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Zebrafish - Do we need another vertebrate model?

We use many organisms as models to answer questions in biology for a number of reasons. Aside from being able to use them for experiments that cannot be performed on humans, model organisms have characteristics that allow manipulation or observation in a particular manner. Often, their biology (their size, reproduction, genetics, behavior) will be relatively simple. This simplifies the performance and interpretation of experiments. Whether an organism becomes a successful model, or not, depends upon its combination of characteristics, and the range of biological questions that can be addressed by using it.

The fruit fly (*Drosophila melanogaster)* and the domestic mouse (*Mus musculus*) are excellent examples of successful model organisms. Both are relatively small and reproduce rapidly and in great numbers. Genetically they are highly manipulable and they can be utilised in experiments ranging from studies of behaviour to molecular biology to population genetics. Mice have an anatomy, physiology and genome similar to humans while *Drosophila* have a comparatively simple genome and can survive even when dramatically mutated.

Despite the genetic utility of mice they have one major drawback - their reproduction is placental. Thus, the number of embryos that can be produced by a mother is limited. The embryos are initially microscopic and the mother must usually be killed to gain access to them. These characters mean that it is very laborious to use mice for mutation screens. In mutation screens, the gonads of adults animals are exposed to mutagens and then their progeny are examined for mutations affecting the character or process of interest. Identification of the genes affected by the mutations allows discovery of what genes control these characters/processes. If the mutations one desires to study affect the development of mouse embryos then it is very difficult to observe their effects without killing the pregnant mouse and, hence, losing the ability to propagate the mutation! Also, mouse embryos that die during development are rapidly resorbed and thus lost to study.

Zebrafish as a new model

Recently, another organism has emerged as a powerful model in which to address questions of vertebrate embryo development - particularly brain development - by mutagenic means. The zebrafish (*Danio rerio*) is a small, hardy, freshwater fish (see figure) originating in northern India. Since it is a vertebrate, its basic embryology and brain structure are similar to that of humans. However, in many ways its reproduction is more similar to that of flies than of mammals. During its short (~12 months) lifespan, one zebrafish female can produce tens of thousands of rapidly developing embryos. The embryos develop externally to the mother and so are easily observed and are not resorbed if they die during embryogenesis. After fertilisation, the basic body plan of the animal develops within 24 hours (equivalent to about 9 days in the mouse). The embryos are also completely transparent which facilitates

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observation of cell fates. The zebrafish egg is macroscopic, allowing easy injection of substances such as cell dyes or transgenes, as well as transplantation of cells. However, due to their limited food supply (the yolk), zebrafish embryos do not grow greatly during development. Con-sequently, techniques for analysis of gene activity in whole embryos such as whole mount *in situ* transcript hybridisation can be used throughout much of the development of the zebrafish embryo, unlike that in mice, where analysis of the whole embryo is limited by its rapid growth.

Zebrafish have been of interest to developmental biologists for at least 40 years (e.g., see Hisoaka and Battle, 1958). However, the current surge in popularity of zebrafish is founded on research on zebrafish genetics performed by George Streisinger in Oregon, USA in the 1970s. In the 1980s, Christiana Nusslein-Volhard (whose work on developmental genetics in *Drosophila* revolutionised this field) was casting around for a suitable vertebrate organism with which to perform a mutation screen to identify genes controlling vertebrate development. Realising that zebrafish represented the best candidate, Dr Nusslein-Volhard applied to zebrafish, essentially, the same mutation strategy that she had used previously with *Drosophila.* She, and a competitor Wolfgang Driever, detected hundreds of genes which, when mutated, disturb embryo development.

The results of their work were published in a single special issue of the journal *Development* in December 1996. Since then, the growth in the number of laboratories using zebrafish to address questions in vertebrate developmental genetics has been exponential. As of December 2000, there were 256 laboratories worldwide registered on the Zebrafish Information Network, see - http://zfin.org/ZFIN/

At the University of Adelaide, I work within both the Department of Molecular Biosciences and the ARC-funded Special Research Centre for the Molecular Genetics of Development. These units host a number of organisms (mouse, chick, zebrafish and *Drosophila*) that are useful models for studying embryo development. Thus, as our research proceeds, we can utilise the particular model organism which presents the most useful characteristics to answer any particular question. This is an organisational paradigm that is increasingly common internationally.

Significance of zebrafish use in embryonic research

The work in my laboratory focuses on two main themes. The first is to understand how the Notch family of receptor proteins interact with each other and associated proteins to control embryo development. The Notch receptors are an ancient and highly conserved family of proteins found in all animals (reviewed by Beatus and Lendahl, 1998). They control communication between neighbouring cells when each cell must adopt a different fate. Thus they are important in creating boundaries within tissues. Loss of control of cell-cell signalling through Notch receptors can result in cancer or dementia in humans (Ellisen *et al.*, 1991; Joutel *et al.*, 1996).

Interestingly, Notch receptors interact with another highly conserved group of proteins, the presenilins, that are the primary sites of mutations causing early onset Alzheimers disease (reviewed by Baumeister 1999). In my laboratory we have cloned parts of all the known Notch and presenilin genes in zebrafish and we are using techniques such as mRNA injection, transgenesis, *in situ* hybridisation and immunohistochemistry to investigate the effects that disruption of Notch signalling and presenilin function has on cell fate and embryo development.

The second research theme in my laboratory is a more general search for the genes that control embryo development, in particular those genes controlling the regionalisation of the brain (e.g., into forebrain, hindbrain etc.) and the genes controlling segmentation in the embryo (e.g., the creation of the precursors of vertebrae called somites). To do this we have been analysing the patterns of transcription in the embryo of genes selected at random. This is performed by generating probes for whole embryo *in situ* transcript hybridisa-

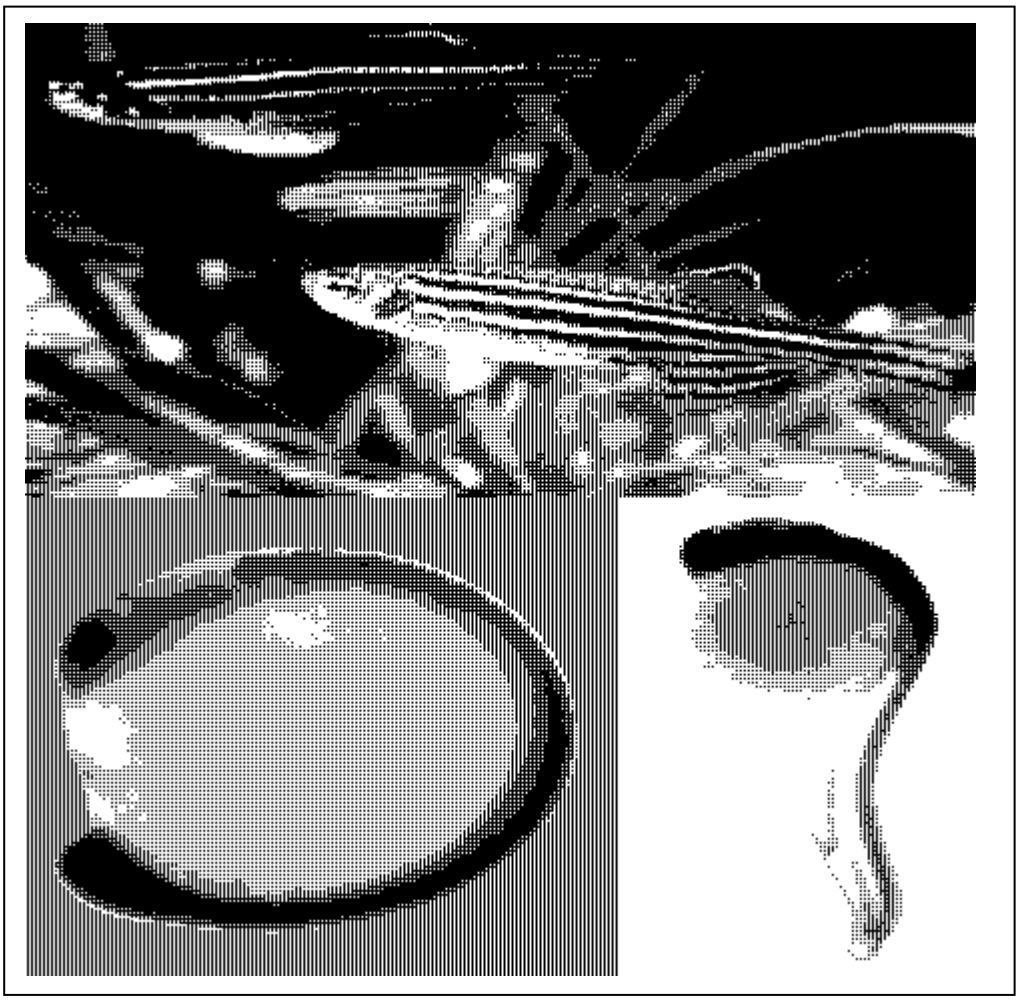

Figure: Zebrafish adults (top panel) and embryos (lower panels) at 12 hours (left) and 24 hours (right) post-fertilisation. The adult fish are 2 - 3 cm in length. The embryos are normally transparent but have been stained to show tissues transcribing a zebrafish Notch gene.

tion using cDNA clones taken from an appropriate library. Probes that reveal a gene transcribed in a restricted pattern often indicate the involvement of that gene in development of that area (see figure).

Our screen has revealed a number of candidate developmental control genes whose function is now being tested. This can be done in a number of ways. By synthesising mRNA from the gene *in vitro* and injecting this into the embryo one can cause expression in inappropriate areas of the protein encoded by the gene. The consequent effects on embryo development and on the expression of other genes can indicate the candidate gene's normal role. Alternatively, expression of the gene's protein product can be blocked by injection of antisense morpholino-oligonucleotides (a type of modified oligonucleotide resistant to degradation) directed against the 5 - untranslated region of transcripts. Thus, the effects of loss of the gene's activity can be analysed.

None of our current work involves the use of mutation screens. This illustrates the versatility of the zebrafish model. The utility of its embryos alone justifies its use to study vertebrate development. Hopefully, the expanding use of zebrafish will relieve somewhat the duress imposed on placental animals for studies of embryo development.

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Editor's note

For more information on fish as laboratory animals, see the following paper:

Casebolt, D.B., Speare, D.J. and Horney, B.S. (1998). Care and use of fish as laboratory animals: current state of knowledge. *Lab. Animal Science* **48 (3)**: 124-135.

Learning, animals and the environment: Changing the face of the future

Joint ANZCCART/NAEAC Conference

Novotel Tainui Hotel, Hamilton, New Zealand

28 - 29 June 2001

Exploring the relationship between ourselves, animals, and the environment is the theme of the conference jointly organised by the Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) and the New Zealand National Animal Ethics Advisory Committee (NAEAC). Issues to be addressed include the interdependence and interconnectedness of all life, the images of science and scientists, relevant legislation, dealing with new technology, fish research and what could and should statistics or the popular media tell us.

In understanding these relationships and challenging our beliefs, this conference will help to map the intricate connections between humans, animals, and the environment. It will therefore be valuable to anyone interested in how we learn, communicate and evolve the relationships between ourselves and the natural world.

This conference will be of special interest to those involved in education, in science in both the public and private sectors, and to those interested in teaching, animal welfare, the environment, ethics and the communication and regulation of community expectations. The program will provide New Zealand, Australian and international perspectives.

ANZCCART aims to provide leadership in developing community consensus on ethical, social and scientific issues relating to the use of animals in research and teaching. NAEAC provides independent advice to the Minister of Agriculture on policy and practices relating to the use of animals in research, testing and teaching. A brochure, including a registration form, is inserted in this issue of *ANZCCART News* or, for on-line registrations and conference updates enter the *Gateway of New Zealand Science* http://www.rsnz.govt.nz/

Research using nonhuman primates: Only Two Rs?

Introduction

Probably no animal-based biomedical research generates so much public concern, and so much argument with groups opposed to animal-based research, as research which uses nonhuman primates (NHPs). The very fact which makes such animals such valuable research subjects, that is, their close resemblance and evolutionary proximity to humans, makes their use so disturbing to many (Hampson *et al*., 1990) . Even when the advantages of such research might appear obvious, a large proportion of respondents in a recent poll would not allow NHPs to be used unless there were absolutely no pain or distress (Aldhous *et al.*, 1999). However, research using NHPs has contributed greatly to understanding of human and animal biology in health and disease (Lewis and Carraway, 1992; NCRR / NIH, 1994); and in particular to public health, in the areas of vaccine development and preparation (King and Yarbrough, 1995).

However, when looking at the number of NHPs used in research over the last twenty years, and at the use of other animals (but excluding rats and mice), it is clear that, in the US for instance, the use of NHPs has remained at around 50,000 animals per year, while use of dogs and cats has dropped dramatically (cats, from 68,482 in 1980, to 23,238 in 1999; for dogs, the figures are 188,783 and 70,541. For NHPs the figures are 56,024 and 54,927 (Anon., 1999), but these may be an underestimate.[1]

Figures for countries other than the US are difficult to obtain because of the lack of a central source (although new regulations in the EEC will remedy this in the future) but it has been estimated that in Europe, in 1994, almost 10,000 NHPs were used, almost 5,000 of those in the UK (Jones, 1996), and sporadic figures suggest that also in Europe, use of NHPs has not declined dramatically (Weber, 1997). Moreover, NHP use in industry, which is substantial in some EC countries, is not included in the above figure.

NHP research and the Three Rs

It seems, therefore, that in research using NHPs, the Three Rs of Russell and Burch (1959) may have become Two Rs. There appears to have been, for twenty years, no notable Reduction overall. But has there been Replacement, or Refinement? Or, to phrase it differently, have the benefits from this research increased, or has perhaps the research itself changed? To investigate this, an analysis was undertaken of the number and nature of publications on research using NHPs, using the listings of such publications by Current Primate References™ (CPR), published by the Primate Information Center of the Regional Primate Research Center in Seattle, from 1980 until the end of 1999. CPR, rather than PubMed, was chosen for the analysis because CPR lists, in more than forty categories, all types of NHP research, from field studies to zoo research, from basic biological processes to disease models. CPR's journal coverage is worldwide and includes some publications in languages other than English (mainly French and German, with minor contributions of Russian and Asian languages).

Included in CPR listings are articles, abstracts, books and book chapters (including symposium proceedings), government reports, bibliographies, dissertation abstracts, news items, and book reviews (the latter were excluded from the analysis). CPR also lists (but not by research category) the species of NHPs used, so that an estimate may be made of which species are used, and how frequently.

Numbers of NHP publications, 1980-1999

From 1980 to 1999, the number of publications has increased dramatically, from 4,135 in 1980 to 6,526 in 1999, peaking at 7,696 in 1996. Thus, although the number of NHPs used has not decreased, there is a substantial increase in the number of publications and more and more varying data appear to be produced. In which categories of research, as listed by CPR, have these increases occurred?

When these categories are divided into major areas such as Experimental (invasive, basic and biomedical research), Behaviour (non-invasive, mainly field studies), Biology (minor invasive, basic research), Primatology, Husbandry, and Other (too few papers to allocate to a definite area), "Experimental" papers make up at least half. This suggests that in the near future, in this area, not only is Reduction unlikely, but so may be Replacement. However, basic biological studies appear to have decreased while notable is an increase in the number of papers dealing with behaviour (see Table 1).

Major research categories, 1980-1999

Which research categories contribute the most papers? The most important ones are shown in Figure 1. Together,

Table 1

Percentage of publications/year in major research areas

Year	1980	1984	1989	1994	1999
Experimental %	64.7	62.5	64	52.8	59.3
Behaviour %	13.6	13.8	12.9	20.7	19.7
Biology %	12.4	12.3	10.1	8.8	7.4
Primatology %	5.6	6.6	6.3	10.6	7.6
Husbandry %	3.7	2.9	4.6	4.8	3.5
Other %	0.2	2	1.5	2.3	2.4

1 NHP research and the three Rs

It must be noted that "used" does not imply "killed". Included, for instance, are 20,000+ NHPs in the US Primate Centers, and animals used in multi-year studies. The animals counted are those in research facilities registered under the Animal Welfare Act and / or in Federal research facilities. However, not all research facilities are registered.

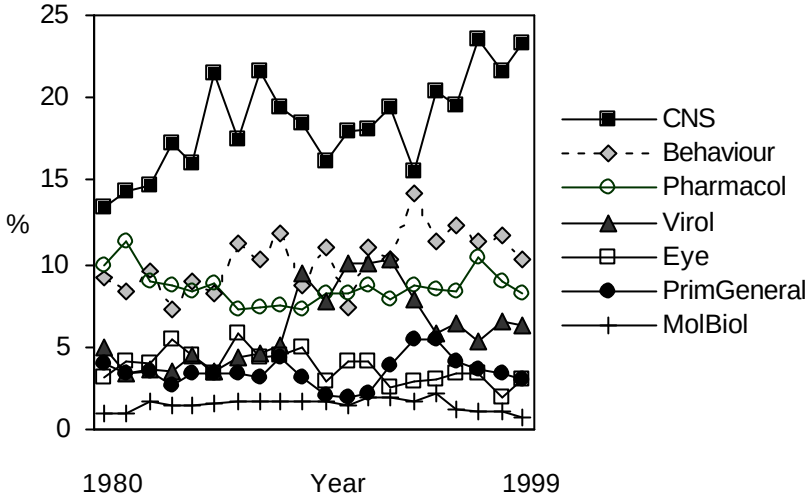

Figure 1: Percentages of total number of publications per year, 1980 - 1999, of most prominent research categories. Together, these seven (out of forty+) categories make up more than half of all publications. Note the importance of the central nervous system (CNS), which in 1999 makes up almost a quarter of the total.

these seven categories make up more than half the total. Papers on the central nervous system (CNS) have the highest percentage every year, increasing to almost a quarter of the total in 1999. Notable also is the peak occurring in virology in the late 1980s: this is associated with the emergence of AIDS (Alter *et al.*, 1984) and the subsequent development of NHP models of HIV infection. Note also the continuing importance of NHPs in pharmacology and therapeutics where numbers of papers have remained steady. As already evident from Table 1, behavioural studies have not only maintained their position but show overall growth, with large increases in some years.

Not shown are some categories that have declined fairly dramatically: these include dental research, cardiovascular research, and metabolism and nutrition. In minor categories where only limited numbers of citations are likely in any year, there nevertheless have been some dramatic changes as well. Substantial increases occurred, for instance, in the category colony management after the Animal Welfare Act in the US was amended, in 1985, to incorporate the provision of psychological well-being of captive NHPs (US Department of Agriculture, 1991), and in Ecology and Conservation, undoubtedly a result of increasing public concern.

From the changes in numbers of papers in major research categories, it may be concluded that perhaps in some, NHPs have been replaced by other species and/or alternatives; it is also possible that some research areas have declined in importance just as others have grown (e.g., virology), or newly emerged with increased technological and computational skills (e.g., molecular biology). This possibility has not been explored.

Use of NHPs in research on the nervous system, 1980-1999

A better idea about specific changes in research practice and use of NHPs may be possible by analysing a specific category such as that on the nervous system (CNS). Not only have publications in this category been the most numerous every year from 1980 to 1999, but the number has increased almost twofold over this period, with citations on the CNS making up almost one quarter of the total number of citations per year (see Figure 1).

The publications on the CNS consist almost exclusively of articles and abstracts. The increase in CNS publications from 1980 to 1999 (from 600 to almost 1600) results not only from an increase in articles (from about 600 to more than 1400), but also from a relatively much larger increase in abstracts (from about 100 to over 600). From this, it may be inferred that although the number of NHPs used per year has not decreased over the period, it seems likely that in the top area of CNS research, more papers are produced, and an active and large research effort is going on, as shown by the many abstacts.

A further analysis may be done by looking only at published *articles* in major journals, since it is these that really represent the pay-off of experiments. Articles were allocated and counted as belonging to certain areas of CNS research, such as anatomy; acute physiology (where animals are killed at the conclusion of the experiment); chronic physiology (where recordings are made, over long periods, in fully conscious, behaving animals), behaviour (including studies of normal behaviour, of animals with experimental CNS damage, and imaging studies); pharmacology; chemistry (including receptor studies); disease models; development; methods and modelling of CNS function; and a small, miscellaneous category.

A full description of these results is beyond the scope of this paper, but some trends will be mentioned. Anatomical studies have declined dramatically, and there have been reductions in acute physiology, but there have been increases in chronic physiology, disease models, and methods and modelling. The decreases reflect changes in the need for particular data (and possibly, in the cost and the number of animals needed), and a change to other animals and better technology in acute physiology. "Chronic" recording, as our only window on what is happening in the conscious brain at the neuronal level, has increased. Such experiments have become more and more sophisticated, both in the types of tasks the animals are taught to perform during recording, and in computer-aided data analysis, and are contributing greatly to our understanding of such higher order functions as cognition, memory, and even attention, decision making and consciousness. The increase in NHPs being used as models of human disease is largely due to the development of an NHP model of Parkinson's disease (Phillips and Burns, 1984) but also to research in Alzheimer's disease. The increase in modelling papers is deceptive since these mostly do not use NHPs at all, instead using computer models of CNS function based on NHP data.

Vision and the motor system are the main areas in CNS research, with other areas being higher order functions like cognition and memory. Not surprisingly, there is frequently overlap between these areas. It seems self-evident that in these areas, there are no adequate non-NHP animal models: NHPs, for instance, share with humans their acute, stereoscopic, and colour vision (Newsome and Stein-Aviles, 1999); as well as a high degree of manipulative skills. Old World monkeys such as macaques, like humans, have a fully oppos-

able thumb and fine distal control of the fingers allowing precision grip (Lemon, 1993) . Another important area is the vestibular system.

Because of these unique biological shared features, replacement by another type of animal is not feasible, and it may well be that more NHPs were used in CNS research in 1999 than in 1980, given the increased number of papers and abstracts. However, the number of NHPs used *per paper* (e.g., in chronic recording studies) appears to have remained steady. Tentatively, it may be concluded that although there has not been a reduction in numbers used, there has been no increase beyond that expected with a larger number of studies. Moreover, when similar studies are compared over the two decade span, there has been a great deal of Refinement: not just in study design and data collection, but also in increased concern with the animals' welfare.

Conclusions

In trying to summarise the trends over the last 20 years in relation to the Three Rs, while there has not been a reduction in the number of NHPs used, nevertheless, there appears to be an increased output of articles, and a wide-ranging and active program of studies, as evidenced by the increased number of abstracts, in many research categories. It seems that NHPs may be used more wisely, and with a better yield of valuable data. What about the other Rs? In terms of Replacement, it appears that in some research categories, NHPs may indeed have been replaced because of substitution of other species (such as dogs), in view of the decline of NHP publications in areas of such importance as the cardiovascular system, while other categories may have declined because of shifts of emphasis on certain types of research. In the largest research category, that of the CNS, there certainly has been Refinement in how and to what purpose NHPs are being used.

It must be kept in mind, however, that experimental studies still make up more than half of all citations. This suggests that indeed, in the foreseeable future, there will be a continued use and need for NHPs in experimentation. Given the high degree of public concern, the ball is in the court of us, who care for and use NHPs, to make sure that care is of the best possible quality rather than the minimum standard required, which may not be the best, and to ensure our ethical and cautious use of these remarkable animals. Furthermore, as scientists, we must be prepared to explain to the public why and how NHPs are used, and that these animals are used in an ethical way. Only thus can the public, whose taxes support research, be convinced it is making an "ethical investment".

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ANZCCART's new address

From 1 February 2001 ANZCCART's office will be in the Department of Environmental Biology at the University of Adelaide. The move followed the relocation of ANZCCART's host Department of Animal Science from the Waite Campus of Adelaide University, where ANZCCART has been located for the past nine years, to the Roseworthy Campus, 50 km from Adelaide.

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Book review

The Use of Animals in Higher Education: problems, alternatives and recommendations

by Jonathan Balcombe

The Humane Society Press, Washington DC, 2000.
ISBN 0-9658942-1-5

Available from Humane Society, Australia for $33 (incl. postage and GST)
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Fax: 02-9973-1729

This book appears at a convenient time when many biology teachers are reassessing their use of animals. It also has a handy format, covering most important aspects of the topic in just 84 pages. Although it follows a slightly unconventional progression of ideas, the book is easy to read, has attractive layout, an effective index, and 300 or so references that are right up to date. So far, so good. To balance the ledger it must also be said that the book has ideological overtones, and advocates a policy for The Humane Society of the U.S. for whom the author works. So while useful, it cannot be regarded as entirely objective and balanced. Due to my own limited experience, I was able to judge only the tertiary teaching content. Secondary education, pre-college courses and science fairs are also analysed, and at a glance look just as well handled as the tertiary component. So the book seems certain to be useful for the whole education industry.

The Introduction spells out the aim "to challenge existing notions pertaining to animals in education", with a special focus on those practices "that incur significant harm" to the animal. Chapter 2 seeks to assess "The Quality and Integrity of Science Education", and expresses concern at the widespread tradition of dissecting animals just for the perceived purpose of "naming structures". Alternative educational approaches, it is argued, offer similar challenges without dissecting animals. The chapter pleads that these alternatives be considered as replacements for dissection, both for their effectiveness and to save animal lives.

However, Chapter 2 also reveals one of several examples of confused reasoning that I feel arise from failure to identify educational aims. Balcombe implies that 'hands-on' dissection could be replaced by 'active' learning, the modern educational technique that demands "additional cognitive processes while learning". But, apart from being 'active', the two techniques are not necessarily similar. Compare their aims using Bloom's classification of common educational objectives (see Kemp and Smellie, 1989, p. 20), which are to gain knowledge ('cognitive'), acquire manual skills ('psychomotor'), and develop appropriate attitudes ('affective'). The special value of hands-on activities is in developing psychomotor skills (as Balcombe admits, p.7) whereas active learning in general makes no demand for manual skills - its goals are mainly cognitive. Balcombe's plea would be more convincing if it recommended dissection when (but only when) manual skills and live animal handling were essential, and promoted 'alternative' methods for learning about body structures and, where possible, body functions too.

The discussion of "Sociological Issues" in Chapter 3 is particularly useful, if contentious. It addresses an aspect of animal biology ignored (perhaps even rejected) during most of my life as a student and teacher, namely the development of appropriate 'attitudes to animals'. This is now the subject of whole books (e.g., Dolins, 1999). Student sensitivities to the well-being of animals being studied have too often been mismanaged. By dismissing student concerns, teachers can alienate good minds that are otherwise keen to learn about animals. Not all students of animal biology need dissecting skills – for example, most nutritionists, ecologists, and epidemiologists do not. If those who need such skills are told why it is important to acquire them, and others are not forced to do so, education can probably proceed both more effectively and with better student motivation.

Teachers deciding how to meet specific objectives in animal biology will be well served by Chapters 4 and 5. Educational assessments are made of various practical exercises that use animals, as well as of the merits of computer packages and programs that do not. The viewpoints of some defenders of animal dissection are also presented, if without much sympathy. Tabulated comparisons are given of student performance after using traditional and 'alternative' methods of learning animal sciences. Unfortunately some of these comparisons are not convincing. Although the impression is given that 'alternatives to dissection' are educationally effective, the limited description of the tests leaves open the possibility that some tests assessed only 'cognitive' gains, not 'manual' skills or 'attitudes'. To be fair, instances are cited where students who had not dissected animals are reported to have good practical skills. The type of test used needs to be especially clear because sceptics will be reluctant to accept that computer programs and models develop laboratory skills, at least until 'virtual reality' instruction on computers is available.

Students training to handle live animals - veterinarians, zoologists, and research-workers in physiology and pharmacology need to acquire skills in laboratory practice (see Dewhurst, 1999, p. 214). In Chapter 5, Balcombe discusses the specific problems of gaining these, noting rather hesitantly, that 'alternatives' are available. He seems to suggest (and I would support him if that is his intention) that 'alternatives' should be used as far as possible, after which expert staff should assist students to acquire the more specialised manual skills and humane ways of applying these to live animals.

Finally, Chapter 6 discusses Law and Policy Issues. Some readers seeking only practical guidance may regard this as a waste of space; some might even see it as a provocative guide to student protest. But ethical review is now part of society, and the chapter establishes important links between society and education. Public knowledge about animals is so widespread that critical attitudes to animal care are already in the minds of many students before they begin training. Perhaps more open discussion of educational goals, of humane yet effective teaching methods, and of the personal agendas of students would help make a resort to law rare or unnecessary. Chapter 6 will assist with this.

The ideas in this book are socially and educationally significant, and it is useful to have them so accessible. So long as readers remain alert to the possible 'spin' on some arguments, they will find the book of considerable value in reaching an informed, rational and compassionate decision about when to use, and not use, animals in their teaching.

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Letters

Comments on the introduction of wild caught animals to the laboratory

The use of wild caught animals for laboratory based experiments is a relatively common technique in ecology (e.g., Wood, 1970; Huang, 1986; Woolley, 1988; Brillhart and Kaufman, 1991, Woolnough and Carthew, 1996). If no breeding colony exists, it is often the only means of obtaining certain types of information regarding the species' ecology and biology. Use of wild caught animals may also provide more accurate and reliable data.

While there has been much published on the maintenance (i.e., housing, feeding and breeding) of captive populations (e.g., Evans, 1982), there seem to be few published articles that outline a suitable protocol for the introduction of wild caught animals to a laboratory environment. Indeed, very few authors actually describe the process they used to introduce animals to the laboratory or the survival rate of captured animals. Here, I present a short commentary on my experiences in introducing wild caught *Ningaui yvonneae* (Dasyuridae : Marsupialia) to captivity.

The *Ningaui yvonneae* is a small insectivorous marsupial which lives for up to 12-18 months (Bos and Carthew, in press). Its conservation status is common, being found across much of southern Australia. Captive specimens from the Eyre Peninsula (South Australia) were to be used in the investigation of the foraging behaviour of the species. Permission was obtained to capture and house up to 20 *N. yvonneae* for these experiments. Animals were housed in Nally tubs (65 x 40 x 30 cm), in which was placed a layer of sterilised sand (1-2 cm deep), a small nesting box (containing sand, leaf litter and a 10 cm section of PVC pipe) and one or two small leafy branches (approximately 20 cm long each). Ningauis were fed daily on Wombaroo™ small carnivore food (1-2 teaspoons) supplmented by 3-4 mealworms/beetles or cockroaches (depending on availability). Live insect prey were placed under the leafy branch to encourage animals to forage. Water was provided *ad libitum*. Animals were housed under reverse lighting conditions (approximately 14 hr day and 10 hr night).

A total of 18 *N. yvonneae* was caught on two occasions. Eight and ten individuals were captured during the periods 2-17 December 1998 and 8-9 March 1999, respectively. The December collection consisted entirely of mature, post-breeding males (aged approximately 14 months). The March collection was composed of two female and eight male ningauis, all of whom were juveniles (aged approximately 5 months).

The survival rate of the two collections differed markedly. Most animals from the December collection survived for 4 to 5 months. This was considered to be successful given their age at the time of collection. In the wild, most of these animals would have died by February (Bos and Carthew, in press). Two of the specimens from the December collection survived until June 1999. The March collection was considered unsuccessful, since most specimens (80%) had died within 8 days of collection. All animals ate well while in captivity and showed no obvious signs of disease. The cause of death was discussed with a veterinarian and was attributed to a delayed stress reaction. The contrast in death rates of the two collections was probably due to different levels of stress resulting from the different protocols used to introduce each collection to the laboratory. These differences, which were unintentional, are listed below.

1. **Time between capture and transportation to the laboratory**. The majority of specimens in the December collection were retained at the study site for 4 to 5 days prior to transport to the University. In comparison, five ningauis from collection two were transported on the day of capture and five after one night in captivity only.

2. **Time between entry into the laboratory and introduction to the reverse lighting room.** The March collection was introduced into the reverse lighting room immediately upon arrival at the University. Ningauis from the December collection were introduced 2 to 3 weeks after their arrival, because the room was initially unavailable.

3. **Difference between field and laboratory temperatures**. Both collections occurred when maximum daily temperatures ranged between 34 and 37^0C. The small animal house is normally kept at a constant temperature of 22^0C. However, for both collections, the thermostat controls were temporarily faulty. For the December collection, laboratory temperatures were higher than normal, around 26-27^0C. In contrast, laboratory temperatures for the March collection were well below normal, at 15-17^0C.

While there is no direct evidence that the different introduction procedures influenced the survival of specimens, the explanation is credible. In my experience, *N. yvonneae* is not a particularly difficult species to house in captivity. It has a high survival rate during short-term field-based captivity and reacts well to handling and investigatory research techniques. Wild caught *N. yvonneae* specimens have also been used by various other authors (e.g., Baverstock and Aslin, 1975; Geiser and Baudinette, 1988; Calver, Bradley and King, 1988; Calver, King, Gardner and Martin, 1991) and have been housed previously at the Roseworthy campus of Adelaide University (Woolnough and Carthew 1996). In this instance, most animals (collected in March/April 1992) survived throughout the year, and a number even survived several years. These specimens had also been collected from the Middleback Ranges, although the precise collection procedure is unknown.

When considered separately, it is unlikely that any one of the above mentioned factors would have impacted on the survival rate of the ningauis. However, in combination, their influence is potentially much greater. The slower introduction to new environments (e.g., Nally tubs and laboratory) and laboratory conditions (constant temperature and reverse lighting) of the December collection may have given animals more time to adjust, thus minimising stress. The different ages of the animals may also have contributed to the different survival rates of the two collections. It is possible that the younger animals of the March collection were more sensitive to capture stress and/or disease. However, I would expect the older specimens to be more susceptible. It is common for Dasyurids of older age to be captured in poor condition (e.g., worn teeth, poor condition of coat, loose fur) especially post-breeding males (e.g., Woolley, 1991; Leung, 1999).

It is important to give due consideration to the capture, transport and introduction protocol of wild animals to a laboratory situation. In particular, animals should be introduced slowly into the laboratory when conditions differ significantly from those in the wild. Ideally, laboratory conditions at the time of entry

should closely mimic those of the field.

I encourage researchers to publish techniques used to acclimatise animals and survival rates of animals in the hope of reducing mortality rates.

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Note

Wombaroo food products are available from:

Native Trading
22A Chasewater Street
Lower Mitcham SA 5062
Tel: 08-8277-7788

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Should an "Animal Welfare Officer" be a veterinarian?

During the recent ANZCCART Workshop for Animal Welfare Officers held in Adelaide (November 2000), one session was devoted to the issue of "Animal Welfare Officers in scientific institutions". The role of such a position, and the skills and qualifications required of the incumbent have been the subject of much debate for some time. It was apparent from the discussion during the workshop that the impasse over a "definition" of an Animal Welfare Officer (AWO) is whether or not the person must have veterinary qualifications. Several factors contribute to the complexity of the issue.

The duties of an AWO vary enormously between institutions. The range includes provision of administrative services to the institutional Animal Ethics Committee (AEC), Executive Officer to the AEC, member of the AEC, management of animal facilities, provision of veterinary services and advice, development of administrative/technical policy and procedures documents on matters relating to the care and use of animals, monitoring the use of animals, collaboration in research protocols, maintenance of institutional compliance with relevant legislation, and training of research and technical staff. It follows that the qualifications of an AWO vary between institutions, with the position embracing administrative, veterinary, managerial, scientific, animal care and/or educational skills.

The statement that "an AWO must be a veterinarian" infers that only a veterinarian can be responsible for animal welfare. There is no doubt that veterinary advice is essential in many areas of animal research. There is also no doubt that most members of the team involved with the care and use of animals for research or teaching purposes are immensely capable of assessing and caring for the welfare of an animal. The team includes animal technicians and researchers, as well as veterinarians. However, when an animal's health or welfare is compromised because of disease or experimental intervention, an assessment of its clinical signs, diagnosis, prognosis and treatment will be necessary. Responsibility for these aspects of animal welfare is most appropriately assumed by the professional trained in this specific discipline, i.e., the veterinarian. Such advice can be provided by an institutional AWO who has veterinary qualifications, a veterinarian employed in another area of the institution (e.g., veterinary faculty), or by an external veterinarian specifically employed by the institution for this purpose.

One approach to the issue of "what is an Animal Welfare Officer?" could be to acknowledge that the inclusion of the term "welfare" in the title is a significant hindrance to agreement for a definition of the position. Resolution may be assisted firstly, by the recognition that animal welfare is not the exclusive property of the veterinarian; and secondly, by the recognition that veterinary qualifications are necessary for the provision of professional advice regarding the clinical aspects of animal welfare, whether this be specific areas of the care and use of animals (e.g., anaesthesia,

surgery), or the diagnosis, prognosis and treatment of an animal's clinical condition. The requirement for veterinary qualifications may be a reasonable basis for separation of the diverse roles of an AWO, rather than a specific requirement for the position. When an institutional AWO's responsibilities do not include the provision of veterinary advice, consideration could be given to the renaming of the position as "Animal Ethics Officer". When an institutional AWO's responsibilities include the provision of veterinary advice, consideration could be given to the renaming of the position as "Veterinary Officer" or "Animal Ethics and Veterinary Officer".

This may be a simplistic treatment of a complex situation. However, removal of the term "welfare" from the title of "Animal Welfare Officer" also removes the unfortunate implication that the ability to assess the welfare of an animal is restricted to only certain members of the team.

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Newly Published

CpG DNA - a new adjuvant

According to a recent paper by Weeratna *et al.*, (2000), a new adjuvant CpG DNA, when compared with other adjuvants used in animal research (Freund's complete and incomplete adjuvants, Titermax Gold) and in humans (alum), had the greatest potential to augment immune responses with minimal side effects at the injection site.

It is a new type of adjuvant, based on CpG technology called Immuneasy™ Mouse adjuvant and has the following benefits:

* negligible tissue damage to mice when administered via intramuscular, intradermal or sub-cutaneous routes;
* high titre of antibody produced (slightly higher than Freunds);
* time saving - prime and boost only once requires less "mouse time";
* ease of use - aqueous solution does not require glass syringes;
* time saving and easy to use - simple add - mix - inject protocol; and
* cost saving - requires only 2 µl of antigen.

For further information contact Ms Rosemary Paxton, Qiagen Pty Ltd., Tel: 03-9489 3666, Fax: 03-9489-3888.

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Animal Experimentation: A Guide to the Issues

Vaughan Monamy
Australian Catholic University, Sydney

Cambridge University Press,
($31.80 Pb / $84.50 Hb)
ISBN 0521667860 / ISBN 0521660939

In 1996, ANZCCART published a monograph by Dr Vaughan Monamy entitled "Animal Experimentation: A Student Guide to Balancing the Issues". Dr Monamy has recently updated and expanded this book for worldwide publication by Cambridge University Press, UK. Released in December 2000, the new book covers issues inherent in the animal experimentation debate. Included are discussions of the history of animal experimentation, the rise in opposition to the practice, the moral status of animals, the regulation of animal experiments in Australia, New Zealand, Britain and North America, and alternative practices based on the Three Rs.

Primarily aimed at life science students, its clarity of style will enable lay people and experts to read it with equal ease. Its balanced treatment covers the arguments that both support and oppose animal experimentation. Students, researchers and animal ethics committee members will find a non-intimidating, readily understood introduction to the principal ethical arguments in the animal experimentation debate.

New ANZCCART Publication

Housing for laboratory rats, mice, guinea pigs and rabbits

by Ann Hargreaves

This 150 page monograph (ISBN 0 958682 35) has been published by ANZCCART following a recommendation from the Australian and New Zealand Society for Laboratory Animal Science (ANZSLAS) that ANZCCART undertake this task.

The Board of ANZCCART commissioned Dr Ann Hargreaves, an animal scientist in Melbourne, to write the monograph. Assistance was provided initially to Dr Hargreaves by a working party from ANZSLAS, but all of the research and writing was done by Dr Hargreaves, to whom ANZCCART and ANZSLAS express their thanks for a difficult task performed very well. It is available at the cost of $A50.00 (+$5.00 GST in Australia) from ANZCCART's Adelaide Office. This includes packing and surface post for Australia and New Zealand.

An order form is inserted in this issue.

ANZCCART awards two prizes

ASCEPT conference 2000, Newcastle

The winner of the ANZCCART Prize ($200) at the ASCEPT 2000 Conference was Hannah Culver, a post-graduate student in the Department of Physiology and Pharmacology at the University of Queensland. Her work involved studying the effect of human tumor necrosis factor and interleukin-1 on inflammatory responses in guinea pig airways and skin *in vivo.*

Her poster and accompanying abstract, along with her discussion with the judges, showed that she had an excellent appreciation of the need for reduction and refinement in experiments using animals and that she had implemented some very appropriate measures to achieve these aims.

Studies *in vivo* are necessary for this work because of the complex set of interacting mediators involved in inflammation. Models are also needed for the testing of novel drugs before introduction into man.

Guinea-pigs were chosen because of their history as a preferred species in studies relating to human airway inflammation and especially plasma protein exudation. The use of the skin model was justified as fewer guinea-pigs were used to obtain information on doses and timing of allergen, mediator or cytokines (up to 16 results could be obtained from each guinea-pig compared with one in airway experiments).

This Prize is awarded annually for the best poster by an honours or postgraduate student at the ASCEPT conference. The work must be in keeping with the objectives of ANZCCART.

ANZCCART's 2000 conference, Adelaide

The 2000 ANZCCART Student Award was won by Rebecca Sargent, a PhD student at the University of Melbourne. Her paper was entitled *The welfare, behaviour and performance of growing pigs in a deep-litter, group-housing system.*

Deep-litter, group-housing systems have been developed as an alternative housing system for growing pigs. Conventionally, growing pigs are kept in a more confined housing system. These systems are indoors and have an automated ventilation system, concrete/slatted floors, liquid manure handling systems (effluent ponds) and smaller group sizes with reduced space allowance/pig. Alternatively, deep-litter group housing systems are naturally ventilated, have a floor base of deep litter, larger group sizes, and the pigs have a greater space allowance.

These deep-litter group housing systems are cheaper to establish and are perceived as being more "welfare-friendly" for pigs, compared to conventional intensive housing systems. However, recent industry records have shown that pigs in deep-litter systems are about 10% less efficient in converting feed to live weight gain, are fatter, and have a higher incidence of bruising and joint damage, compared to conventionally housed pigs.

This annual Award covers travel, registration and accommodation at the conference to a maximum of $1,000. The student gives a short paper at the conference and the written paper is published in the conference proceedings.

Notice of a joint conference (AVERT and ANZCCART)

14-16 May 2001

Melbourne, Australia

The nature of pain

During the Pan-Pacific Veterinary Conference to be held in Melbourne in May 2001, AVERT (Australian Veterinarians in Ethics Research and Teaching, a Special Interest Group of the Australian Veterinary Association), in conjunction with ANZCCART and Boehringer Ingelheim Pty Ltd, is presenting an in-depth examination of the nature of pain. The program will commence with an ethical overview of the responsibility of humans to animals in pain. This will be followed by a systematic consideration of pain generation and transmission, the effect of pain on animals and their response to it. Papers will review the latest available therapeutic mechanisms of the prevention and amelioration of pain in a variety of species.

The AVERT/ANZCCART program is aimed at assisting conference delegates to develop processes for assessing whether pain is present and evaluating the effectiveness of the pain control methods employed. Leading Australian and international speakers will address all of these issues in what promises to be an exciting and stimulating three day program of interest to many areas of the Australian and New Zealand veterinary and research communities. Speakers will include Dr Karol Matthews (Canada), Dr Christina Dart (Sydney) and Professor David Mellor (New Zealand). All persons are welcome to attend.

The program will run from 14 to 16 May 2001. Further details will be available in the next Newsletter, or can be obtained from the Australian Veterinary Association website at http://www.ava.com.au/ (click on "conferences").

News

International course on Laboratory Animal Science - Utrecht, The Netherlands

May 14 - 25, 2001

A two week intensive course on laboratory animal science will be held at the Department of Laboratory Animal Science. University of Utrecht, Utrecht, The Netherlands in May 2001. This course has been held each year since 1993.

The objective is to present basic facts and principles that are essential for the humane use of animals and for the quality of research. The contents of the course are in line with recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) regarding the training of the young scientist whose research involves the use of vertebrate animals.

The course may also be of interest for those who intend to set up a similar course at their location.

For information and application forms please contact:

Prof. L.F.M. van Zutphen or
Mr Stephan van Meulebrouck
Department of Laboratory Animal Science
Faculty of Veterinary Medicine
PO Box 80.166
3508 TD Utrecht
The Netherlands

Phone: 31-30-2532033
Fax: 31-30-2537997

Email: pdk@las.vet.uu.nl

What's new at ILAR?

The Institute of Laboratory Animal Research of the US National Research Council has published *Definition of Pain and Distress and Reporting requirements for Laboratory Animals: Proceedings of the workshop held April 22, 2000.*

The aim of this ILAR/NIH joint workshop was to provide feedback from the scientific community to the USDA regarding the lack of a functional definition of distress as well as the efficacy of continuing to use current categories to report pain and distress. The speaker areas of expertise and perspectives ranged from scientific research to animal welfare policy, protocol review and relevant organisation or institutions.

Copies are available directly from ILAR. Visa and Mastercard orders are acceptable by phone at 1-202-334-2590 or by fax at 1-202-334-1687. The cost is $US15 and $US20 (foreign).

Another publication available from National Academy Press is - *ence cost containment in animal research facilities.*

This is the second report of the National Research Council's Committee on cost of and payment for animal research, in which the committee presents its conclusions and recommendations regarding cost containment methods for animal research facilities. This follows the Committee's initial report that examined interpretation of governmental policy concerning institutional reimbursement for overhead costs of animal research facilities. Copies can be obtained from the National Academy Press by credit card payment (call 1-800-624-6242) or on the internet on www.nap.edu/bookstore

Newly Published: Volume 42(1) of ILAR Journal.

The articles in this issue cover laboratory animal allergy and complement and update the 1997 ILAR publication *Occupational Health and Safety in the Care and Use of Research Animals.* The issue provides an overview of the science and pervasiveness of laboratory animal allergy, specific information on controlling exposure, recommendations for replacement evaluation and medical surveillance of workers, and assessment and treatment of common symptoms such as allergic rhinitis, conjunctivitis and asthma.

To obtain a copy of this issue or a subscription through the ILAR Associates program, contact the Journal office (email: ilarj@nas.edu) or visit the website http:www.national-academies.org/ilar

2001 SCAW Conference

Best Practices for Research Animal Well-being

ANZCCART's US counterpart, the Scientists Center for Animal Welfare (SCAW), will organise a conference on *Best Practices for Research Animal Well-being* in Baltimore on May 17-18, 2001.

Topics to be covered include:

* impact of noninvasive technology on animal research;
* human euthanasia trends and practices for research animals;
* animal welfare confronts transgenics;
* umane and appropriate anaesthesia and analgesia;
* medical imaging in laboratory animals as a refinement; and
* how to achieve best practices and comply with the law, policies and other requirements.

Further information about this conferences is available by email at info@scaw.com, or on the SCAW website, www.scaw.com

ANZCCART News is published quarterly by the Australian and New Zealand Council for the Care of Animals in Research and Teaching Limited.

It is a publication for researchers and teachers; members of animal ethics committees; staff of organisations concerned with research, teaching and funding; and parliamentarians and members of the public with interests in the conduct of animal-based research and teaching and the welfare of animals so used.

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