ANZCCART NEWS

Australian and New Zealand Council for the Care of Animals in Research and Teaching

The National Statistics Package — background and progress

In 1989, the Australian Senate Select Committee on Animal Welfare recommended that a summary of the animals used in teaching and research should be produced by each State and Territory and that these should be collated on a national basis. This recommendation has been extremely difficult to put into practice for several reasons. The definition of "Research and Teaching" varies between States. In some cases, wildlife environmental studies are not included. Similarly, animals held in schools and kindergartens may or may not be included. To make the problem even more difficult, States required different information and reported data in different formats which were not compatible. Despite the difficulties, it has been acknowledged since 1989 that there is a need for a clear, concise and accurate mechanism for reporting such data.

A statistical collection system must be accurate, simple, concise, easy to use and easy to collate. As the level of computer literacy is also highly variable, any computerised system has to be simple. Detail had to be minimised but essential data had to be collected for the figures to be meaningful. The biggest impediment to the collation of statistics has been the sheer volume of projects considered by Animal Ethics Committees (AECs). The development of databases, particularly software development kits which are compatible with both the Windows and Macintosh environments, has made an almost impossible task much easier. Before a database could be developed, consensus had to be reached on what data were to be collected and in what detail. The revision of the Code provided the vehicle for determining what was to be included. A set of parameters was defined and endorsed by the NHMRC Code Liaison Group, and the National Consultative Committee on Animal Welfare (NCCAW), in conjunction with the States and other interested parties.

New South Wales and Victoria have produced annual statistical reports for some years. However, the methodologies used have been different. Collation was difficult because animals were counted several times if projects were approved by more than one committee, or if one animal was used for several purposes. Terminology also presented problems. A rat could be recorded as a Hooded Wistar, a *Rattus norwegicus* or simply as a rat in different returns. To overcome these problems, the NHMRC Code Liaison Group recommended that standard data be collected across the nation using common names and be collated in a consistent manner.

A trial version of a database statistics package was developed and the South Australian AECs agreed to test the system during the 1995/96 financial year. Feedback from those Committees, the NHMRC Code Liaison Group, NCCAW and other interested parties on a national basis was obtained. With the assistance of funding provided by the NHMRC, the database was then modified and refined.

The package has been endorsed by the NHMRC Animal Welfare Committee and distributed to all States and Territories for acceptance and implementation.

The database will be trialed on a national basis during 1997. Each State and Territory will be responsible for collating its own data and ANZCCART will collate the data on a national level. If all deadlines are met (which is probably optimistic), the first national summary will be completed for the 1997 year by mid-1998.

The package, and the accompanying instruction book *A Beginner's Guide to the Animal Use Statistics Package* will evolve over the next few months as people try it out and pin-point any difficulties they discover. By the end of the year, an upgraded version of the package will be incorporated into the home page of the ANZCCART Web site. This will enable AECs and researchers to download the database without having to obtain and load disks. Similarly, collation of data will be even easier.

For many years, there has been considerable public interest in animal based research. People want to know what sort of animals are used, what is done to them, how many animals are involved and why the research is done. The statistics package aims to answer those questions in terms which are meaningful and relevant. Within the limits of commercial confidentiality and individual privacy, the public has a right to know how its money is spent and what actually happens to the animals involved. By using simple language and identifying the key issues, the database will be able to answer those questions. Through all stages of development, the emphasis has been on developing an accurate, clear and simple mechanism to obtain meaningful data relating to animal use in research and teaching.

We now have the ability to act on the recommendation which the Senate Select Committee made eight years ago. The financial and moral assistance given by the NHMRC has made that ideal possible. The comments received from animal welfare groups, researchers and other interested parties have made the data relevant to the people who will use it. The availability of the ANZCCART Web site has made it accessible. The package will evolve according to feedback received. Everything is in place but it can only work with your participation and support.

Deborah Kelly
Dept of Environment and Natural Resources
Adelaide, SA

Polyclonal antibody production and adjuvants - a dilemma

Polyclonal antibody production is a complex issue and sometimes seems to be incompatible with animal welfare requirements of using procedures that cause minimal harm to experimental animals and using as few animals as possible. The purpose of this article is to suggest strategies to produce antibodies without violating animal welfare requirements. The suggestions refer to the production of polyclonal antibodies only and are based on the assumption that savings in time, money or labour are not valid reasons to compromise animal welfare. The strategies suggested here are aimed at achieving the goal of an experiment (e.g., an antibody suitable for the intended application) rather than producing an antibody of highest specificity and affinity.

Choice of adjuvant

This is probably the most important single factor affecting animal welfare. The dilemma is that many adjuvants can be noxious to animals and produce significant pathological lesions. The choice of adjuvant is made even more difficult by the large number of adjuvants available. Most adjuvants will fit into one of the following categories:

- Mineral oils (e.g., Montanides, Freund's adjuvant);
- Aluminium salts;
- Saponins (e.g., Quil A);
- Gamma-inulin;
- Peptides; or
- Combinations (complete Freund's adjuvant, ISCOMs, Algammulin, Montanides).

Unfortunately, most information on the efficacy of adjuvants and their pathological effects is anecdotal, making the rational choice of the appropriate adjuvant rather difficult.

Adjuvants are required because many antigens used to induce antibodies are only weakly immunogenic and require an adjuvant to boost the immune response. This is particularly true for some of the modern recombinant proteins or subunit vaccines. Vaccination of sheep with a recombinant parasite protein showed that it was difficult to induce antibodies without the use of adjuvants (Deol *et al.*, 1995). The results are illustrated in Figure 1, which shows antibody titres against a recombinant parasite protein (the larvae of the dog tapeworm *Taenia ovis*, the causative agent of sheep measles) achieved in sheep with and without adjuvants. Mean antibody titres in the group which received no adjuvant (saline group) were very low. Freund's adjuvant produced the highest antibody titres, being more than twice as high as the titres achieved with Alum and Algammulin. This excellent performance of Freund's adjuvant is the reason why it is often referred to as the gold standard. However, further immunisation experiments conducted at Agriculture Western Australia using various adjuvants and a conventionally purified bacterial protein as an antigen (*Dermatophilus congolensis*, the causative agent of lumpy wool disease) showed that Freund's adjuvant is not always the best choice (Figure 2). Several adjuvants (Montanide, Quil A, Auspharm, ISCOMs and even Alum) produced higher antibody titres than Freund's adjuvant (T.M. Ellis, personal communication).

These experiments illustrate that, although Freund's adjuvant is generally regarded as a potent adjuvant for most antigens, it is not always the best choice. The optimal adjuvant seems to be dependent on the antigen and the intended application (Kenney *et al.*, 1989).

Freund's and other adjuvants, particularly mineral oils, are known to cause local reactions at the injection site (Mallon *et al.*, 1991; Johnston *et al.*, 1991; Broderson, 1989). This is particularly true if complete adjuvant is given more than once to the same animal (Laufer *et al.*, 1959; Steiner *et al.*, 1960). Accidental injection in man can result in severe reactions (Chapel and August, 1976). Extreme care is therefore necessary when handling complete Freund's adjuvant to avoid accidental injection or contact of the adjuvant with eyes.

In an immunisation experiment conducted at Murdoch University using the experimental antigen Keyhole Limpet Hemocyanin, we compared antibody titres and lesions produced by the following three adjuvants: Freund's incomplete adjuvant (FIA), Montanide ISA 50 and Algammulin (Figure 3). The mean antibody titres produced in the Montanide group were again higher than in the Freund's adjuvant group. However, Freund's adjuvant produced significant lesions (six out of seven animals had multiple nodular lesions larger than 10 mm in diameter). Montanide ISA 50 also produced multiple large lesions in some animals, but the overall lesion score was lower. Algammulin produced only small single nodular lesions with a diameter of less than 10 mm. This confirmed that Freund's adjuvant can produce significant local reactions at the injection site. However, it also showed that other adjuvants, often considered as alternatives to Freund's adjuvant, can produce significant granulomatous lesions. It is interesting to note that immunisation with complete Freund's adjuvant seems to be well tolerated in chickens without development of local granulomatous reactions (Gassman *et al.*, 1990; D. Palmer, unpublished observation).

From a practical view, the choice of the adjuvant is still empirical. A good strategy would be to start with the least noxious adjuvant if the antigen is readily available or the quality of the required antibody is not very critical. Antigens that are difficult to purify or are available only in small amounts may justify the use of more potent (but also more noxious) adjuvants such as Freund's or Montanide.

Immunisation protocol

Although there have been numerous studies on the effect of the immunisation protocol on the antibody response (Hu *et al.*, 1989; 1990a; 1990b) there is little convincing evidence that the final antibody titre is significantly affected by the antigen application site (intravenously, intramuscularly, subcutaneously, intraperitoneally, intradermally) or the length of the booster intervals. For most practical applications the protocol to follow is one that causes the least harm to animals. The tradeoff for minimising the stress to animals (long booster intervals, intramuscular or subcutaneous injections) may be that it takes longer to achieve good antibody titres.

The intravenous route usually requires large amounts of antigen and is not suitable for most adjuvants. Booster injections given intravenously

Elsewhere in this issue ...

Figure 1: **Comparison of mean antibody titres in 4 groups of 5 sheep immunised with recombinant *Taenia ovis* antigen in saline (no adjuvant), Freund's complete and incomplete adjuvants (FCA/FIA), Alum, and Algammulin.**

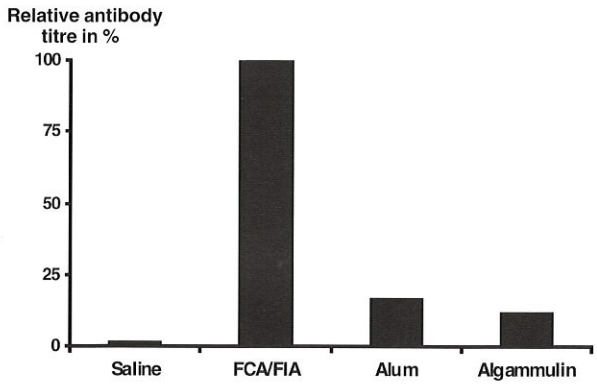


Figure 2: **Comparison of mean antibody titres in sheep (3-5 animals per group) immunised with purified *Dermatophilus congolensis* proteins in saline (no adjuvant), Freund's complete and incomplete adjuvant (FCA/FIA), Quil-A, Montanide ISA 206, Montanide ISA 50, Alum, Auspharm, GERBU and ISCOMs.**

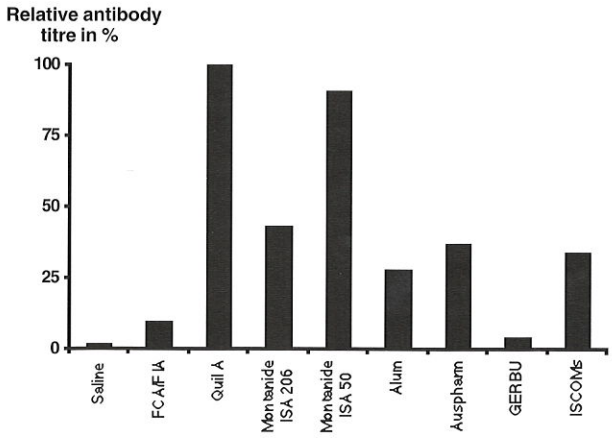
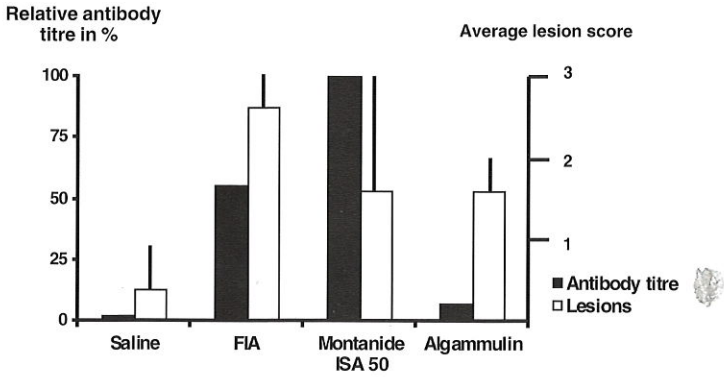
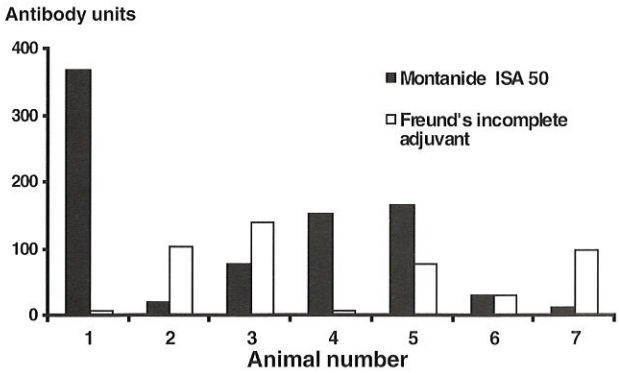


Figure 3: **Comparison of antibody titres and lesions (average and maximum scores) in 4 groups of 7 sheep immunised with Keyhole Limpet Hemocyanin in saline (no adjuvant), Freund's incomplete adjuvant (FIA), Montanide ISA 50 and Algammulin.**



Lesion score 1 = single granuloma smaller than 10 mm in diameter
Lesion score 2 = single granuloma larger than 10 mm in diameter
Lesion score 3 = multiple granulomas larger than 10 mm in diameter

Figure 4: Comparison of antibody titres in individual sheep immunised with Keyhole Limpet Hemocyanin in Freund's incomplete adjuvant and Montanide ISA 50.



can be dangerous, because they can elicit immediate hypersensitivity reactions. The intravenous route is sometimes chosen for a booster injection for monoclonal antibody production to stimulate spleen cells prior to fusion.

The intramuscular injection is only an option in larger animals but requires good knowledge of the anatomy to avoid damage to adjacent structures (nerve bundles, major blood vessels, tendons, joints) by irritant and noxious adjuvants. Damage to muscle tissue can be considerable and the cause of severe pain. However, in the hands of an experienced operator it is a safe way of delivering an antigen.

Intraperitoneal injections are sometimes used for monoclonal antibody production in mice, but should not be used for polyclonal antibody production. The procedure can be dangerous and, in combination with irritant adjuvants, very painful.

Similarly, the intradermal route can cause painful ulceration and it is not suitable as a routine procedure. Its use requires special justification. Intradermal injection delivers the antigen to a different compartment of the immune system and therefore can be an efficient way of inducing an antibody response against minute amounts of antigen. It should only be considered if everything else failed.

Subcutaneous injections are convenient for the investigator and safe for the experimental animals. They should be used whenever possible except in poultry, where the intramuscular injection into the breast (pectoral) muscles is probably easier and safer than subcutaneous injections. An injection site should be chosen that can be readily observed and treated, should lesions develop. In smaller laboratory animals it is important to avoid sites that are normally used for handling and restraining. Multiple injection sites are sometimes necessary, mainly to reduce the volume of an irritant adjuvant per site. There is probably little gained, from an immunological point of view, by distributing the antigen over multiple sites, although this is disputed by some.

Intervals between booster injections should be long, at least 4 weeks or preferably longer. Initial immunisation with 2-3 booster injections followed by a rest for 3-4 months before a final booster injection (without adjuvant) often results in a dramatic increase in the antibody titre (memory response). It is always worthwhile to make use of this memory response, because it often results in antibodies with high titre and affinity.

Number of animals

Analysis of antibody titres in individual sheep immunised with antigen in Freund's adjuvant or Montanide ISA 50 showed that four out of seven sheep produced good antibody titres (Figure 4). This means that at least one out of three animals should provide a good antiserum, an observation shared by others (Garvey *et al.*, 1977; Sigel *et al.*, 1983). This ratio is probably even better in inbred strains of animals . Thus, for most antigens and animal species, the immunisation of three animals will be sufficient for the production of useful antibodies. The number of animals can be significantly reduced by sequential rather than simultaneous immunisation of animals (Hanly *et al.*, 1995). There is a good chance that the first animal used will produce a good antiserum. This strategy also reduces the amount of antigen required, an important consideration when dealing with antigens that are scarce. Antibody production by sequential immunisation can take longer, but minimises the number of experimental animals used.

Animal species

The animal species used for the production of antiserum is unimportant for most applications. Secondary antibodies against most common animal species are now available from many commercial suppliers. There are only a few specialised applications, where antibodies from a certain animal species are preferred. A good example is the precipitation properties of horse antiserum for immuno-electrophoretic applications or as antitoxins for therapeutic purposes. Chicken antibodies do not cross-react with immunoglobulins from mammalian species and don't bind to proteins A and G, which can be an advantage or disadvantage depending on the intended application. However, some adjuvants are not tolerated by all species. Horses and dogs, for example, are very sensitive to Freund's adjuvant and Quil A (Dalsgaard *et al.*, 1990; Hanly *et al.*, 1995).

An important aspect in the choice of animal species is the amount of antibody that can be harvested. The more antibody obtainable, the less likely that an experiment has to be repeated at a later date. This minimises the number of experimental animals used in the long term. It also allows the generous distribution and supply of antisera to other institutions and researchers, avoiding unnecessary duplication of experiments involving animals. The amount of immunoglobulins that can be obtained from the rabbit, chicken and sheep in a 4 week period (two blood collections) is shown in Table 1. Assuming similar antibody titres, a chicken therefore produces five times and a sheep 10 times more antibodies

Table 1: Immunoglobulin yield over a period of 4 weeks (2 blood collections) in different animal species.

Species	Volume collected in 4 weeks	Amount of immunoglobulins	Immunoglobulin isotype
Rabbit	30 mL	300 mg	IgG
Sheep/goat	400 mL	4 g	IgG_1+IgG_2
Chicken	100 mL egg yolk (~20 eggs)	1-2 g	IgY

than a rabbit in the same time frame. Chicken antibodies come in a conveniently packaged form in the chicken egg, from which they can be easily purified (Svendsen *et al.*, 1995). Chicken eggs can be collected over long periods of time without any harm to the animal.

Recommendations

The strategies discussed above lead to the following practical recommendation for polyclonal antibody production:

- choose the least harmful adjuvant;
- never use complete Freund's adjuvant twice in the same animal;
- inject the smallest possible volume of adjuvant (use concentrated antigen);
- choose an injection site where local pathological responses cause least pain, can be readily observed and treated if necessary;
- use long booster intervals (at least four weeks);
- take advantage of the antibody memory response;
- minimise animal numbers by sequential rather than simultaneous immunisations of animals;
- choose animal species that yield large amounts of antibodies (sheep, goats, chickens); and
- plan ahead and be patient; successful production of a good antiserum can take 6-12 months.

Antibody production is a complex biological process and it is not always possible to follow the recommendations outlined above. Procedures and protocols might have to be changed depending on the antigen and the intended use of the antibodies. This is acceptable as long as good scientific reasons can be presented to justify it.

Dieter Palmer, Anne Masters
and Harjit Deol*
Animal Health Laboratories, Agriculture
Western Australia, South Perth WA

* Biotechnology Programme, School of Biological and Environmental Sciences, Murdoch University, Murdoch WA 6150

Editor's Note:

ANZCCART will soon be publishing a monograph on *The Use of Immunoadjuvants in Animals*, which incorporates discussion and recommendations from the workshop it held at the Walter and Eliza Hall Institute, Melbourne, in October, 1994.

References

Broderson, J.R. (1989). A retrospective review of lesions associated with the use of Freund's adjuvant. *Laboratory Animal Science* **39:** 400-405.

Chapel, H.M. and August, P.J. (1976). Report of nine cases of accidental injury to man with Freund's complete adjuvant. *Clinical and Experimental Immunology* **24:** 538-541.

Dalsgaard, K., Hilgers, L. and Trouve, G. (1990). Classical and new approaches to adjuvant use in domestic and food animals. *Advances in Veterinary Science and comparative Medicine* **35**: 121-160.

Deol, H.S., Palmer, D.G., Dunsmore, T. and Carnegie, P.G. (1995. The influence of gamma inulin and Algammulin on the immune response in sheep to a recombinant antigen of *Taenia ovis*. *Vaccine* **13:** 429-433.

Garvey, J.S., Cremer, N.E. and Sussdorf, D.H. (1977). Methods in Immunology, 3rd edition. W.A. Benjamin. Inc. Reading. Mass.

Gassmann, M., Thömmes, P., Weiser, T. and Hübscher, U. (1990). Efficient production of chicken yolk antibodies against a conserved mammalian protein. *FASEB Journal* **4**: 2528-2532.

Hanly, W.C., Artwohl, J.E. and Bennet, B.T. (1995). Review of polyclonal antibody production procedures in mammals and poultry. *ILAR Journal* **37:** 93-118

Hu, J-G., Ide, A., Yokoyama, T. and Kitagawa, T. (1989.) Studies on the optimal immunisation schedule of the mouse as an experimental animal, the effect of antigen dose and adjuvant type. *Chemical Pharmacology Bulletin* **37:** 3042-3046.

Hu, J-G., Yokoyama, T. and Kitagawa, T. (1990a). Studies on the optimal immunisation schedule of experimental animals. IV. The optimal age and sex of mice, and the influence of booster injections. *Chemical Pharmacology Bulletin* **38:** 448-451.

Hu, J-G., Yokoyama, T. and Kitagawa, T. (1990b). Studies on the optimal immunisation schedule of experimental animals. V. The effects of the route of injections, the content of mycobacteria in Freund's adjuvant and the emulsifying antigen. *Chemical Pharmacology Bulletin* **38:** 1961-1965.

Johnston, B.A., Eisen, H. and Fry, D. (1991). An evaluation of several adjuvant emulsion regimens for the production of polyclonal antisera in rabbits. *Laboratory Animal Science* **41:** 15-21.

Kenney, J.S., Hughes, B.W., Masada, M.P. and Allison, A.C. (1989). Influence of adjuvants on the quantity, affinity, isotype and epitope specificity of murine antibodies. *Journal of Immunological Methods* **121**: 157-166.

Laufer, A., Tal, C. and Behar, A.J. (1959). Effect of adjuvant (Freund's type) and its components on the organs of various animal species: a comparative study. *British Journal of Experimental Pathology* **40**: 1-7.

Mallon, M.M., Graichen, M.E., Conway, B.R., Landi, M.S. and Hughes, H.C. (1991). Comparison of antibody response by use of synthetic adjuvant system and Freund's complete adjuvant in rabbits. *American Journal of Veterinary Research* **52:** 1503-1506.

Sigel, M.B., Sinha, Y.N. and VanderLaan, W.P. (1983). Production of antibodies by inoculation into lymph nodes. *Methods in Enzymology* **93:** 3-12.

Steiner, J.W., Langer, B., and Schatz, D.L. (1960). The local and systematic effects of Freund's adjuvant and its fractions. *Archives of Pathology* **70:** 424-434.

Svendsen, L., Crowley, A., Østergaard, L.H., Stodulski, G. and Hau, J. (1995). Development and comparison of purification strategies for chicken antibodies from egg yolk. *Laboratory Animal Science* **45:** 89-93.

Thank you ...

Thank you to all those who have replied to the overhaul of the *ANZCCART News* mailing list. The response has been marvellous, with most of you expressing the desire to keep receiving the newsletter any way you can!

Your feedback on how much you enjoy *ANZCCART News* is much appreciated. If you have any further queries regarding the mailing of the newsletter, please don't hesitate to contact us.

Cathy Lineham
Administrative Assistant

Book Reviews

Animals in Education: The Facts, Issues and Implications

By Lisa Ann Hepner

Richmond Publishers, PO Box 91683, Albuquerque NM, 1994.
ISBN 0 96 39418 0 1. Price: $US 12.95 plus postage $US2.75.

Although the title suggests a wide-ranging examination of animals in education, in fact this book deals almost exclusively with dissection at the high school and college level in the U.S. There is only one chapter which touches on other uses of animals in the fields of psychology, medicine and veterinary science.

This is a heart-felt book. Hepner makes it clear at the outset that she is opposed to dissection and other forms of animal exploitation. The first chapter describes her own struggle for the right to study biology without dissection. There are a further two chapters of stories from other students describing similar struggles. Personally I find the book rather heavy on these anecdotes, although the stories clearly show the anguish which is caused to compassionate students by intransigent teachers. Most of these students withdrew from courses rather than dissect, and so initially enthusiastic students were lost to the discipline.

Hepner presents some survey data to support the impressions given by the personal accounts. For example, a large survey of high school students in the Albuquerque Public School System showed that only about one quarter of students were confident teachers would not penalise them if they chose alternatives to dissection. Among teachers, about one quarter always informed students of alternatives prior to dissection, while a similar number never discussed alternatives unless pressed by students. In a nation-wide university survey about half the respondents indicated they would refuse to make allowances for students with ethical objections to dissection. In some quarters there is clearly still resistance to alternatives, and this negative attitude is conveyed to students. It would be interesting to have comparative data in Australia.

On the other hand, in one of the appendices Hepner presents legislation from those U.S. states and public school systems which by law require teachers to make available alternatives to students.

A chapter on ethical considerations again has a very personal focus; for more philosophical treatments Hepner refers the reader to Peter Singer and Tom Regan. Her basic position is that killing animals for dissection fails to respect their inherent worth as individuals. In this context animals are presented as "specimens" or "tools" rather than living, feeling individuals. She is concerned at the desensitising message conveyed by dissection, a concern echoed in the statements quoted from science educators. For example: "...dissection is a desensitizing activity reflecting a morally bankrupt view that non-human animals exist only to be exploited by humans" (Thomas Bickleman), or again: "In times when we are trying to reduce violence in our society, the practice of harming and killing sentient creatures to conduct an educational exercise seems out of place" (Barbara Orlans).

A chapter on educational issues summarises studies showing that students learn at least as much by using alternatives as they do through doing dissections. Hepner's list of studies is not exhaustive; there are further studies which could be cited, and which support her conclusion that dissection is not necessary. In this chapter Hepner also queries why "hands-on" is always interpreted to mean "dissection" by traditional teachers. Surely "hands-on" simply means active involvement with concrete materials, and therefore includes a wide range of activities other than dissection.

I found the chapter on alternatives the most interesting. Hepner describes not only products such as computer programmes, but also experiments students can conduct on themselves, and investigations of local environments. In one particularly interesting example, a school set up a refuge for sick and injured wildlife, funded by a recycling programme. In conjunction with caring for and rehabilitating animals, students also studied their appearance, behaviour, diet and habitat. In another example, students rehabilitated a local creek and re-introduced a fish population, enabling them to study the life cycle of the fish and the interdependence of animals and their physical environment. In these examples, biology truly becomes the study of life, rather than the study of dead things.

In conclusion, I am not convinced that there is enough substance in the topic of dissection alone to fill a book, which is perhaps why Hepner included so many personal experiences. However, I still think the book could be particularly useful to students who do not wish to dissect. It provides evidence to support their case, gives guidelines on how to approach educational authorities, and lists support organisations and companies producing alternatives.

Suzanne Pope
Adelaide, SA

Feline and Canine Infectious Diseases

Library of Veterinary Practice. By R.M. Gaskell and M.Bennett.

ISBN 0 632 03446 7. Published by Blackwell Science, PO Box 378, Carlton, Vic. 3053, 1996. 186 pages. Price: $80.00.

This concise, affordable, soft cover book is the latest in a series of publications by Blackwell Science on specific topics in small animal medicine. Other titles in the series of interest to readers of this Newsletter are "Diseases of Domestic Rabbits" and "Diseases of Domestic Guinea Pigs".

The text originated as lecture notes to veterinary undergraduates at Liverpool University in the UK, where the principal authors teach. Each disease or condition covered is described with regard to aetiology, pathogenesis and clinical signs, treatment and control. In some instances the descriptions are brief but a comprehensive list of recent references is partial compensation. The layout is somewhat confusing as the book is divided into five sections - Major Feline Infectious Diseases; Major Canine Infectious Diseases; Miscellaneous Viral Infections; and Miscellaneous Bacterial Infections; Fungal, Algal and Protozoal Infections. This division does not necessarily reflect the current importance and frequency of incidence of the diseases. Feline abscess and feline infectious anaemia are relegated to "Miscellaneous". Cryptococcosis and Giardia get less than a third of a page each, perhaps reflecting the UK experience. Apart from this the layout is clear and attractive using dot points, tables and well-executed figures to emphasise a point. There are no illustrations, presumably to reduce costs. Articles on Feline Coronavirus, Feline Immunodeficiency Virus, Kennel Cough and Canine Infectious Gastroenteritis are excellent and give

Variables in Animal Based Research: Part 1. Phenotypic Variability in Experimental Animals

Ivor Harris
University of Queensland
PO Box 125, Kenmore Qld 4069

ANZCCART Facts Sheet

ANZCCART
PO Box 19
Glen Osmond SA 5064 Australia
Tel: 08–8303 7393 Fax: 08–8303 7113
E-mail: anzccart@waite.adelaide.edu.au

Introduction

The use of animals in scientific research introduces an important source of potential variability into the results to be obtained. It is most important for the sources of this variability to be understood so that it can be prevented, minimised or controlled. This variability may be both within batches of animals and between batches. Variability within batches, will at the least, necessitate using an increased number of animals in order to obtain statistically significant results, or may prevent results being obtained at all. Variability between batches may be variability over time or variability from place to place. This, in turn, affects the repeatability of the research results, and if the results are not repeatable by the same or other investigators, they are useless. Variability may be induced by factors inherent to the animals themselves, or by factors inherent to the experimental procedures, including experimental stress.

This is illustrated in the figure on page two.

This Facts Sheet will address the issues of variability due to animal inherent factors, and a subsequent Facts Sheet by another author will address variability due to experimental factors.

From an experimental viewpoint the phenotype of an animal includes all experimentally measurable characteristics (other than the genotype and strictly genetic characteristics). Thus, the degree of phenotypic variability within or between animal groups contributes largely to the degree of variability in measured experimental results. Just as the phenotype comprises components reflecting genotype, environmental influences, and genotype/environment interaction (G/E); so the total phenotype variability comprises variabilities originating from the same three components (Lang and Vessell, 1976; Festing, 1976).

Therefore, to describe the sources of phenotypic variability in experimental animals, the genotype, environment, and G/E components of the total variability can be separately described.

Genetic variability

The degree of genetic variability is largely determined by the genetic type of the strain of animal used. Traditionally there are three main types, outbred, inbred and F_1 hybrid. To these three could be added congenic inbred, recombinant inbred and the various types of transgenic and knockout animals on an inbred or outbred background.

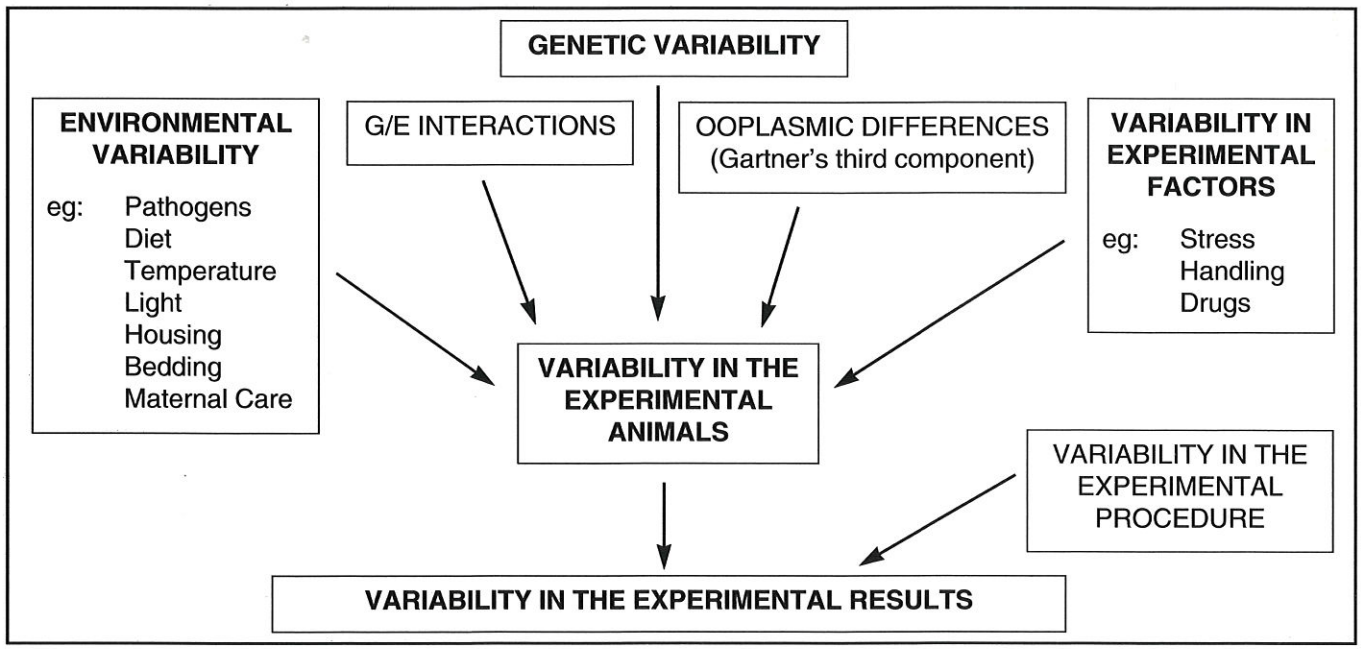
Outbred animals

Examples are Swiss, Quackenbush and CD-1 mice and Wistar and Sprague-Dawley rats. Outbred animals should be bred via a mating scheme which precludes matings between related animals, or at least between litter mates (e.g., Poiley Crossover scheme) (Poiley, 1960). For this reason they should not be random bred and this term should not apply. This is aimed at retaining maximum heterozygosity within the population, and they should be used for purposes where isogenicity is not required. They will be characterised by a high degree of heterozygosity and heterogeneity. Most outbred strains are uniformly albino and this may be the only thing that is uniform about them! There are also multicoloured strains. Compared with inbred animals, they are larger in size, more prolific breeders, more robust and resist stress better. These characteristics should be considered when deciding whether or not to use outbred animals in a particular experiment, as these can be very useful advantages.

Another useful feature of outbred animals when compared with inbred animals is that they show a lower level of phenotypic sensitivity and variability in response to environmental variables. This reflects the fact that they are more biologically fit animals (see later discussion under inbred animals). They are cheaper and easier to produce due to being more highly prolific.

A very important point to remember about outbred animals is that there will be a great amount of sub-line differentiation between colonies at different locations, and within one colony at different times, due to their having a high degree of heterozygosity and not being isogenic (Hayakawa *et al.*, 1980). Similarly, their general phenotypic characteristics cannot be defined to anything like the degree that is possible with inbred animals. Swiss mice or Wistar rats from different sources will be genetically quite different (Cui *et al.*, 1993). This is in addition to the fact that there will be a high degree of heterozygosity within the group in the first place.

Over time, a closed outbred breeding colony will tend to become inbred to some degree. The rate at which this happens depends on the size of the starting population. The increase in the inbreeding co-efficient (the percentage of



gene pairs which were originally heterozygous, but have become homozygous through inbreeding) is given by the formula (Festing, 1987):

Increase in inbreeding coefficient per generation

$$= \frac{1}{16 \times \text{no. of males in starting population}} + \frac{1}{16 \times \text{no. of females in starting population}}$$

Therefore it is important that a new outbred colony is started with as many initial breeders as possible, or that it is re-started every few years, or that breeders of one sex are replaced — otherwise the outbred animals are steadily becoming inbred animals with no control over selection pressures and no knowledge of what characteristics are becoming fixed by homozygous gene pairs.

Outbred animals should not be used where control of the degree of genetic variability is required. They are better used where isogenicity, phenotypic uniformity and constancy of characteristics are not required, but larger size, robustness, resistance to stress, cheapness or a population wide spread of results (e.g., in toxicology trials) are required.

Inbred animals

Examples are BALB/c, C57BL/6 mice and DA or PVG/c rats. Inbred animals are bred from consecutive full sibling or parent-offspring matings. The effect of such matings between highly related animals is to increase the degree of homozygosity in the offspring, and, as the process is sequentially repeated, the offspring become increasingly more closely related. After 20 generations of inbred matings, the inbreeding coefficient exceeds 98%, i.e., more than 98 per cent of the gene pairs which were originally heterozygous have become homozygous.

The very high degree of homozygosity provides isogenicity, which should be fixed indefinitely over the generations as long as the inbreeding continues. This in turn provides isograft (organs, tissues or tumours) acceptance within a strain or between strains that possess the same histocompatibility genes. It provides comparability of results obtained using the same strain from colony to colony, institution to institution, country to country and over time.

Theoretically, there is long- term genetic stability. In practice there will be slow sub-line differentiation between colonies (Matsumoto *et al.*, 1989; Papaioannou and Festing, 1980). This is due to the low level (i.e., 0 - 1 per cent) of residual heterozygosity in the starting population, the possibility of genetic contamination and, of course, spontaneous mutation. Ideally, colonies should be replaced periodically from a common national or international source. Annual monitoring of serum enzyme phenotype by electrophoretic examination will provide a safeguard against genetic contamination and is essential (Watts *et al.*, 1977; Adams, 1989).

Inbred animals should show no response to genetic selection. Selecting future breeders in the breeding colony from larger than average litters is practically convenient, but of no genetic significance. Inbred animals show various manifestations of inbreeding depression, as a result of general biological unfitness. This reflects the presence of a number of marginally harmful recessive genes in the homozygous state, which in the outbred animal are likely to be heterozygous and therefore masked by a normal dominant gene. This causes reductions in size, growth rate, fertility, vigour, resistance to stress (e.g., experimental stress or disease) and increased disease susceptibility. They also show more phenotypic variability than outbred animals in response to environmental variability. This highlights the importance of a high quality specific pathogen-free (SPF) environment for inbred colonies. Paradoxically, the early inbred mouse strains developed in the 1950s showed a higher extent of total phenotypic variability than outbred strains available at the time. This was because of the poor quality non-SPF animal house environments in use

(McLaren and Michie, 1956; Biggers *et al.*, 1958).

Characteristics with a low heritability will still show considerable phenotypic variability in inbred animals unless the contributing environmental variables are known and very well controlled. For example, tumour incidence in aged DBA mice is 90% environmental and 10%genetically controlled and unless environmental factors, particularly diet, are strictly controlled, the population variability may be high (Riley, 1981).

Phenotypic variation within an inbred strain is likely to be due to any of the following factors:

- behavioural variability e.g., in levels of maternal care;
- litter size;
- within-litter competition;
- developmental variabilities;
- variations in pathogenic burden or normal gut flora species present; and
- seasonal nutritional variability or poor food mixing.

(Gartner *et al.*, 1988).

F_1 hybrids

F_1 hybrids must always be produced by the first generation cross between two inbred strains. They are completely isogenic, but heterozygous at gene loci where the parent strains differ. They possess excellent phenotypic uniformity, better than inbred animals, as they are not as responsive to environmental variation. They possess excellent long-term genetic stability — again, better than inbred animals, as they will only change if the same recessive mutation occurred in both parent strains.

The presence of heterosis or hybrid vigour overcomes all the deleterious effects of inbreeding depression. In particular, F_1 hybrids are prolific and resist stress well. They will accept grafts or tumour transplants from both parent strains. They are more expensive than inbred animals as the two parent strains must be maintained to provide F_1 breeder replacement. All the desirable characteristics are lost in an F_2 generation, which becomes highly variable as the heterozygous genes segregate. A female A X male B F_1 hybrid may vary from a female B X male A F_1 hybrid although genetically they are identical. This reflects different uterine and maternal environments.

The relative qualities of outbred, inbred and hybrid strains are illustrated below.

Characteristic	**outbred**	**inbred**	**F1 hybrid**
homozygosity	+	+++	+
isogenicity	+	+++	+++
heterozygosity and vigour	++/+++	+	+
ease of production	+++	++	+
resistance to environmental variability	+++	+	+++
phenotypic uniformity	+	++	+++
comparability over time and distance	-	++	+++

Environmental variability

Environmental variables can be grouped under the following headings:

- Physical variables;
- chemical variables;
- biological variables; and
- behavioural variables.

(Newton, 1978; Baker *et al.*, 1979; Clough, 1984; Weihe, 1988).

In each case, the effects should be considered at two levels, micro-environment (cage level) and macro-environment (room level) (Woods, 1980). In many cases these levels will vary significantly.

Physical variables

- Temperature

The thermoneutral zone (the temperature range within which thermoregulation requires no energy expenditure) of a healthy adult rodent is 18-24°C. Within this range, thermoregulation only involves minimal behavioural adaptation, e.g., huddling, lying in contact with another or stretching out alone (Weihe, 1976; Murakami and Kinoshita, 1977). The effect of single caging in this regard may be significant for metabolic rate measurements. Neonates have no autonomous thermoregulation in the first week, but their appropriate thermoneutral zone is 30-34°C. This is normally achieved within the micro-environment of a well structured nest. Lactating females have a higher basal metabolic rate and show a preference for slightly lower ambient temperatures.

Old, sick or experimentally stressed animals have generally poor homeostasis, including thermoregulation. Variability in ambient temperature is more likely to result in changes in body temperature, resulting in further stress. The effect of warming such animals for the purpose of tail vein injection should be considered.

Uniformity of temperature throughout a room will depend on the effectiveness of the room ventilation (Reeb, 1997). There is an effect due to heat conduction within a rack of rodent cages from the body heat of the animals — the cages at the top and middle of the rack may be up to 5°C hotter than the bottom cages. The insulation effect of plastic cage bases is significant. Cage temperatures are more uniform and closer to room temperature with metal cage or wire grid cage floors. Environmental temperature has well-recognised effects on drug metabolism. For example, the mouse LD50 for chlorpromazine is 11 mg / kg at 13°C and 350 mg / kg at 28°C (Weihe, 1973).

- Humidity

Humidity variations are less significant than temperature variations. Variations in relative humidity at low temperatures are less significant that at higher temperatures. Low relative humidity causes higher dust levels, increased levels of respiratory infections, and

ringtail in baby mice and rats. High relative humidity lowers resistance to infection. The effect of increased relative humidity (and increased temperature and decreased gas exchange) in filter-topped cages is appreciable. Relative humidity will, of course, be higher in solid-floored cages than wire-grid floored cages.

Experience indicates that controlling the relative humidity of the air supply through the air-conditioning system is not necessary for general animal house purposes in a climate like that of Brisbane, where in winter the air, even after heating to the required temperature, still does not have a low relative humidity. On the other hand, in a cool dry winter climate like Canberra, the heated winter air has a very low relative humidity, which can cause problems. Breeding rodents can control the relative humidity in the cage micro-environment by nesting.

- Light

Variability can occur in intensity, spectral wavelength distribution and photo-period. Intensity may vary by up to 100 times between top and bottom of a cage rack. There is less variability with transparent cages. Albino animals are susceptible to phototoxic retinal degeneration under normal lighting conditions (e.g., over 60 lux) or with 24 hour continuous lighting (Semple-Rowland and Dawson, 1987).

Different bands of fluorescent tubes may have a large variability in wavelength distribution. Simulated natural wavelength lights with a normal ultraviolet component result in increased calcium absorption and gonad weights, and decreased skeletal development and dental caries. Standardised illumination in rooms will of course be greatly affected by natural light coming through windows.

Photoperiod effects are most important and affect most physiological and biochemical parameters, e.g., haematology, plasma steroids, enzyme activity, drug metabolism and toxicity, susceptibility to infection, duration of anaesthesia and time to death after irradiation (Weihe, 1976; Bellhorn, 1980).

A 12/12 light / dark cycle results in a 4 day oestrous cycle in mice, while a 16:8 cycle produces a 5 day cycle. A 22/2 cycle results in continuous oestrus and continuous dark results in anoestrus (Wollnik, 1989).

- Noise

Rodents communicate at 10-70 KHz, compared with the human audible range of up to 20 KHz. Inaudible higher frequency noises can be important e.g., a running tap produces up to 70 decibels (DB) between 2 KHz and 250 KHz and a squeaky wheel produces up to 100 DB at 40-60 KHz (Clough, 1988).

Prolonged noise over 100 DB, or 160 DB short term, causes inner ear damage in rodents. Known effects include cochlear damage, hypertension, changes in immune response and tumour resistance, reproductive changes, audio-genic seizures, atypical drug responses and blood chemistry changes (Gamble, 1982; Sales *et al.,* 1988). Many regard a stable background noise level (e.g., radio) of around 50 DB while noisy work is taking place as desirable.

Chemical variables

- Diet and nutrition

It is not possible to guarantee an absence of both within-batch and between-batch dietary variabilities with commercial rodent diets, particularly with regard to seasonal availability of ingredients and seasonal differences. This can only be prevented by making chemically defined diets from pure ingredients. This is likely to be a significant cause of phenotypic variability in inbred rodents.

The presence of various contaminants is also a source of variability. These include heavy metals (lead and cadmium), insecticides (organophosphates and chlorinated hydrocarbons), aflatoxins, oestrogens, excess antioxidants and vitamins and pyrrolizidine alkaloids. All of these have been confirmed or suspected in Australia. Their effects include lowered resistance to infection, lowered antibody production and higher susceptibility to endotoxins.

Diet sterilisation is also a source of variability. Mice fed an autoclaved diet may show higher cell-mediated immune responses due to overstimulation with a vitamin A derivative (Medawar *et al.*, 1979). Heat damage to heat labile vitamins is another potential variable with autoclaving food.

- Water

Water sterilisation with acid to a pH of 2-3 or hyperchlorinated with hypochlorite is a source of variability (Herman *et al.*, 1982). There is variability in cellular immune responses in mice on acidified water, and in mice drinking tetracycline medicated water. High chlorine levels also affect *in vivo* macrophage function.

- Cage bedding

Some wood shavings may contain aromatic hydrocarbons which induce hepatic microsomal enzymes and are unsuitable in toxicity studies (Vessell *et al.*, 1976; Weichbrod *et al.*, 1988). They are also possibly carcinogenic. The possibility exists of wood shaving contamination with pesticides, heavy metal preservatives (Gibson *et al.*, 1987), polychlorinated biphenyls, paints and aflatoxins (Wirth, 1983). The dust content, ammonia generation and absorption properties of different types of bedding vary significantly (Potgeiter and Wilke, 1996; Thigpen *et al.*, 1989).

- Other chemical contaminants

Detergents and disinfectants should be thoroughly rinsed from cage surfaces. Nitrates, nitrosamines and trihalomethanes in tap water may all induce hepatic microsomal enzymes.

- Noxious gases

Ammonia commonly exceeds 25 ppm in rodent cages when

bedding changes are becoming due. At this time the room ammonia level may be less than 10 ppm. Ammonia above 25 ppm in the cages is a potent co-irritant in inhalation toxicity studies and can act synergistically with respiratory pathogens. Above 50 ppm, it depresses cell-mediated immune responses and lowers hepatic microsomal enzyme activity. Above 100 ppm it causes inflammatory thickening of the tracheal mucosa (Targowski *et al.*, 1984; Schaerdel *et al.*, 1983). Filter-top cages increase ammonia levels by 50-100%. The effect of through-cage ventilation rates on ammonia accumulation in the cage is important. Wire grid-floored cages have approximately 90% of the room ventilation rate, while solid floored cages have at best 60% of the room ventilation rate.

Biological variables

- Pathogens

Any pathogens present in a population are likely to be present at variable infection rates within a colony and over time. Apart from the direct effect of the clinical or subclinical disease manifestations on research results, such effects will also be highly variable (Fox, 1983). This is a strong argument for using animals of a known health or microbiological status. Many rodent viral infections are subclinical, yet still have significant effects on many processes, particularly cell-mediated immune responses. Diseases such as mycoplasmosis, which are common in conventional animals, have similar effects.

- Normal flora

Commensal gut flora species may be highly variable within a conventional population, e.g., the presence or absence of gut methanogens. The same can apply to microbiologically defined animals unless the range of species present is severely restricted, in which case this may then be a cause of significant variation between colonies. Gut flora can have considerable effects on drug metabolism, nutrition and carcinogenicity studies. The effects of antibiotic administration on normal flora may also need to be considered.

Behavioural variability

- Pheromone effects

Exposure to pheromones of the same sex and of the opposite sex has important effects, and the degree of exposure may be variable within a group or between groups. Within 5 minutes of exposure of a male mouse to an oestrous female or her urine, the luteinising hormone output of the male doubles, causing elevated testosterone levels. The Bruce, Whitten and Lee-Boot effects on mice are well known (Cunliffe-Beamer and Les, 1987) and can probably be mediated by airborne pheromone transmission in heavily stocked rodent rooms, without requiring direct exposure to the opposite sex animals or their used bedding. In a juvenile female mouse, exposure to male urine may accelerate, and female urine may inhibit, puberty and oestrus. Re-circulation of pheromones (and noxious gases) by air-conditioning systems which incorporate any re-used air is an excellent reason why such systems should not be used in animal houses.

- Handling

Handling rats frequently in early life leads to lowered susceptibility to gastric ulcers and tumours (Russell, 1971). Handling and stroking of rabbits fed 2% cholesterol leads to 60% less atherosclerosis (Jezierski *et al.*, 1993).

- Housing

Rodents show isolation stress when singly housed (Rose, 1993). A singly housed rat after 24 hours has lowered cortisone levels. Prolonged single housing leads to raised cortisone, aggression, induction of hepatic microsomal enzymes, lowered cellular immune responses, larger adrenal glands and an increased volume of ascitic fluid with ascitic tumours.

High population density also causes psychosocial stress, particularly in the lower ranked animals (Peng *et al.*, 1989). This can cause increased adrenal weight, lowered weight gain (Peters and Festing, 1990), lowered thymus and gonad weight, increased granuloma responsiveness, lowered infection resistance and increased drug sensitivity (Riley, 1981). Overcrowding also causes increased heat, moisture, respiratory gases, ammonia (Eveleigh, 1993) and increased activities involved in behavioural thermoregulation.

There are many effects due to cage factors (Lewis, 1980; Hirsjarvi and Valiaho, 1987; Corning, 1991). Cage micro-environment is affected by cage size, design and material (Keller *et al.*, 1989), bedding, population, husbandry procedures (Saibaba *et al.*, 1996) and position of the cage in the rack. Body weight gain is highest in the middle of a rack and lowest on top, while air temperature is highest in the top and middle and lowest at the bottom.

Cage filter tops increase variability in the micro environment, which may have greater impacts than the diseases they are intended to prevent in the first place. Ammonia is increased by 50-100%, temperature by 1- 2°C, relative humidity by 10% and air exchange rate is decreased by 50-90% (Muller and Weihe, 1983). Standardisation of group size and husbandry procedures is therefore more important with filter-top cages than normal cages.

For low stress housing, the following is recommended (Riley, 1981): no air recirculation; sound-proofed shelving; elimination of high pitched sounds; elimination of drafts; precision light control (duration and intensity); segregation of sexes from pheromones; segregation of experimental animals; minimal stress handling techniques and avoidance of wire grid-floored cages.

Environment / genotype interactions

Environment effects on genetic drift

Different environments, and particularly different husbandry practices with respect to selecting future breeders, may cause genetic divergence over several generations, particularly with outbred animals. Selecting

future breeders in outbred colonies from large litters or from female breeders with good breeding histories will have an obvious selection pressure effect which will benefit colony production, but may have other unintended effects. By selecting from a limited number of the breeders in the previous generation, it will also increase inbreeding, which may over time have the opposite effect of causing a fall in productivity.

Impact of environmental variability on phenotype

As discussed earlier, inbred animals are far more susceptible than outbred animals to phenotypic variability induced by minimal environmental variability.

Gartner's postulated third component

Additionally, Gartner (1990) described a possible third component (besides genotype and environment) creating random variability, as a possible reason for the limited success of 30 years of effort to standardise laboratory animals. He argued that reduction of genetic variability by using inbred strains and reduction of environmental variability by highly standardised husbandry in laboratory animals did not remarkably reduce the range of random variability in quantitative biological traits. Neither did a tremendous increase in the environmental variability (i.e., living in a natural setting) increase it. Therefore the post-natal environment cannot be that important as a source of random variability. Utilising methods established in twin research, only 20-30% of the range of the body weight in inbred mice was directly estimated to be of environmental origin. The remaining 70-80% was due to a third component creating biological random variability, in addition to the genetic and environmental influences. This third component is effective at or before fertilisation and may originate from ooplasmic differences. It is the most important component of the phenotypic random variability, fixing its range and dominating the genetic and environmental components.

In other studies (Gartner and Baunack, 1981), the degrees of similarity between monozygotic twin mice and between isogenic dizygotic twins, from two ova of one mother, all of a very highly inbred mouse line, were compared. Monozygotic twins were produced by mechanical separation of eight cell embryos into halves. All were then transplanted at the eight cell stage into foster mothers. The monozygotic twins had a greater degree of similarity than the isogenic dizygotic twins. This suggests that the differences between dizygotic twins may be due in part to non-genetic influences on the zygote as far as the third cleavage stage.

References

Adams, M. (1989). ASLAS Newsletter, December 1989, pp. 3-4.

Baker, H.J., Lindsey, J.R. and Weisbroth, S.H. (1979). Housing to control research variables. In: The Laboratory Rat Vol 1. (eds. Baker *et al.*) Academic Press, New York. pp. 169-188.

Bellhorn, R.W. (1980). Laboratory Animal Science, **30:** 440-450.

Biggers, J.D. , McLaren, A. and Michie, D. (1958). Nature, **192:** 77-80.

Clough, G. (1984). Environment factors in relation to comfort and well-being of laboratory rats and mice. In: *Standards in Laboratory Animal Management*. UFAW, UK. pp.7-24.

Clough, G. (1988). Housing and welfare of laboratory rodents. In: *New Developments in Biosciences: Their Implications for Laboratory Animal Science*. (eds. Beynen, A.C. and Solleveld, H.A.). Martinus Nighoff, Dordrecht. pp. 239-244.

Corning, B.F. and Lipman, N.S. (1991). *Laboratory Animal Science,* **41**: 498-503.

Cui, S., Chesson, C. and Hope, R. (1993). *Laboratory Animals,* **27**: 116 - 123.

Cunliffe-Beamer, T.I. and Les, F.P. (1987). The Laboratory Mouse. In: *The UFAW Handbook on the Care and Management of Laboratory Animals*, 6th Edition. Longman Scientific and Technical, Harlow, UK. pp. 289 - 290.

Eveleigh, J.R. (1993). *Laboratory Animals* **27**: 156-160.

Festing, M.F.W. (1976). *Nature,* **263**: 230-232.

Festing, M.F.W. (1987). Introduction to Laboratory Animal Genetics. In: *The UFAW Handbook on the Care and Management of Laboratory Animals*, 6th Edition. Longman Scientific and Technical, Harlow, UK. p. 69.

Fox, J. G., (1983). *Progress in Experimental Tumour Research* **26**: 208-240.

Gamble, M.R. (1982). *Biological Reviews,* **57**: 395-421.

Gartner, K. (1990). *Laboratory Animals,* **24**: 71-77.

Gartner, K. and Baunack, E. (1981). *Nature.* **292**: 646-647.

Gartner, K., Iglauer, F., Mensing, K and Vellever, R. (1988). *New Developments in the Biosciences - Their Implications for Laboratory Animal Science*. (Eds. Beynen, A.C. and Solleveld, H.A.). Martinus Nighoff. pp. 121-126.

Gibson, S.V., Besch-Williford, C., Raisbeck, M.F., Wagner, J.E. and McLaughlin, R.M. (1987). *Laboratory Animal Science*. **37**: 789-791.

Hayakawa, J., Koizumi, T. and Sakai, S.N. (1980). *Laboratory Animals* **14**: 233-236.

Hermann, L.M., White, W. and Lang, C.M. (1982). *Laboratory Animal Science* **32**: 603-608.

Hirsjarvi, P.A. and Valiaho, T.U. (1987). *Laboratory Animals* **21**: 95-97.

Jezierski, T., Mekking, P. and Wiepkema, P.R. (1993). *Laboratory Animals*. **27**: 235-239.

Keller, L.S., White, W.J., Snider, T.M. and Lang, C.M. (1989). *Laboratory Animal Science,* **39**: 237-239.

Lang, C.M. and Vessell, E.S. (1976). *Federation Proceedings of the Federation of American Societies for Experimental Biology,* **35b:** 1123-1164.

Lewis, L. L. (1980). *Laboratory Animal*, March-April pp. 53-58.

Matsumoto, K., Yamada, T. and Yamada, J. (1989). Laboratory Animal Science, **39** : 474.

McLaren, A. and Michie, D. (1956). *Journal of Genetics*, **54**: 440-455.

Medawar, P., Hunt, R. and Mertin, J. (1979). *Proceedings of the Royal Society of London*, **B**, **206**: 265.

Muller, H.R. and Weihe, W. H. (1983). *Z. Versuchstierk*, **25**: 277-283.

Murakami, H. and Kinoshita, K. (1977). *Journal of Applied Physiology* **43**: 573-576.

Newton, W.M. (1978). *Advances in Veterinary Science and Comparative Medicine* **22**: 1-28.

Papaioannou, V.E. and Festing, M.F.W. (1980). *Laboratory Animals,* **14**:11-13.

Peng, X., Lang, C.M., Drozdowicz, C.K. and Ohlsson-Wilhelm, B.M. (1989). *Laboratory Animals*, **23**: 302-306.

Peters, A. and Festing, M. (1990). *Laboratory Animals,* **24**: 273-279.

Poiley, S.M. (1960). *Proceedings of the Animal Care Panel,* **10**: 159-166.

Potgeiter, F.J. and Wilke, P.I. (1996). *Laboratory Animals*, **30**: 79-87.

Reeb, C.K. (1997). *Contemporary Topics*, **36**: 74-79.

Riley, V. (1981). *Science,* **212**: 1100-1109.

Rose, M.A. (1993). *ANZCCART News*, **6**: 10-11.

Russell, P.A. (1971). *Psychology Bulletin*. **75**: 192-202.

Saibaba, P., Sales, G.D., Stodulski, G. and Han, J. (1996). *Laboratory Animals,* **30**: 13-21.

Sales, G.D. Wilson, K.J., Spencer, K.E.V. and Milligan, S.R. (1988). *Laboratory Animals,* **22**: 369-374.

Schaerdel, A.D. White, W.J., Lang, C.M., Dvorchick, B.H. and Bohner, K. (1983). *Laboratory Animal Science* **33**: 40-46.

Semple-Rowland, S.L. and Dawson, W.W. (1987). *Laboratory Animal Science*, **37**: 289-293.

Targowski, S.P., Klucinski, W., Babiker, S. and Nonnecke, B.J. (1984). *Infection and Immunity*, **43**: 289-293.

Thigpen, J.E., Lebetkin, E.H., Dawes, M.L., Clark, J.L., Langley, C.L., Amyx, H.L. and Crawford, D. (1989). *Laboratory Animal Science*, **39**: 60-62.

Vessell, E.S., Lang, C.M. and White, W.J. (1976). *Federation Proceedings of the Federation of American Societies for Experimental Biology*, **35**: 1125-1132.

Watts, C.H.S., Baverstock, P.R. and Cole, S.R. (1977). *Australian Journal of Experimental Biology*, **55**: 347-353.

Weichbrod, R.H., Cisor, C.F., Miller, J.G., Simmonds, R.C., Alvares, A.P. and Veng, T. (1988). *Laboratory Animal Science*, **38**: 296-298.

Weihe, W.H. (1973). *Annual Reviews in Pharmacology*. **13**: 409-425.

Weihe, W.H. (1976). "The effect on animals of changes in ambient temperature and humidity". In: *Control of Animal House Environment* (ed. McSheedy, T.) Laboratory Animals Ltd, London. pp. 41-50.

Weihe, W.H. (1988). "Housing conditions and experimental results". In: *New Developments in Biosciences: Their Implications for Laboratory Animal Science* (eds. Beynen, A.C. and Solleveld, H.A.) Martinus Nighoff, Dordrecht. pp. 245-253.

Wirth, H. (1983). *Laboratory Animals*, **17**: 81-84.

Wollnik, F. (1989). *Laboratory Animals*, **23**: 107-121.

Woods, J.E. (1980). *Laboratory Animal Science*, **30**: 407-413.

ANZCCART's Facts Sheets

This series commenced in Winter, 1993 (Volume 6, Number 2) and now numbers 15 issues. Each of the following facts sheets is available as an offprint.

- **The Mouse. Part 1 and Part 2**
  by Catheryn O'Brien and Margaret Holmes

- **The Rat.**
  by David Pass and Graham Freeth

- **Experimental Techniques and Anaesthesia in the Rat and Mouse**
  by Steven Marshall, Angela Milligan and Ray Yates

- **The Common Marmoset (*Callithrix jacchus*)**
  by Julie Clarke

- **The Guinea Pig (*Cavia porcellus*)**
  by Denise Noonan

- **The Laboratory Rabbit**
  by Ivor Harris

- **The Laboratory Cat**
  by Tony James

- **The Sheep**
  by David Adams and Michael McKinley

- **Australian Marsupials: Tammar Wallaby, Fat-tailed Dunnart and Brushtail Possum**
  by Louise McKenzie, Clive Chesson, Rory Hope, Janine Duckworth and Lynne Meikle.

- **Restraint and Handling of Captive Wildlife**
  by Andrew Tribe and Derek Spielman

- **The Domestic Chicken**
  by Philip Glatz, Kim Critchley and Christine Lunam

- ***In Vitro* and Other Non-Animal Experiments in the Biomedical Sciences**
  by Bas Blaauboer

- **The Dog as an Experimental Animal**
  by Mary Bate

- **Variables in Animal Based Research: Part 1. Phenotypic Variability in Experimental Animals**
  by Ivor Harris

For further information, contact ANZCCART's Adelaide office.

the reader a concise but comprehensive update on these important diseases. The epidemiology and importance of the carrier state in feline respiratory disease are also required reading for anyone managing a cat colony.

Some of the diagnostic tests mentioned may not be available in Australia and clinicians should check with their diagnostic laboratory before submitting samples.

The small section at the end on vaccination is one of the most useful and could be expanded in a later edition. A summary of control measures in similar format would also be helpful and make this a more valuable text for anyone involved in running a kennel or cattery.

In summary, this book fills a niche in the market as an affordable text on the topic and would be particularly valuable for undergraduates or for those with little clinical experience with dogs and cats. It is not intended as a reference text for laboratory managers but could prove useful as a supplementary handbook. More detailed information will still be needed, especially for problem cases.

Julia Nicholls
Prospect Road Veterinary Clinic
Adelaide SA

Letters

Laboratory animal use in practical classes

The use of laboratory animals in practical classes has historically been an important part of the teaching of pharmacology. However, contemporary ethical and societal standards demand that academics reconsider this traditional method of teaching. Academics are also facing the challenge of increasing class sizes and reductions in funding, forcing us to use financial and classroom resources more rationally.

Several years ago, academic staff within the School of Pharmacy and Biomedical Sciences at the University of South Australia faced this dilemma. We had, up to that time, run the traditional pharmacology teaching practicals such as organ bath experiments or whole animal experiments monitoring blood pressure and heart rate. However, it was obvious that the pressures mentioned above would force a change in our teaching method.

During the last three years, we investigated and purchased programs from the range of ready-to-use courseware provided by the PCCAL(website address http://www.coacs.com /PCCAL/price1.htm) consortium and the pharma-CAL-ogy (website address: http://cbl.leeds.ac.uk/raven/pha/phCAL.html) consortium, both in the United Kingdom. Additionally, we started to write our own teaching aids using Asymetrix Tool book, and have developed experimental simulation material for use by both pharmacy and environmental toxicology students.

Whereas the practical component of the subject pharmacology was previously totally reliant on the use of experimental animals, these have now been completely replaced by computer simulations. The response from students has been extremely positive. They are unanimously in favour of the new "practicals", particularly as they can use these programs repeatedly and at their own pace to clarify and reinforce concepts. We have also started to develop other pharmacology subject material in the same way. We have thus used the impetus of reducing animal use in teaching to diversify our teaching methodologies.

Our Pharmacy course in particular has a very high percentage of students from a non-English speaking background. Their response to this method of presenting subject material has been particularly encouraging but the student contingent on the whole reflects favourably on the introduction of computer-aided learning (CAL) as a significant part of their subject material.

Apart from the cost of setting up a suite of computers within the school, costs of running the program have been minimal. Expenses of materials, animals and of two staff required to set up the traditional practical sessions have been totally eliminated whilst student outcomes have not been compromised.

I would be very keen to find out whether other universities are adopting similar measures in their teaching programs.

Ieva Stupans
David Adams
School of Pharmacy and Medical Sciences
University of South Australia
Adelaide, SA

Lay descriptions in ethics applications

I was glad to read the comments on lay descriptions as outlined by Graham Nerlich (*ANZCCART News* **10**(2): 7). He provides a range of perspectives and helpful advice on this matter which, gently, or so it seemed to me, places the onus of responsibility squarely on the shoulders of the scientists making application to animal ethics committees. That is where it should properly reside.

I would state the position more forcefully than Graham Nerlich. We scientists have an obligation to provide intelligible lay descriptions of the purposes of our work and how they are to be achieved. Any member of an animal ethics committee who finds a lay description too technical should, in my view, feel completely free to request that another lay description be provided before an application is considered further.

It is the scientists' responsibility to satisfy the members of their ethics committee - lay, independent and scientific - that the proposed work is worthwhile, properly conceived in scientific terms and that the cost or harm to the animals involved is both minimal and acceptable in light of the expected benefits. Imperative to achieving this are clear lay descriptions of the 'why' and the 'what' of the proposed work - why is it to be done and precisely what is to be done to the animals, how harmful or noxious it is likely to be and what measures are there to minimise any noxiousness.

Many scientists hold the view that they cannot simplify descriptions of what they do to make its purposes and character understandable to lay people. My experience is otherwise. For about 25 years I have assisted animal-based scientists to explain their work in non-technical terms. I have found that once they overcome an uneasiness that explaining their work without recourse to jargon and technical terms will make it, or them, seem less scientific, they find the activity both illuminating and satisfying. It is illuminating because in relinquishing technical descriptions, they clarify for themselves the principles of their work, articulated in simple terms, and that almost invariably leads them to feel they have a far better grasp of what they are doing than they did before they undertook the exercise . It is satisfying because in providing acceptable lay descriptions they can communicate, often for the first time, some of their enthusiasm and excitement for their work, and can inspire others who are not scientists to share their sense of its value.

Increasingly we animal-based scientists are being required by lay persons to justify what we do. Those of us

who can do so only by recourse to technically difficult language feel uneasy when so challenged because the language creates a barrier to understanding. Those of us who can use lay terms to describe both the 'what' and the 'why' of our work in a way that is clear and convincing can and do feel confident, because it is then easier to convincingly outline the ethical justification for our work, as Graham Nerlich pointed out.

Developing a facility in the use of lay terms to describe our work is therefore not only helpful to members of the animal ethics committee as they deliberate on our applications to undertake particular pieces of work, it can also improve the clarity of our own thinking in ways that improve communication with our colleagues and friends as well as with the lay public.

David Mellor
Vice-Chairman of ANZCCART (NZ)
Massey University, New Zealand

Light on the subject of lay descriptions

We read Graham Nerlich's letter on the point of lay descriptions (*ANZCCART News* **10** (2):7) with great interest. On the premise that concrete examples may sometimes provide a useful focus for discussion, we sought acceptable and unacceptable lay descriptions that had been submitted in the past to our Animal Ethics Committee. Finding a good summary was straightforward. Identifying a poor one was not so easy because of the issue of confidentiality - and so we created one.

Illuminating the project — a good lay summary

"Abscesses cause both persistent and recurrent forms of infection in people, with the potential for serious disease and even death. Abscesses can arise spontaneously, through trauma (for example, penetrating knife wounds to the abdomen: a memorable study published in the USA described the outcome of hundreds of these seen in one Chicago hospital), or through surgery. In this last instance, surgery for appendicitis or for removal of a colon cancer will inevitably result in the spillage of normal gut bacteria and faecal fibre into an otherwise sterile site, the peritoneal cavity. Antibiotics given prior to exposure to the infecting bacteria can assist in prevention of infection, but may be of little use in patients with an immune system compromised by other illness, advanced age, or drug treatments. In addition, antibiotics alone are of little value in treating established abscesses.

The aim of our work is to understand the early events in abscess formation, in order to identify biological processes that might be the target of drugs other than antibiotics. Currently-available evidence suggests that fibrin deposition at the site of infection is a key element in abscess formation. This project aims to define the mechanisms leading to fibrin deposition, and to develop strategies for manipulating this to prevent abscess formation or accelerate its treatment.

Our central model involves the initiation of abscesses inside the abdomen of mice by injecting them with bacteria and a faecal fibre substitute, bran. Whilst mimicking a potentially serious and life-threatening human infection, the model itself does not produce serious illness or death in mice. This we attribute to the fact that the mice are otherwise normal (in contrast, many humans with life-threatening infection may have a compromised immune system, and don't readily control the infection), and the bacterial doses used are relatively moderate. The aim is to induce abscess formation, not overwhelming peritonitis (widespread inflammation in the abdomen) or septicaemia (entry of bacteria to the bloodstream). Nevertheless, there is evidence of a mild peritonitis in experimental animals in the first day after infection (for example, the animals don't move around as much as normal). Also, there may be the risk of more serious infection. Therefore, the mice will be monitored regularly on the first day after challenge, and every 1-2 hours in the first 6 hours, in order to ensure that animals with possible significant infection are identified, monitored and if necessary euthanased. Whilst the experiments described don't require the mice to be kept for a long time, it is known that the abscesses gradually disappear from the abdomen over weeks, again with no evidence of illness or death.".

A helpful lay summary such as the one above is couched in plain language and includes the following:

- a clear statement of the specific problem to be investigated;
- a brief explanation of the overall context of the experiment, including a realistic summary of the likely outcomes and benefits of the work; and
- an overview of what will actually happen to the animals (not always necessary, but often very useful).

Keeping the Committee in the dark — a poor lay summary

"This study will test the ability of intracerebral injections of glutamate receptor antagonists to reduce apoptotic cell death in a rat model of stroke. Stroke is a major cause of disability in humans. The findings of the proposed investigations are likely to lead to the development of successful treatments for this major neurological disorder."

The above lay summary, strictly the figment of one scientist's imagination, was invented to display many of the flaws commonly encountered by Animal Ethics Committees. The problems inherent in this particular description may be summarised as follows:

- the description is too short: the author has made no real effort to communicate the details of the project to the Committee;
- the first sentence contains three undefined technical terms ("intracerebral"; "glutamate receptor antagonists", "apoptotic");
- no general experimental details have been provided to allow the Committee to evaluate what will happen to the animals; and
- an overblown claim has been made for the likely outcome of the work.

We accept that there are many ways to construct a useful lay description, but perhaps relatively few in which to write a poor one. Avoiding the common pitfalls described above may help researchers to allow their Animal Ethics Committees to help them.

Keryn Williams, John Finlay-Jones,
Neil Sims and Elizabeth Close
Flinders University of South Australia
Adelaide, SA

Access to dogs for teaching purposes

In view of the current concerns over supply of dogs from pounds and charities there are two points that are rarely mentioned, but that require stating here.

First, it is important to make the distinction between animals used for research and those utilised for teaching purposes. With animals used for teaching purposes a further distinction needs to be drawn . That is, between those dead bodies of animals, cadav-

ers, which are utilised for anatomy sessions and those used for survival surgical practicals, even if the dogs are only kept alive until the end of surgery and not allowed to survive the anaesthesia.

It is important to note that in TAFE, animal bodies used in practical sessions involving anatomical instruction are always obtained from pound animals that are already dead and will soon be incinerated — the strays. This is a tragic waste of life and an indictment on our society. However, this can be redeemed to some extent if a few are utilised as a teaching resource to benefit others of their species by advancing knowledge and training of the next generation of animal health workers.

Another argument which could be advanced is that there is no real philosophical difference between this and the medical schools in our universities, where human bodies are used for anatomical instruction of undergraduate medical students. It could also apply to the system for body organ donations of corneas and other organs many people proudly display on their driver's licence.

The second point is that universities are not the only tertiary institutions that require access to pound animals. It is often forgotten that TAFE Colleges around the country require cadavers to teach subjects such as anatomy, surgical skills (although never survival surgery, rather clipping and skin preparation) and clinical pathology techniques in a wide variety of courses covering veterinary nursing, animal technology, animal house attendants and kennel and cattery operations. It is essential that these students gain experience with real animal bodies, albeit already dead, since they need to do the same in their work places.

This group constitutes a large number of para-professionals who also work with animals on a daily basis in the broader animal health industry. For example, at Richmond TAFE College, which has a relatively small animal care section, we service about 120 students in various animal care courses each year. These courses are offered in at least four other TAFE Institutes in NSW and this is mirrored in every other State.

I should restate that the only animals that we source have been euthanased at the pound under normal circumstances. We do not house the animals until needed and we do not breed animals specifically for this purpose.

In summary, it is becoming increasingly difficult for all tertiary teaching institutions to procure real life animal models for teaching practical subjects. This valuable and under utilised resource, dead pound animals (strays which are unloved and unowned largely due to ignorance or lack of concern) might in death, provide a benefit for others of their species as well as to future animal health care professionals — veterinarians, veterinary nurses and others. This could give some value and purpose to these waifs in death that was denied to them in life.

David Horan
Western Sydney Institute of TAFE
Richmond Campus
Sydney, NSW

Editor's Note:

The NSW Minister for Agriculture has questioned the practice of council pounds supplying dogs to universities for medical and veterinary testing and research.

The September issue of the *Australian Veterinary Journal* (**75**(9): 625 and 629 - 630) includes comment on this topic from the AVA President, Dr Roger Clarke, as well as responses from five other persons.

Newly published

Conservation biology and the preservation of biodiversity in Australia: A role for zoos and the veterinary profession, by J.D. Kelly and A.W. English.

Australian Veterinary Journal **75**(8): 568-574.

It has only been in the past 20 to 30 years that the veterinary profession in Australia has become involved with zoos, commencing with the appointment of Dr Ted Finnie as veterinarian at Taronga Zoo in 1968. All major public zoos and wildlife parks now employ at least one veterinarian, whose responsibilities sometimes extend to curatorial work.

This has coincided with the emergence of the new discipline of conservation biology and the recognition of the important role played by zoos. The authors of this review paper, both veterinarians, bring much experience in these fields. Dr John Kelly has been Director and Chief Executive of the Zoological Parks Board of NSW since 1987 and is responsible for Taronga Zoo in Sydney and the Western Plains Zoo at Dubbo. Dr Tony English is with the Department of Animal Health at the University of Sydney and some years ago founded the veterinary special interest group of the AVA, Australian Association of Veterinary Conservation Biologists, which has over 300 members.

The paper defines biodiversity as having three key areas — genetic diversity, species diversity and ecosystem diversity. Conservation biology is stated as having two goals — to investigate human and other impacts on biological diversity and to develop methods of preventing the extinction of species.

It then reviews the human impact on the Australian environment since aboriginal settlement and emphasises the loss of biodiversity from the effects of agriculture and other development, as well as from introduced herbivores and pest species, both animal and plant.

The authors' solutions are not new — the setting aside of carefully chosen areas of land to protect particular habitats and the species which inhabit them is the main recommendation. This needs legislative protection, together with allocation of resources to manage them, and in particular, to control pest species.

Reintroduction of vertebrate species to the wild has generally not been successful, usually because the underlying problems (such as feral cats and foxes) are still present. In all situations, governments and conservation authorities must consider the underlying social, economic and political causes.

The difficult question of commercial utilisation of wildlife is covered, with reference being made to the IUCN strategy of sustainable development, which applies only to renewable resources, with utilisation at rates within their capacity for renewal.

Discussion of the role of zoos in preservation of diversity stresses the need to maintain genetic material in captivity, where it is not possible for species to survive in the wild. Dr Bill Conway, one of the authors of the Species Survival Plan of the World Zoo Conservation Strategy, has estimated that all of the world's zoos can maintain a minimum of 500 and a maximum of 2000 vertebrate species. This must be combined with *in situ* management of protected areas of reserves, together with cost-effective education, research and habitat preservation programs.

The paper concludes with discussion of the Conservation Research Centre at Taronga Zoo as a case study

The moribund state as an experimental endpoint, by Linda Toth

Contemporary Topics in Laboratory Animal Science **36**(3): 44-48; May, 1997.

The author discusses experimental animal protocols associated with high mortality rates. These often state that animals will be euthanased if they become moribund. However, effective implementation of this depends on the definition of moribund. Toth considers how the moribund state can be assessed objectively. This is difficult, as some clinical signs, while indicative of illness or distress, may not be useful in predicting when an animal will die.

She recommends the use of specific physiological or clinical variables relevant to the particular experimental paradigm. One example includes the phases of weight change following induction of central nervous system tumours in rats. The third phase, one of weight loss, is a good indicator of imminent death. Another example is the use of hypothermia, e.g., in mice with acute experimentally-induced bacterial infections.

The use of such criteria in experimental procedures facilitates the implementation of timely euthanasia prior to the onset of clinically overt signs of being moribund and therefore reduces pain, distress and suffering in the experimental animals.

Fat rats and carcinogenesis screening, by Michael Festing

Nature **388**: 321-22 (24 July, 1997).

This article stresses the need for toxicologists to use isogenic strains of laboratory animals in the toxicity testing of chemicals. While the importance of strain differences is recognised in most other biological disciplines, Festing states that virtually all toxicity testing is done using outbred stock of genetically undefined, heterogeneous animals.

He argues that toxicologists' problems are compounded by the use of genetically labile outbred stocks that have become heavier as a result of selection for faster growth and which do not live long.

To put this problem in perspective, Festing points out that more than 70,000 animals are used each year for toxicological testing. He argues that poor experimental design is leading to inaccuracies that make validation of alternative *in vitro* or short-term tests very difficult.

Festing believes that in the future it should be possible to develop *in vitro* tests that are sufficiently accurate to make animal testing unnecessary. At present, validation of data is affected by the quality of the animal data, which must be improved.

Proceedings of the Second World Congress on Alternatives and Animal Use in the Life Sciences, held in Utrecht, The Netherlands, from 20 to 24 October, 1996

The Proceedings, published by Elsevier Press, entitled *Animal Alternatives, Welfare and Ethics*, are volume 27 in the series *Developments in Animal and Veterinary Sciences* (1997), ISBN 0 444 82424 3 and were edited by LFM Van Zutphen and M Balls.

The 1260 page volume includes plenary lectures, papers or workshop summaries by a number of Australian and New Zealand persons, including R Baker, A Brennan, R Einstein, N Johnston, D Mellor, M Rose, W van Dok and R Yates.

Animal laboratory exercises in medical school curricula, by Mitchell Wolfe, Neal Barnard and Suzanne McCaffrey

Alternatives to Laboratory Animals **24**: 953-956 (1996).

The authors sent a questionnaire on the use of animal laboratory exercises and suitable alternatives to the chairpersons of the physiology, pharmacology and surgery departments of each of the 126 US medical schools. When compared with previous studies, the results showed that the number of medical schools reporting the use of animals in physiology courses had declined from 50 per cent to 41 per cent, the number using animals in pharmacology courses had declined from 25 per cent to 16 per cent, whereas the number of schools using animals for surgery classes had increased from 20 per cent to 30 per cent. Of the 53 schools which responded to the questionnaire, 49 per cent did not use laboratory animals in any of these disciplines. Computer programs and films were the most commonly used non-animal alternatives in physiology and pharmacology, with operating room experience the most common alternative in surgery courses.

Institutional Animal Care and Use Committees (IACUCS)

Lab Animal **26**(5), May, 1997.

There are four articles in this issue about IACUCS, the US and Canadian equivalent of Animal Ethics Committees. They are:

- *Do pressure and prejudice influence the IACUC?*, by Jerald Silverman, who developed a questionnaire to determine whether an IACUC's deliberations can potentially be influenced by pressures and perceptions that it may not even recognise.

- *The SCAW IACUC Survey Part I: Preliminary Results,* by Lee Krulisch and Joy Mench. This paper provides preliminary results from the study conducted by the US Scientists Center for Animal Welfare (SCAW). The study was designed to help institutions that use animals in research, testing and education and to help the IACUCS at those institutions better understand how other IACUCS function. The survey data are still being analysed and only a summary is provided here.

- *The SCAW IACUC Survey Part II: The Unaffiliated Member,* by Peter Theran. In the US IACUC system, the unaffiliated member represents the general community on the IACUC and is the only appointee not employed by the institution. This paper provides a copy of the survey form and a brief analysis of the responses.

Respondents recorded similar concerns to those expressed by some category D members on Australian AECs; that is, although they generally valued the opportunity to sit on the IACUC, some had concerns about the value of their contribution and whether their IACUC was effective in improving animal welfare.

- *Resource: IACUCS and the World Wide Web,* by Ken Boschert. This is a one page summary of IACUCS and other organisations which participate in the World Wide Web in the USA, Canada and Norway.

New service available for database searching

Dr Wendy van Dok, a Melbourne toxicologist, has established a company, Scientific Information Services (SIS), to provide a scientific search, processing and delivery service specialising in information that will replace, reduce and refine scientific experiments involving animals. SIS uses the DIALOG and Datastar database warehouses to ensure international coverage of primary journals, conference proceedings, books, news and reports. It is thus able to access millions of documents in over 650 databases. The DIALOG warehouse includes Medline, Agricola, CAB Abstracts and Biosis.

In addition to searching for information on the three Rs, SIS will also search for:

- data on any scientific subject;
- specialist views on the ethical implications of specific areas of science and technology;
- codes of practice, regulations and guidelines that relate to scientific research;
- biographical information on scientists;
- statistics;
- historical information about the development of a field of science;
- information on Australian, New Zealand and foreign institutions and universities.

Search results can be provided very quickly be email and translations can be made from scientific to lay English. SIS can summarise, review and compile a bibliography on a topic.

For further information, contact Dr van Dok on: tel: (03) 9802 7211, fax: (03) 9802 0772; email: wvandok@netspace.net.au or by post at GPO Box 2579W, Melbourne Vic. 3001.

NAEAC 1996 Annual Report

The New Zealand National Animal Ethics Advisory Committee (NAEAC), has as its mission statement "To provide independent, high quality advice to the Minister of Agriculture on policy and practices relating to the use of animals in research, testing and teaching". Its functions also include being conscious of the needs of:

- animal ethics committees;
- educational institutions;
- research institutions; and
- industry.

NAEAC works closely with ANZCCART in New Zealand and co-sponsored ANZCCART's 1997 Conference in Auckland and the Animal Welfare Officers' Workshop immediately preceding it. NAEAC is working with ANZCCART to explore the establishment of a system of confidential, voluntary reviews of institutional AECs in New Zealand. Such reviews will assist institutions in their quality assurance management procedures for the use of animals in research, teaching and product testing.

The NAEAC report covers the issues of blood harvesting from livestock, xenotransplantation and genetic manipulation of animals. The appendices include a list of organisations with an approved code of ethical conduct and a summary of animal usage, species by species, in the years 1992 to 1996. The total number of animals used in 1996 was 264,516. The report is available free of charge from NAEAC, Ministry of Agriculture, PO Box 2526, Wellington, New Zealand.

Coming up ...

Australasian Society for HIV Medicine Conference
13 - 16 November, 1997
Adelaide, SA
Contact: ASHM Secretariat,
GPO Box 2609, Sydney NSW 2001
Tel: 02 9241 1478
Fax: 02 9251 3552
Email: reply@icmsaust.com.au

5th IFTS Scientific Conference International Federation of Teratology Societies
'Stress Proteins and Apoptosis in Prenatal Development, Cancer and Medicine'
16 - 19 November, 1997
Swiss-Grand Hotel, Bondi, NSW
Contact: Assoc Prof David Walsh
School of Anatomy, University of NSW
Tel: 02 9385 1069
Fax: 02 9313 6252

Australian Society for Medical Research Annual Conference
23 - 26 November, 1997
Adelaide, SA
Contact: Ms Jenny Blanchard
PO Box 153, Nairne, SA 5252
Tel / Fax: 08-8388 6164
Email:
dleaver@medicine.adelaide.edu.au

Australasian Wildlife Management Society Conference
25 - 27 November, 1997
University of New England,
Armidale, NSW 2351
Contact: Dr Graeme Moss
Tel: 02 6773 3287
Fax: 02 6773 2769
Email: gmoss@metz.une.edu.au

Australasian Society for Clinical and Experimental Pharmacology and Toxicology Conference
30 November - 3 December, 1997,
Canberra
Contact: Conference Secretariat
93 Victoria Ave., Albert Park
Vic. 3206
Tel: 03 9690 6744
Fax: 03 9690 7155
Email: wsm@latrobe.edu.au

14th Australasian Biotechnology Conference "Food and Health for the 21st Century"
19 - 23 April, 1998, Adelaide
Contact: PO Box 153,
Nairne SA 5252
Tel / Fax: 08-8388 6164
Email: Biotech.Conf@flinders.edu.au

Australian Veterinary Association Annual Conference
18 - 22 May, 1988, Sydney
Contact: Mrs Doreen Culliver,
AVACOS, 7 Phipps Place,
Deakin ACT 2600
Tel: 02 6285 3600
Fax: 02 6285 3913

INVITOX 98
10th International Workshop on In Vitro Toxicology
14 - 18 September, 1998
Winchester, UK
Contact: Dr Diane Benford
School of Biological Sciences
University of Surrey
Guildford, Surrey GU2 5XH, UK
Fax: 44 1 483 300 374
Email: d.benford@surrey.ac.uk

ANZCCART News on The Web

Each issue of ANZCCART News is available electronically on ANZCCART's home page, together with an up-to-date index of all articles published in the newsletter since its inception in 1988, on http://www.adelaide.edu.au/ANZCCART/.

An index of the first five volumes was published as an insert in ANZCCART News **6**(1) (Autumn, 1993).

News from overseas

Europe

Revised status for animals

The European heads of government agreed in June to give new status to animals. The protocol states that in future the development of European Union policies on agriculture, transport, research and the internal market will "pay full regard to the welfare of animals" and that animals will be recognised as "sentient beings". They were previously classified as "agricultural products". The new protocol should affect all the industries which use animals in each of the 15 EU member states (Source: *Veterinary Record*, 28 June, 1997).

International course on laboratory animal science — Utrecht, June, 8-19, 1998

A two week intensive course on laboratory animal science will be held at the Department of Laboratory Animal Science, University of Utrecht, The Netherlands in June 1998.

The objective of this course is to present basic facts and principles that are essential for the humane use of animals and for the quality of research.

The contents are in line with recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) regarding the training of young scientists 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:
Professor LFM van Zutphen or Mrs Marianne Albers, Department of Laboratory Animal Science, Faculty of Veterinary Medicine, PO Box 80.166, 3508 TD Utrecht, The Netherlands.
Tel: 31-30-253 2033, fax: 31-30-253 7997, email: pdk@pobox.ruu.nl.

Germany

The August 1997 issue of the *EBRA Bulletin* includes an update on current issues in animal research in Germany. The article, by Ivar Aune, states that animal research issues have diminished in importance in the past three years as social problems have risen in Germany since unification with East Germany. The German Government is currently considering changes to the Animal Welfare Act. Animal rights activists are lobbying to have animal welfare included as part of the national Constitution, by having it included in the constitution of German States.

The Netherlands — statistics on animal experimentation

The number of animals used in experimental procedures in The Netherlands in 1995 was 632,714, a decrease of 6 per cent on the previous year. There was a continued decrease in the number of mice and rats used, although other rodents increased in numbers used.

The number of primates used increased from about 500 to 841, due to their use in the safety evaluation of human or veterinary products. The number of dogs, horses, reptiles, amphibians or fish used also increased. The data were published in *FRAME News* (May, 1997, p. 18), which commented that, while the overall decrease in the number of animals used was encouraging, the increases in certain categories were of concern and needed to be addressed.

Canada

The Canadian Council on Animal Care (CCAC) newsletter *Resource* (**21**(1), Spring - Summer, 1997) reviewed the operation of the CCAC in a number of articles. The CCAC is the primary agency in Canada responsible for establishing and monitoring standards of experimental animal care and use. The CCAC Council last February redefined CCAC's programs, which include its traditional coverage of animals used in research, teaching and testing, with the addition of animals used in the production of biological products; the capture, production or transportation of animals used for research, teaching, testing or manufacturing; the production of biological products and agricultural quality improvement programs.

The editorial by the CCAC Director, Dr Donald Boisvert, goes on to describe the criteria for CCAC's operations and for ensuring that its programs meet the needs of the community and of its clients.

The CCAC has recently issued *Guidelines on Transgenic Animals* to assist Animal Ethics Committees to review transgenic protocols and to provide guidance for animal care facilities which house these animals and for the researchers who create and use them.

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.

Contributions to *ANZCCART News* are welcomed and should be sent to:

Dr RM Baker, Executive Officer, ANZCCART,
PO Box 19, Glen Osmond, SA, 5064.

Tel. 61-8- 8303 7393: Fax. 61-8- 8303 7113
E-mail address: anzccart@waite.adelaide.edu.au
http://www.adelaide.edu.au/ANZCCART/

or

Mrs G Sutherland, Executive Officer, ANZCCART New Zealand
PO Box 598, Wellington, New Zealand

Tel. 64-4-472 7421: Fax. 64-4-473 1841
E-mail address: anzccart@rsnz.govt.nz
http://www3.rsnz.govt.nz/ctees/anzccart/

ISSN 1039–9089