, present and future

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"Our story must begin with Charles Hume, for the whole of this grand enterprise began as a twinkle in his eye, which often twinkled" (Russell 1995). Fifty-one years ago, in 1954, besides important achievements in other fields of animal welfare, Hume and the Universities Federation for Animal Welfare (UFAW) had brought out the first edition of *The UFAW Handbook on the Care and Management of Laboratory Animals*, edited by Alastair Worden (Worden 1947), who was already preparing a greatly enlarged second edition with William Lane-Petter (Worden & Lane-Petter 1957). Hume had already contributed to the first edition of the Handbook an article on statistical analysis, which is clearly relevant to actual experimentation (Hume 1947); he now had the brilliant and totally original idea of starting a *general* study of humane technique in actual experimentation. The late Rex Burch and I were appointed to undertake this project; the moment Rex walked into my office I knew that here was not only the perfect colleague but also a life-long friend, and I sadly miss him.

Hume was our inspiration and our guide throughout, and we had much help from his then colleagues at UFAW. From across the Atlantic we received help and support from the Animal Welfare Institute, and Christine Stevens made repeated visits to encourage our work.

In 1957, I organised for UFAW the first Symposium on *Humane Technique in the Laboratory* — UFAW has held many such symposia in recent years. We owe much here to William Lane-Petter, who combined the posts of Director of the Laboratory Animals Bureau (later Centre), Secretary of the Research Defence Society (which he committed to promoting laboratory animal welfare as well as defending scientists), and Co-Editor of the second edition of the Handbook. He was also on the UFAW Committee to which Rex and I reported: the Chairman was Sir Peter Medawar, who was equally renowned as a great scientist (Nobel Laureate for his wonderful work on tissue grafts) and for his passionate concern for animal welfare; I had been fortunate enough to have him as a tutor at Oxford University. Lane-Petter now kindly arranged for the papers of the 1957 Symposium to be published as Volume 6 of the Collected Papers of the Laboratory Animals Bureau.

The Three Rs are present in essence, but not explicitly as such, in a short paper published in 1955 (Russell 1955), and I formally announced them at the Symposium (Russell 1957), so we must have evolved them in the interim. But neither Rex nor I, though we always thought of improvements beside replacement, could recall how, or more exactly when, they appeared. "We have to conclude that like Topsy in *Uncle Tom's Cabin* they just 'growed'" (Russell 1995); of course they were developed in full in our book *The Principles of Humane Experimental Technique* (Russell & Burch 1959, reprinted 1992).

There appeared to be no response to our book until 1978, when David H Smyth published his book on *Alternatives to Animal Experiments*; he gave this name to *all three* of our Rs. "The concept of alternatives appears in a publication in 1971 by Jack Hegarty, the Honorary Treasurer of FRAME"; there is more on this organisation later. "According to Sir William Paton, it probably 'crystallised', or 'growed', in the 1960s, the age of alternative medicine, alternative comedy, and alternative culture" (Russell 1995).

Alan Goldberg (2004) has pointed out that the term '*alternatives*' is unfortunate, because it obviously suggests only one R — *replacement* (after all, in the field of *reduction*, it is ridiculous to apply the term to good experimental design and analysis, because it is only alternative to bad design and analysis). Goldberg has experienced the resulting misunderstandings of this term as Director of CAAT — The Johns Hopkins University Center for Alternatives to Animal Testing, Baltimore; an organisation which has done, and continues to do, wonders for *all Three Rs*. I have been pleased to note that in the last couple of years this confusing term appears to be on the way out. Besides the title of this issue of *Animal Welfare*, the organisation just set up by the British Government, to promote Three Rs research and application, is called The National Centre for the Replacement, Refinement and Reduction of Animals in Research (Balls & Combes 2004).

"D H Smyth's 1978 book is obviously the work of a first-rate scientist deeply concerned with animal welfare. He had contributed to the UFAW Symposium of 1976, and an important new *in vitro* technique was developed in his laboratory. It is therefore most striking that his book is full of negative statements and predictions about the future of computers and tissue cultures as possible replacement

by Alan Goldberg, who hosted the first in Baltimore, where he and his wife Helene, and his CAAT colleagues, gave a kind welcome to Claire and me. The second one was hosted by Bert van Zutphen in Utrecht in 1996. The third was hosted by Michael Balls in Bologna in 1999 — we shall see later what he was doing in Italy. The fourth was hosted by Andrew Rowan of the Humane Society of the United States, distinguished, among many other scientific contributions to animal welfare, as the chief historian of the Three Rs. The fifth one will be held in Berlin this summer, hosted by Horst Spielmann. "These great occasions have been enormously important in stimulating the astonishing growth of our subject" (Russell 2004b), and their Proceedings have been, in themselves, a major contribution to its already vast literature (Goldberg *et al* 1995; van Zutphen & Balls 1997; Balls *et al* 2000; Balls *et al* 2004b).

At this point, it is worth mentioning two statements. In 2000, the European Science Foundation (ESF), in its statement on Use of Animals in Research, declared that it "strongly endorses the principles of the Three Rs" (with reference to our book). "This means that efforts ought to be taken to *replace* the use of live animals by non-animal alternatives, to *reduce* the number of animals used in experiments to the minimum that is required for obtaining meaningful results, and to *refine* procedures, so that the degree of suffering is minimised." "The ESF is a non-governmental association of 67 leading national funding agencies and other organisations that carry out and promote research from 23 European countries" (Anon 2000b).

In 1999, the participants of the Bologna Congress unanimously agreed on the 'Three Rs Declaration of Bologna', which includes the following statements: "In their book, Russell and Burch stated that 'the greatest scientific achievements have always been the most humane and the most aesthetically attractive, conveying that sense of beauty and elegance which is the essence of science at its most successful...'. The participants in the 3rd World Congress on Alternatives and Animal Use in the Life Sciences strongly endorse and reaffirm the principles put forward by Russell and Burch in 1959. Humane science is a prerequisite for good science, and is best achieved in relation to laboratory animal procedures by the vigorous promotion and application of the Three Rs. The Three Rs should serve as a unifying concept, a challenge, and an opportunity of reaping benefits of every kind — scientific, economic and humanitarian" (Anon 2000c). A major principle of our book was the close positive correlation of humaneness with scientific effectiveness, so I was delighted to see this principle so firmly asserted in the Declaration of Bologna.

We may now consider in turn each of the Three Rs. The numerousness and diversity of experimental procedures present a host of specific problems for finding *replacements* in the various media, such as tissue cultures, cultures of more than one tissue, organ cultures and micro-organisms. In addition, it is now important to explore the potential for replacement in totally new techniques developed long after 1959 — for example, genomics (Balls 2002; Anon 2005; Bhogal *et al* 2005), proteomics (Evers & Gray 2001), microarray analysis (Schena 2003) and molecular profiling (Stoughton & Friend 2005). In fact there is a vast amount of work being done around the world to develop specific requirements and generally useful principles. This is obvious from a glance at issues of, for instance, *Alternatives to Laboratory Animals* (*ATLA*), *Netherlands Centre Alternatives Newsletter*, or the *Swiss 3R-Info-Bulletin*, or the four *World Congress Proceedings*, or, for those with computers, the CAAT websites eg http://caat.jhsph.edu and http://altweb.jhsph.edu.

When replacements are developed for procedures in research, they are normally eagerly welcomed. They generally save expense and often save time. In the 71st Stephen Paget Lecture of the Research Defence Society in 2001, Sir Richard Sykes, Chairman of GlaxoSmithKline, observed "in replacement, technical and genomic advances mean that information that would have been slow or impossible using animals can be obtained in hours, using *in vitro* techniques" (Richmond 2002). In some research fields rapid progress, or even *any progress*, would have stopped dead without replacement. In our 1957 Symposium, the papers were already arranged in three groups. In the replacement group, Kingsley Sanders listed the huge advantages of tissue culture for virology, and showed that this science would have got nowhere without this replacement. "The animal virologist", he concluded, "has every cause to rejoice at his liberation from the hazards and uncertainties of animal experiment. At this point — to quote *Alice in Wonderland* — 'one of the guinea-pigs cheered, and was removed by an officer of the court'" (Sanders 1957).

However, when replacements are developed for tests of the potency and toxicity of drugs and biologicals, they have to be validated before they can be accepted by the regulators responsible for the health and safety of human beings and their domesticated animals. It was therefore of vital importance for replacement that in 1991 the European Union established in Ispra, Italy, the European Centre for the Validation of Alternative Methods (ECVAM), which began work in 1993 with Michael Balls of FRAME as Head — which is why he was able to host the 3rd World Congress in Italy. In the first decade or so, it is quite astonishing how much ECVAM achieved under Michael Balls' direction and inspiration (Russell 2003).

They did much work on replacement itself, they validated various replacements and got them accepted by the regulators, and above all, they developed a rational and rigorous form of validation, as an "unambiguous algorithm for converting" physiochemical or *in vitro* data into "predictions of a pharmacotoxicological endpoint in animals or humans" (Balls 2002, 2003). "*In vitro* methods are often *better* than *in vivo* ones — not surprisingly, because few, if any, of the *in vivo* methods have ever been validated by the rigorous methods of validation developed by ECVAM in recent years" (Russell 2004a). In addition to all this, ECVAM held literally dozens of very useful workshops on various subjects.

as Hume (1957a) pointed out, the proper procedure is not to use a 'job lot' of outbred animals, but a number of crosses between different inbred lines, and to allow for the variance between them. Clearly, for both these purposes, it is essential to have a number of inbred lines available.

Here again, Michael Festing has repeatedly emphasised the value of these principles, bringing them to the attention of as many experimenters as possible (Festing *et al* 1972; Festing 1995b, 2003). And he has done an invaluable service to help those prepared to practice genetic control by producing his *International Index of Laboratory Animals* (Festing 1993).

Turning now to environmental control, we come to the greatest discovery ever made in Three Rs research, by the late Michael Chance; another sadly missed friend and colleague, and one of the greatest of all ethologists. When Rex and I were working on our project, at my suggestion UFAW also supported the laboratory work of Michael Chance, who was initiating the study of laboratory animal ethology. By 1957 he was able to announce his great discovery at our Symposium (Chance 1957; see also Chance & Russell 1997). Hitherto it had always been supposed that to make animals uniform it was only necessary to keep them in the same environment. Chance discovered that *some environments are more favourable to uniformity than others*. The most uniform populations of all were those kept in *an environment optimal for their well-being*. In this respect, the goal of reduction is precisely the same as the goal of *refinement*; Chance well understood this.

We originally envisaged refinement as minimising pain and distress, and by 1959, discomfort (Russell 1959). It is now clear that we must aim positively at optimal well-being, for the following reason. "The major discovery of anatomy and physiology in the last half-century has been that of the extraordinarily subtle, comprehensive and intimate linkages and interactions between the somatic nervous system, the organ of behaviour, and the autonomic nervous system and the endocrine system, which control events within the body" (Russell & Burch 1959, reprinted 1992). It was already clear in the 1950s which parts of the brain were chiefly involved in these linkages — the hypothalamus in all vertebrates and the limbic system in mammals. These connections are capable of "converting distress caused by the physical, behavioural or social environment into physiological stress bound to disturb experimental results.... More is known now about the pathways to and from the limbic system, and the corticotropin-releasing factor in the hypothamalus (discovered in 1955) was isolated in 1981 and has since been the subject of numerous studies — some *in vitro* — and related substances have been found in lower vertebrates" (Russell 1997). In man, this is the basis for the discipline of psychosomatic medicine, which is equally important in the veterinary context (Russell 2002).

We may now consider the physical environment, with factors such as light and sound, the behavioural environment, the extent to which the animal is free to deploy its species-specific behaviour, and the social environment, the presence or absence of the company of conspecifics (Russell 2002). It is found that deviation from the well-being optimum, which may appear slight to us, in any of these three aspects of the environment, will cause surprisingly severe pathological effects on the animal's physiology, which on the one hand betray the presence of considerable distress and at the same time play havoc with experimental results. For instance, "caging monkeys in isolation causes a decline in the number and function of the T cells so vital for immunity" (Russell 1999). The identity of good science and humane science is particularly obvious here. It is also clear that, with all the factors involved, the identification of these deviations, and the finding of refinements to correct them, in all the species used in the laboratory, with their different needs and habitats, amounts to a vast programme of research; and, just as with replacement, there is by now a correspondingly vast literature, though, I think, scattered in more different journals. And all this, as we have seen, is equally important for reduction, for the deviations also increase variety.

The size of the literature shows that many good scientists are working on this aspect of refinement. Viktor and Annie Reinhardt are notable for their work with primates (Reinhardt & Reinhardt 1991, 2001a,b, 2002; Reinhardt 1997, 1999, 2004; Reinhardt & Rossell 2001). But for an alphabet addict, it is irresistible to mention the Three Ms of FRAME — *M*ichael Balls, for replacement, *M*ichael Festing, for reduction, and David *M*orton, for refinement. Besides contributing to other aspects of refinement, David Morton has done a great deal of research finding the ill-effects of deviation from the optimum and refinements to correct them, and has repeatedly publicised the need to deal with such deviations, both scientific and humanitarian (Morton 1990, 1992, 1997, 1998a; Morton *et al* 1993; Morton & Townsend 1995).

Another problem for refinement arises from the need to collect blood in many experiments. It has been common practice to restrain the animals during this procedure, but it has been shown that restraint causes serious pathological effects, disastrous for experimental results and that must involve severe distress (Russell 2002). Fortunately there are two alternatives to restraint — telemetry (Morton *et al* 2003) and (reward) training the animals to present a limb (Reinhardt & Cowley 1990, 1992; Reinhardt 2003).

Another important aspect of refinement is the assessment of pain. Phyllis Croft, the discoverer of the cardiac pain reflex, who was also working for UFAW, spoke on the problem at our 1957 Symposium (Croft 1957a). Recently, David Morton has done important work on the assessment of pain and distress (Morton & Griffiths 1985; Morton 1995).

Obviously a very important aspect of refinement is the control of pain by the proper use of anaesthetics and analgesics. Phyllis Croft, then a leading expert on this, spoke on this subject too at our 1957 Symposium (Croft 1957b). Nowadays, the leading expert is Paul Flecknell, and he makes very important contributions to this aspect of refinement (Flecknell 1987).

Perhaps the greatest challenge of all to refinement is the matter of humane endpoints. In toxicity testing, and in testing the potency of biologicals, animals have generally been left to die in severe distress, as in the notorious LD50.

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