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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 lifelong 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

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Table 1 Information on some further organisations specifically devoted to the Three Rs.

Country	Acronym	Name	Foundation date
USA	CAAT	Center for Alternatives to Animal Testing	1981
Germany	ZEBET	Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments	1989
Japan	JSAAE	Japanese Society of Alternatives to Animal Experiments	1989
The Netherlands	NCA	The Netherlands Centre for Alternatives	1994

methods, especially in toxicity testing" (Russell 2004b). I have repeatedly shown how rapidly and completely all these negative predictions were shown to be false (Russell 2004b).

After 1959, Rex was engaged in his work as a microbiologist, and I was engaged with my late wife Claire in our studies of animals and human behaviour, and the relations between them. The first news we had of any notice at all being taken of our book was in 1990, when the Humane Society of the United States asked our permission to call a new prize for contributions to laboratory animal welfare the Russell and Burch Award. Subject to UFAW's approval, we naturally agreed. UFAW gave its blessing, and in the following years a number of distinguished scientists in the Three Rs field received this international award; a great stimulus to work of this kind.

Rex and I then found, to our amazement and delight, that after two decades of little or no notice, the Three Rs had suddenly taken off in about 1980. In the last quarter of a century, their progress has been astonishing. The Three Rs have entered the legislation of a number of countries and the memorable Council Directive 86/609/EEC of 1986, and there are excellent histories of the legislative developments by Alan Goldberg, and two distinguished Three Rs scientists of the Humane Society of the United States, Andrew Rowan and Martin Stephens (Rowan 1994; Stephens *et al* 2001); Rowan is the leading historian of laboratory animal welfare (eg Rowan 1984). There are now organisations specifically devoted to the Three Rs in many countries around the world.

The first of these organisations was FRAME in Britain, which was founded in 1969, as the Fund for the Replacement of Animals in Medical Experiments. Eventually, in spite of its name, under the leadership of Michael Balls, FRAME became firmly committed to *all Three Rs*, and they symbolised this commitment in the most generous way by calling their new building in Nottingham Russell and Burch House.

In my recorded speech to the New Orleans Congress (Russell 2004b) — I cannot now travel abroad — I listed some other Three Rs organisations, which are shown in Table 1.

In Cuba, ProALT, founded in 2000, is promoting the Three Rs all over Latin America (Perez & Murillo 2000).

"A very useful type of organisation is the National Platform for coordinating ministries, universities, industry and welfare societies in the promotion of the Three Rs. The German and Dutch platforms were founded in 1987. A Belgian platform was founded in 1999, by His Royal Highness Prince Laurent, who has done so much for science and animal welfare, and who honoured us by attending and speaking at the Utrecht Congress in 1996" (Russell 2004b). There are now eleven such platforms (van der Valk 2004).

If at my age (80) I may be excused a little autobiography, the worldwide Three Rs activities have made possible some very pleasant foreign contacts for me. I have made many visits to the Netherlands, accompanied until her death by my late wife Claire, where we were welcomed by our friends Bert and Annemarie van Zutphen, and other Dutch friends working in the Three Rs field. In 1997, we were welcomed to Prague and Hradec Králové by our friends Mirek and Zuzana Cervinkovi, who have long been both practising and promoting Three Rs research. In 2004, I had the honour of writing (in English) the preface to a book in Chinese on the Three Rs, edited by He Zheng-Ming of the Beijing Society of Alternatives to Laboratory Animals, which is doing much to promote the Three Rs in the People's Republic of China (He 2004).

A very important development is the introduction of courses in laboratory animal science and the Three Rs for scientists intending to experiment on animals (van Zutphen 1991; van Zutphen & van der Valk 1995). This began in the Netherlands in 1985, and Britain and Sweden followed suit in 1986 and 1988, respectively. This is largely due to the efforts of Bert van Zutphen, who taught the Dutch course for many years, and who, with his colleagues V Baumans and AC Beynen, edited an excellent textbook on this essentially new subject (van Zutphen *et al* 1993).

Another important development has been the establishment of a Chair in Alternatives at the University of Utrecht, the first Professor being Coenraad Hendriksen, distinguished for his application of the Three Rs to the testing of biologicals (vaccines, sera etc) (Anon 2000a). I understand there are by now two such Chairs in Germany, thanks to the efforts of Horst Spielmann of ZEBET (Spielmann 2002).

There have been many important small conferences; in my speech for New Orleans I listed as examples Sydney in 1993, Hradec Králové and Utrecht in 1994, London in 1998, Brussels in 1999, and Baltimore, Prague, Santiago de Cuba and Warsaw in 2001 (Russell 2004b), and there have been many more since then.

Most exciting of all have been the World Congresses, with literally hundreds of contributors from dozens of countries; the most striking measure of the amount of worldwide activity in the field. The Congresses were launched in 1993

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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.

In 1995, Michael Balls had the kindly and imaginative idea of arranging an ECVAM workshop on the Three Rs at Rex Burch's work-place, Sheringham, Norfolk, so that Rex, who could not travel for health reasons, could meet a large number of people working in the Three Rs field. Although we had kept in touch with letters and phone calls, Rex and I had not seen each other, except briefly in 1991, since 1959, and my late wife Claire had only seen him in that brief visit, so it was a wonderful time for us, as well as a very interesting and fruitful workshop (Balls *et al* 1995). We were all the more grateful for this when Rex died in the following year — it had been our last chance.

Our second 'R', *reduction*, has sometimes caused misunderstandings because, of course, replacements bring down the number of animals used. We defined it as "reduction in the number of animals used to obtain information of given amount and precision", which implies that *some* animals are being used. Reduction in our sense is perfectly defined by the title of a joint meeting of FRAME and the Laboratory Animal Science Association (Balls *et al* 2004a) — *Reducing the Use of Experimental Animals where No Replacement is Yet Available*.

The crucial function on which the need for reduction turns is this: the greater the variation between the members of an animal population in the response being studied or tested, the more animals are needed to comprise a representative sample of the population. This is obvious when one reflects that if all the animals of a population were perfectly identical, any one of them would represent all the others. If there is appreciable variation in a population, we can still keep the size of the sample used to a *minimum* — too many wastes the superfluous animals, but too few wastes all the animals in whole experiments. To achieve the goal of the minimum, we use correct statistical design and analysis of experiments.

Unlike the concept of replacement, which, as we have seen, opens up a vast field of specific experiments, reduction of this kind only requires the proper application of certain well-established principles. These are clearly set out in two books available to use in 1959 — Fisher (1942) and Emmens (1948) — and we were able to state all the main principles of the subject for experimenters on animals; we even included the then recent technique of sequential analysis, which I had practiced with a colleague (Russell & Burch 1959, reprinted 1992). Of course, statistical method continues to improve, but that is a matter for the statisticians.

What is needed here, then, is a grand educational exercise to ensure that as many experimenters as possible use the principles correctly. For this, very high expertise in both statistics and biomedical science is required. Though a number of people have contributed, there is no doubt that the leader in this enterprise is Michael Festing, who is connected with FRAME.

By studying a large number of papers in the biomedical field, Festing found an appalling number of elementary errors in design and analysis sufficiently gross to invalidate — and therefore waste — whole experiments. He had done everything possible to improve matters; by publishing repeated papers explaining the urgent need for using the methods correctly and also repeatedly explaining the methods, fully brought up to date, tailored for biomedical experiments, and specifically oriented to calculating the use of a minimum of animals (Festing 1994a,b, 1995a, 1996, 2000; Festing & Lovell 1996; Festing *et al* 2002; Festing & Weigler 2003). He has always insisted on the need to consult a professional statistician *before* starting an investigation, so that the design is correct. (This was the main point I made in a course in Elementary Statistics, which I taught for 24 years in the Department of Sociology at the University of Reading.) At the Sheringham Three Rs Workshop (Balls *et al* 1995), where Festing led our discussions of reduction, we agreed that every experimenter should have a named professional statistical adviser, unless fully statistically qualified him- or herself. If this were made compulsory, it would surely greatly promote reduction.

However, it is perfectly possible actually to make animal populations more uniform. When we consider the things that can vary between individual animals, we can begin with the phenotype, the complex of anatomical structures and basic physiological processes in the adult animal; this is the product of the interaction of the developmental environment with the genotype, which is the complex of the alleles present at every locus. In experimentation, we are normally concerned with something more transient — *a physiological response* — and Rex and I named this the dramatype (Russell & Burch 1959, reprinted 1992). The dramatype is the product of the interaction of the immediate environment with the phenotype.

The immediate environment includes the background environment of acute experiments, the continuous background of chronic (eg nutritional) experiments, and the maintenance environment of all adult experimental animals — so *The UFAW Handbook on the Care and Management of Laboratory Animals*, now in its 7th edition (Poole 1999), is itself a very important contribution to humane experimental technique. Because the same principles apply to the immediate and the developmental environment — which is, of course, the immediate environment for experiments on immature animals — I need not consider them separately, and will deal generally with the environment. Evidently, then, we can control variation and approximate to uniformity by manipulating either the genetic or the environmental situation, or of course preferably both.

The control of genetic variation was already discussed at our 1957 Symposium (Lane-Petter & Bloom 1957). The principles were clearly established by the time of our 1959 book, so here, as with design and analysis, and unlike the replacement situation, the need is not for extensive research but for education and the diffusion of the correct methods. The most uniform populations are those of F1 crosses between inbred lines. The crosses are as homogeneous as their parents, but highly heterozygous, hence they have several ways of synthesising important molecules and are buffered against differences in early environment, which could drive their metabolism in different directions. Sometime we are interested in variation itself; in that case,

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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.

It is obviously desirable to find a physiological, biochemical or immunological variable that can show the effects of the poison or infection before suffering begins; the animals can then be painlessly killed. In 1957, Hume showed that this is scientifically preferable as well as humanitarian, in a criticism of the LD50 (Hume 1957a). He mentioned this at our Symposium (Hume 1957b). Recently, David Morton has done much work on this (Morton 1998b, 1999, 2000; Morton & Hau 2003). There is now an excellent book on the whole subject of humane end-points, edited by David Morton and Coenraad Hendriksen, the first Professor of Alternatives, distinguished for his application of the Three Rs to the study and testing of biologicals (Hendriksen & Morton 1999).

This completes my summary of the Three Rs in past and present. At 80 years of age, with a feeling that I have rather done my bit in this field, and with Rex no longer with us, it is a great joy to know that hundreds of fine scientists, all over the world, are pursuing research in and promotion of the Three Rs. UFAW began it all, and despite the demands of the many other aspects of animal welfare science to which it is committed, UFAW has quietly and steadily continued to pursue and promote humane experimental technique. It was a great pleasure when James Kirkwood, who has done so much in many fields of animal welfare science, invited me to introduce this Three Rs issue of the distinguished UFAW journal, *Animal Welfare*, which I am sure will be a major contribution to humane experimental technique.

References

Anon 2000a Professor of alternatives appointed at the NCA. *NCA Newsletter 9*: 7

Anon 2000b *ESF Statement on the Use of Animals in Research.* European Science Foundation: Strasbourg, France

Anon 2000c The three Rs declaration of Bologna. In: Balls M, van Zeller A-M and Halder ME (eds) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* p 15. Elsevier: Amsterdam, The Netherlands

Anon 2005 Dutch Government touts genomics for reducing animal use. *Lab Animal Europe 5(2)*: 4

Balls M (ed) 2002 *Alternatives to Animal Experiments: Progress made and Challenges Ahead. ATLA 30, Suppl 2*

Balls M (ed) 2003 *Biostatistical Methods and the Development, Validation and the Application of* In Vitro *Toxicity tests. ATLA 31, Suppl 1*

Balls M and Combes RD 2004 The UK national centre for the three Rs: pathway to progress or mere fig leaf? *ATLA 32*: 61-64

Balls M, Festing MFW and Vaughan S (eds) 2004a *Reducing the Use of Experimental Animals where No Replacement is Yet Available. ATLA 32, Suppl 2*

Balls M, Firmani D and Rowan A (eds) 2004b *The Three Rs at the Beginning of the 21st Century: Proceedings of the Fourth World Congress on Alternatives and Animal Use in the Life Sciences. ATLA 32, Suppl 1*

Balls M, Goldberg AM, Fentem JH, Broadhead CL, Burch RL, Festing MFW, Frazier JM, Hendriksen CFM, Jennings M, van der Kamp MDO, Morton DB, Rowan AN, Russell C, Russell WMS, Spielmann H, Stephens ML, Stokes WS, Straughan DW, Yager JB, Zurlo J and van Zutphen LFM 1995 The three Rs: the way forward. The report and recommendations of ECVAM workshop 11. *ATLA 23*: 838-866

Balls M, van Zeller A-M and Halder ME (eds) 2000 *Progress in the Reduction, Refinement and Replacement of Animal Experimentation.* Elsevier: Amsterdam, The Netherlands

Bhogal N, Grindon G, Combes R and Balls M 2005 Toxicity testing: creating a revolution based on new technologies. *Trends in Biotechnology 23*: 299-307

Chance MRA 1957 The contribution of environment to uniformity: variance control, refinement in pharmacology. *Laboratory Animals Bureau, Collected Papers 6*: 59-73

Chance MRA and Russell WMS 1997 The benefits of giving experimental animals the best possible environment. In: Reinhardt V (ed) *Comfortable Quarters for Laboratory Animals, 8th Edition* pp 12-14. Animal Welfare Institute: Washington DC, USA

Council 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. http://europa.eu.int/comm/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf (accessed 4 October 2005)

Croft PG 1957a The criteria for a humane technique. *Laboratory Animals Bureau, Collected Papers 6*: 19-22

Croft PG 1957b Aspects of anaesthesia. *Laboratory Animals Bureau, Collected Papers 6*: 75-77

Emmens CW 1948 *Principles of Biological Assay.* Chapman & Hall: London, UK

Evers S and Gray CP 2001 Application of proteome analysis to drug development and toxicology. In: Pennington SR and Dunn MJ (eds) *Proteomics* pp 225-236. BIOS Scientific Publishers: Oxford, UK

Festing MFW 1993 *International Index of Laboratory Animals, 6th Edition* pp 238. Michael FW Festing, PO Box 301, Leicester LE1 7RE, UK

Festing MFW 1994a Reduction of animal use: experimental design and quality of experiments. *Lab Animal 28*: 212-221

Festing MFW 1994b Are animals experiments well designed? In: Bunyan J (ed) *Welfare and Science* pp 32-36. Royal Society of Medicine Press: London, UK

Festing MFW 1995a Reduction of animal use and experimental design. In: Goldberg AM, van Zutphen LFM and Principe ML (eds) *The World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing* pp 43-49. Mary Ann Liebert: New York, USA

Festing MFW 1995b Use of a multi-strain assay could improve the MTP carcinogenesis bioassay programme. *Environmental Health Perspectives 103*: 44-52

Festing MFW 1996 Are animal experiments in toxicological research the right size? In: Morgan BJT and Williams DA (eds) *Statistics in Toxicology* pp 3-11. Oxford University Press: Oxford, UK

Festing MFW 2000 Reduction, model development and efficient experimental design. In: Balls M, van Zeller A-M and Halder ME (eds) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* pp 721-727. Elsevier: Amsterdam, The Netherlands

Festing MFW 2003 Laboratory animal genetics and genetic quality control. In: Hau J and van Hoosier GL (eds) *Handbook of Laboratory Animal Science, 2nd Edition* pp 173-203. CRC Press: Boca Raton, Florida, USA

Festing MFW and Lovell DP 1996 Reducing the use of laboratory animals in toxicological research and testing by better experimental design. *Journal of the Royal Statistical Society: Series B (Statistical Methodology) 58*: 127-140

Festing MFW and Weigler BJ 2003 Experimental design and statistical analysis. In: Hau J and van Hoosier GL (eds) *Handbook of Laboratory Animal Science* pp 327-350. CRC Press: Boca Raton, Florida, USA

Festing MFW, **Kondo K, Loosli R, Polly SM and Speigel A** 1972 International standardised nomenclature for outbred stocks of laboratory animals. *ICLA Bulletin 30*: 4-17

https://doi.org/10.1017/S0962728600029596 Published online by Cambridge University Press

Festing MFW, Overend P, Gaines Das R, Cortina Borja M and Berdoy M 2002 *The Design of Animal Experiments.* The Royal Society of Medicine Press: London, UK

Fisher R 1942 *The Design of Experiments*. Oliver and Boyd: Edinburgh, UK

Flecknell PA 1987 *Laboratory Animal Anaesthesia*. Academic Press: London, UK

Goldberg AM 2004 Animals and alternatives: societal expectations and scientific need. *ATLA 32*: 545-551

Goldberg AM, van Zutphen LFM and Principe ML 1995 *The World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing.* Mary Ann Liebert: New York, USA

He ZM (ed) 2004 *Alternate Methods to Animal Experiment* (in Chinese). Beijing Academic Publications: Beijing, China

Hegarty J 1971 Alternatives. In: Godlovitch S, Godlovitch R and Harris J (eds) *Animals, Men and Morals*. Gollancz: London, UK

Henriksen CFM and Morton DB (eds) 1999 *Humane Endpoints in Animal Experiments for Biomedical Research*. The Royal Society of Medicine Press: London, UK

Hume CW 1947 A conspectus of the elements of statistical analysis. In: Worden AN (ed) *The UFAW Handbook on the Care and Management of Laboratory Animals, 1st Edition* pp 283-352. Baillière Tindall and Cox: London, UK

Hume CW 1957a The strategy and tactics of experimentation. *The Lancet* 23 November 1957: 1049-1052

Hume CW 1957b The strategy and tactics of experimentation. *Laboratory Animals Bureau, Collected Papers 6*: 49-50

Lane-Petter W and Bloom JL 1957 Control of genetic variation. *Laboratory Animals Bureau, Collected Papers 6*: 51-57

Morton DB 1990 Adverse effects in animals and their relevance to refining scientific procedures. *ATLA 18*: 29-39

Morton DB 1992 A fair press for animals. *New Scientist 134*: 28-30

Morton DB 1995 The post-operative care of small experimental animals and the assessment of pain by score sheets. In: Johnston ME (ed) *Proceedings of Animal in Science Conference: Perspectives on Their Use, Care and Welfare* pp 82-87. Monash University: Melbourne, Australia

Morton DB 1997 A scheme for the recognition and assessment of adverse effects. In: van Zutphen LFM and Balls M (eds) *Animal Alternatives, Welfare and Ethics* pp 235-241. Elsevier: Amsterdam, The Netherlands

Morton DB 1998a The use of score sheets in the implementation of humane endpoints. *Proceedings of the Joint ANZCCART/NAEAC Conference on Ethical Approaches to Animal-Based Science* pp 75-82. 19–20 September 1997. Auckland, New Zealand

Morton DB 1998b The importance of non-statistical design in refining animal experimentation. *ANZCCART News 11(2)*: 12

Morton DB 1999 Humane endpoints in animal experimentation for biomedical research: ethical, legal and practical aspects. In: Hendriksen CFM and Morton DB (eds) *Humane Endpoints in Animal Experimentation for Biomedical Research* pp 5-12. The Royal Society of Medicine Press: London, UK

Morton DB 2000 A systematic approach for establishing humane endpoints. *ILAR Journal 41(2)*: 80-86

Morton DC and Griffiths PHM 1985 Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record 116*: 431-436

Morton DB and Hau J 2003 Welfare assessment and humane endpoints. In: Hau J and van Hoosier GL (eds) *Handbook of Laboratory Animal Science, 2nd Edition* pp 457-486. CRC Press: Boca Raton, Florida, USA

Morton DB and Townsend P 1995 Dealing with adverse effects and suffering during animal research. In: Tuffery AA (ed) *Laboratory Animals — An Introduction for Experimenters* pp 215-231. John Wiley: Chichester, UK

Morton DB, Hawkins P, Bevan R, Heath K, Kirkwood J, Pearce P, Scott L, Whelan G and Webb A 2003 Refinements in telemetry procedures. *Laboratory Animals 37*: 261-299

Morton DB, Jennings M, Batchelor GR, Bell D, Birke L, Davies K, Eveleigh JR, Gunn D, Heath M, Howard B, Koder P, Phillips J, Poole T, Sainsbury AW, Sales GD, Smith DJA, Stauffacher M and Turner RJ 1993 Refinements in rabbit husbandry. *Laboratory Animals 27*: 301-329

Perez LU and Murillo G 2000 Promotion of alternative methods in Latin America and the Caribbean: challenges and hopes. In: Balls M, van Zeller A-M and Halder ME (eds) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* pp 1395-1400. Elsevier: Amsterdam, The Netherlands

Poole TB (ed) 1999 *The UFAW Handbook on the Care and Management of Laboratory Animals, 7th Edition*. Blackwell Science: Oxford, UK

Reinhardt V (ed) 1997 *Comfortable Quarters for Laboratory Animals, 7th Edition*. Animal Welfare Institute: Washington DC, USA

Reinhardt V 1999 Pair-housing overcomes self-biting behaviour in macaques. *Laboratory Primate Newsletter 38*: 4

Reinhardt V 2003 Working with rather than against macaques during blood collection. *Journal of Applied Animal Welfare Science 6*: 189-197

Reinhardt V 2004 Common husbandry-related variables in biomedical research with animals. *Laboratory Animals 38*: 213-235

Reinhardt V and Cowley D 1990 Training stumptailed monkeys to cooperate during in-home cage treatment. *Laboratory Primate Newsletter 29*: 9-10

Reinhardt V and Cowley D 1992 In-homecage blood collection from conscious stumptailed macaques. *Animal Welfare 1*: 249-255

Reinhardt V and Reinhardt A 1991 Impact of a privacy panel on the behaviour of caged female rhesus monkeys living in pairs. *Journal of Experimental Animal Science 34*: 55-58

Reinhardt V and Reinhardt A 2001a Legal space requirement stipulations for animals in the laboratory. Are they adequate? *Journal of Applied Animal Welfare Science 4*: 143-149

Reinhardt V and Reinhardt A 2001b *Environmental Enrichment for Caged Rhesus Macaques (*Macaca mulatta*) — Photographic Documentation and Literature Review, 2nd Edition*. Animal Welfare Institute: Washington DC, USA

Reinhardt V and Reinhardt A (eds) 2002 *Comfortable Quarters for Laboratory Animals, 9th Edition*. Animal Welfare Institute: Washington DC, USA

Reinhardt V and Rossell M 2001 Self-biting in caged macaques: cause, effect and treatment. *Journal of Applied Animal Welfare Science 4*: 285-294

Richmond C 2002 Annual Meeting: Welfare and Science. *Research Defence Society News, January*: 8-9

Rowan AN 1984 *Of Mice, Models and Men*. State University of New York Press: Albany, NY, USA

Rowan AN 1994 Looking back 33 years to Russell and Burch: the development of the concept of the Three Rs (alternatives). In: Reinhardt CA (ed) *Alternatives to Animal Testing: New Ways in the Biomedical Sciences, Trends and Progress* pp 1-11. VCH: Weinheim, Germany

Russell WMS 1955 A research on the history and progress of humane experimental techniques. *UFAW Courier 11*: 16-20

Russell WMS 1957 The increase of humanity in experimentation: replacement, reduction and refinement. *Laboratory Animals Bureau, Collected Papers 6*: 23-25

Russell WMS 1959 On comfort and comfort activities in animals. *UFAW Courier 16*: 14-26

Russell WMS 1995 Speech at the Awards Luncheon. In: Goldberg AM, van Zutphen LFM and Principe ML (eds) *The World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing* pp 71-80. Mary Ann Liebert: New York, USA

Russell WMS 1997 Shooting the clock. *Science and Animal Care 8(3)*: 1-2 and inset

Russell WMS 1999 Reduction and refinement in biomedical experiments. *Research Defence Society News, January*: 12-13

Russell WMS 2002 The ill-effects of uncomfortable quarters. In: Reinhardt V and Reinhardt A (eds) *Comfortable Quarters for Laboratory Animals, 9th Edition* pp 1-5. Animal Welfare Institute: Washington DC, USA

Russell WMS 2003 Alternatives to animal experiments: progress made and challenges ahead. *ATLA 31*: 216-218

Russell WMS 2004a The use of non-human animals in research: a guide for scientists. *ATLA 32*: 119-120

Russell WMS 2004b Nine glorious years. In: Balls M, Firmani D and Rowan AN (eds) *The Three Rs at the Beginning of the 21st Century: Proceedings of the Fourth World Congress on Alternatives and Animal Use in the Life Sciences. ATLA 32, Suppl 1A*: 3-10

Russell WMS and Burch RL 1959 (reprinted 1992) *The Principles of Humane Experimental Technique*. Universities Federation for Animal Welfare: Wheathampstead, UK

Sanders FK 1957 Tissue cultures as substitutes for experimental animals. *Laboratory Animals Bureau, Collected Papers 6*: 35-43

Schena N 2003 *Microarray Analysis*. John Wiley: Hoboken, NJ, USA

Smyth DH 1978 *Alternatives to Animal Experiments*. Scolar Press in association with the Research Defence Society: London, UK

Spielmann H 2002 A chair on alternatives? *ALTEX 19*: 69-73

Stephens ML, Goldberg AM and Rowan AN 2001 The first forty years of the alternatives approach: refining, reducing and replacing the use of laboratory animals. In: Salem DJ and Rowan AN (eds) *The State of the Animals 2001* pp 121-135. Humane Society Press: Washington DC, USA

Stoughton RB and Friend SH 2005 How molecular profiling could revolutionise drug discovery. *Drug Discovery 4*: 345-350

van der Valk J 2004 Editorial. *Netherlands Centre Alternatives 17*: 1

van Zutphen LFM 1991 Education and animal experimentation. In: Hendriksen CFM and Koëter HBWM (eds) *Replacement, Reduction and Refinement. Present Possibilities and Future Prospects* pp 119-125. Elsevier: Amsterdam, The Netherlands

van Zutphen LFM and Balls M (eds) 1997 *Animal Alternatives, Welfare and Ethics*. Elsevier: Amsterdam, The Netherlands

van Zutphen LFM and van der Valk J 1995 Education and training: a basis for the introduction of the Three Rs alternatives into animal research. *ATLA 23*: 123-127

van Zutphen LFM, Baumans V and Beynen AC (eds) 1993 *Principles of Laboratory Animal Science*. Elsevier: Amsterdam, The Netherlands

Worden AN (ed) 1947 *The UFAW Handbook on the Care and Management of Laboratory Animals, 1st Edition*. Baillière Tindall and Cox: London, UK

Worden AN and Lane-Petter W (eds) 1957 *The UFAW Handbook on the Care and Management of Laboratory Animals, 2nd Edition*. Universities Federation for Animal Welfare: Wheathampstead, UK

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