News

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Genetically modified mice: past, present and future

Introduction

Genetically modified mice have been used in basic research for the past 20 years. In the early 1980s the first transgenic mice were produced and over the past decade an increasing number of gene-targeted mice have been generated (Gordon and Ruddle, 1981; Koller and Smithies, 1992). Transgenic mice carry additional genetic information, transgenes, which are introduced via micro-injection of DNA into fertilised eggs. Gene targeted animals, such as knockout mice, are created through the genetic manipulation of embryonic stem (ES) cells, which are then used to generate a new mouse strain. These genetic alterations can be precisely controlled.

The role of genetically modified mice in science today

Genetically modified mice are studied in all major areas of biomedical research:

- basic research;
- applied research;
- drug target validation;
- drug production; and
- transplantation research.

Basic research

In basic research, transgenic mice (gain of gene function) and knockout mice (loss of gene function) are generated to study the biological function of a particular gene. In contrast to traditional *in vitro* assays, no prior knowledge of gene function is required. A knockout mouse may show defects or phenotypic changes in some tissues due to the deletion of the gene under investigation. These phenotypic changes allow a biological function to be assigned to the gene. Assigning function to genes is one of the major challenges that will be faced after the sequencing of the human genome is completed. The human genome project (HUGO) is the worldwide effort to decipher the genetic code of man (Bentley, 2000). The genetic code, however, is only the beginning of understanding human biology, as it will be equally or more important to uncover the physiological function or role of each individual gene. Transgenic and knockout mice are the best models that are currently available to study gene function *in vivo*. They are the most sophisticated tools in what is called functional genomics, the task of translating a DNA sequence into a biological function. Functional genomics would describe the majority of research undertaken at academic institutions where the driving factor is the quest to find out what a particular gene does *in vivo*. One such example is the discovery of the biological function for the leukaemia inhibitory factor (LIF). LIF-deficient mice revealed that LIF was responsible for the implantation of the early mouse embryo into the uterus (Stewart *et al.*, 1992), a finding that was not too unexpected, but one which would have been very difficult to demonstrate in any system other than the live animal. Other examples include the discovery of the biological functions of the Sek1 protein in the development of the nervous system (Dottori *et al.*, 1998). This research has developed the transgenic and gene targeting technology and is still one of its major users and developers. One of the more recent developments is the conditional gene targeting system, which allows deletion of a gene in only one tissue in the mouse at a specific time during development, overcoming the obstacle of embryonic lethality (Kuhn *et al.*, 1995).

Applied research

Applied research utilises genetically modified mice to generate mouse models to study the mechanisms of, for example, inherited human disorders. These are the result of changes within the DNA sequence of a certain gene (mutations), or the reorganisation of chromosomes (e.g., translocations and inversions). Mutations as well as chromosomal reorganisations can be recapitulated in the mouse using gene targeting techniques. The resulting mouse models are used to research the underlying mechanisms of human disease with the intention to develop a basis for either therapy and/or cure. The generation of a mouse with mutations found in the

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Facts Sheet

This issue contains the facts sheet on Development and use of transgenic rodents in preclinical research — practical issues.
The facts sheet is also available as an offprint.

human condition cystic fibrosis (CF) is one of many examples (van Doorninck *et al.*, 1995). Although the observed pathology might not always be identical to that in humans, the mouse model can nevertheless be used to study disease mechanisms and potential treatments. Gene therapy was successfully tested in CF-mice before it was considered for treatment in humans (Alton *et al.*, 1993). A further example is the SCL gene that was identified in conjunction with a particular white blood cell cancer in humans. In order to elucidate its physiological role in this cancer, SCL-deficient mice were generated (Robb *et al.*, 1995), which confirmed that SCL plays an important role in the development of the red and white blood cells. These and other examples demonstrate the advantage that genetically modified mouse models have when compared to other *in vitro* or `non-genetic' *in vivo* systems. Genetically modified mice are the only disease model that mimic the human condition at a molecular level.

Drug target validation

Drug target validation is the process whereby biotechnology and pharmaceutical companies confirm that particular genes are associated with a disease. These genes then become candidates for therapeutic use. In other words a certain gene is validated as a target for drug validation. The vast amount of sequencing data as well as computing power available to date has made it possible to discover novel genes through database mining. This identifies human genes based on sequence similarity with known genes from the fly, fish or even worm. Many of these genes can potentially be involved in diseases. One of the many challenges is to identify these disease genes. Drug target validation presents one of the major bottlenecks in current drug discovery (Drews, 1999). One way to validate a gene as a drug target is by its over-expression in a transgenic mouse and / or by its complete ablation in a knockout mouse. Cathepsin K deficient mice demonstrated that cathepsin K protein is, as was predicted, involved in osteopetrosis, thereby confirming it as a potential drug target (Gowen *et al.*, 1999), in the same way as the LIF protein is a potential target for contraceptive drugs, for reasons mentioned above.

Drug design and production

Genetically modified mice can also be used in the last step of managing a disease, the implementation of treatment. Mice as well as other animals, such as pig, cow and sheep, can be made transgenic in order to produce foreign proteins. In some cases these foreign proteins are human proteins that are isolated, purified and used as therapeutics in the clinic. A prime example is human monoclonal antibodies (humAb). Antibodies are proteins produced by specific white blood cells, the B lymphocytes. Antibodies recognise, bind and neutralise invading bacteria and viruses. They are the molecular protection that is generated after an immunisation. In order to produce humAb as therapeutic agents, mice have been created that can generate humAb. In these humAb-mice, the mouse antibody genes have been deleted by gene targeting and the human antibody genes were induced as transgenes (Jakobovits *et al.*, 1993; Mendez *et al.*, 1997). Immunisation of humAb-mice results in the generation of human antibodies instead of mouse antibodies. These humAb are purified and used to treat diseases such as rheumatoid arthritis and breast cancer.

Transplantation research

Transplantation aims to provide patients in need of replacement organs with viable alternatives because of the insufficient numbers of donor organs. The development of mouse as well as human ES cells might soon help solve this problem (Gossler *et al.*, 1986; Shamblott *et al.*, 1998; Thomson *et al.*, 1998). ES cells, in combination with recent success in animal cloning, hold great promise to allow the custom design of tissue as required (Wilmut *et al.*, 1997; Wakayama *et al.*, 1998). Although it will take several years before clinical trials in humans can be started, the research on mouse and rat models is already very promising. It has been demonstrated that mouse ES cells can be made to differentiate *in vitro* into glial cells, cells that form the insulating cover sheet around nerve cells. These glial cells were then transferred into rats where they went on to wrap around and insulate nerve cells (Brustle *et al.*, 1999). These results show great potential for the treatment of neuro-degenerative diseases such as Parkinson's disease or repair of spinal cord injury. ES cells could one day provide an unlimited source of replacement tissues for treating human diseases, replacing the immune system cells in chemotherapy patients, supplying pancreas islet cells in diabetes and tissues for organ transplants.

Animal ethics and welfare

In all aspects of genetically modifying animals the welfare of these animals and the ethical responsibility of the people involved in handling them are of major importance. Guidelines have to be followed and suffering and pain of animals have to be avoided at all times. However, the outcome of a genetic modification is not as easily predictable as sometimes desired. In some cases the resulting phenotype is quite unexpected and does not confirm initial predictions. In many ways this only emphasises the power of the technology. Initial phenotype predictions are usually based on scientific observation made in experimental systems other than the whole mouse and therefore might not reflect the actual physiological reality. In contrast, the phenotype of a genetically modified mouse is the definitive result of what a gene or its protein product does in a complete organism. It is for that reason that results obtained from such animal models have biological significance and are unlikely to be artefactual, as is so often the case for results; for example, those obtained from immortalised cell lines in tissue culture.

The production of a novel genetically-modified mouse strain usually requires between 50 and 100 mice. This might seem a large number of animals, but the number of experimental animals needed for the phenotypic analysis is far lower when compared to classical animal experimentation and at the same time produces biologically more meaningful results. The total animal usage is therefore lower. Furthermore, these animals are often shared all over the world among research groups, again facilitating more rapid results and ultimately minimising the number of animals used. It is therefore generally believed that the down-stream benefits far outweigh the larger number of animals required to establish a novel mouse strain.

Conclusion

Genetically modified mice have become very prominent tools in biomedical research over the past 20 years. Initially these mice were only available to a small number of specialised laboratories which generated them in their own facilities. Many centralised facilities as well as commercial suppliers now custom-design and generate genetically modified mice, which has made them widely available within the research community. It is no longer acceptable to publish the function of a gene without having analysed a knockout or transgenic mouse. Knockout mice in particular have become the gold standard in functional genomics and drug target validation for academic institutions, as well

as for the biotechnology and pharmaceutical industry. In addition, transgenic mice are used to produce therapeutics and serve as models in transplantation studies.

While this article has focused on examples of genetically modifying the mouse, as this is the major and most experimentally advanced animal in biomedical research, genetic modification is possible in sheep, cow, rat and other species but not to the same extent. Rat ES cells, for example, are not yet available and cloning experiments have not been successful. For a more detailed discussion on the technical aspects of genetically modifying the mouse please refer to the facts sheet in this news- letter.

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Editor's Note

For further information on the ethical and welfare aspects of transgenic animals, see p.10 of this newsletter.

ANZCCART Conference 2000

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Farm Animals in Research - can we meet the demands of ethics, welfare, science and industry?

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A brochure and registration form will be available as an insert in ANZCCART News, 13:3, September, 2000.

For continuing updates on information regarding Conference 2000, please check our website:

http://www.adelaide.edu.au/ANZCCART/

Serological evidence for the presence of herpes B virus in macaques in Australia

Introduction

Herpes B virus (*Herpes virus simiae, Cercopithecine herpesvirus 1*) infection has recently been confirmed in a group of crab-eating macaques (*Macaca fascicularis*) held at Taronga Zoo. As part of a health assessment prior to export to a zoo overseas, seven crab-eating macaques were tested for herpes B virus. Five of the seven animals were seropositive for the virus. Stored sera from two out of four other crab-eating macaques held in the collection also tested positive for herpes B virus. Antibodies were detected using a herpes B virus specific ELISA. Although of little consequence for macaques, the virus has significant zoonotic potential.

Although previously suspected to be present in macaques in Australia, herpes B virus infection had never before been confirmed. The difficulty in obtaining a diagnosis of herpes B virus infection stems from the lack of suitable diagnostic tests in Australia. A limited number of laboratories in the USA and Europe provide testing for herpes B virus.

Macaques, particularly crab-eating (cynomolgus, long-tailed) macaques, rhesus macaques (*M. mulatta*) and pig-tailed macaques (*M. nemestrina*), are common non-human primates held in zoos, fauna parks, research facilities, circuses and private hands in Australia. Crab-eating macaques have been held in Taronga Zoo's collection since 1914.

Herpes B virus occurs as a common, latent, and asymptomatic infection of Asian macaques (*Macaca spp.*). It has been demonstrated by viral isolation and serology to occur in rhesus macaques (*M. mulatta*), bonnet macaques (*M. radiata*), Japanese macaques (*M. fuscata*), stump-tailed macaques (*M. arctoides*), Formosan rock macaques (*M. cyclopis*), and cynomolgus macaques (*M. fascicularis*).

Infection with B virus in primates other than macaques is usually fatal (Mansfield and King, 1998).

Infection has been reported in the patas monkey (*Erythrocebus patas*), black and white colobus (*Colobus abyssinicus*), capuchin monkey (*Cebus apella*), common marmoset (*Callithrix jacchus*), and humans. During a recent outbreak of B virus infection in a group of DeBrazza's guenons (*Cercopithecus neglectus*) several animals presented with classic herpes vesicles on the nares, oral cavity, and conjunctiva and signs of respiratory involvement prior to death (Bielitzki, 1999; Loomis *et al.,* 1981).

Transmission of herpes B virus among macaques occurs primarily through sexual contact or biting. Animals are usually seronegative at birth. The incidence of infection in juvenile macaques is usually low, yet may reach up to 80-90% in adult animals and may vary with the conditions under which animals are kept (Ott-Joslin, 1993). Seroconversion occurs soon after primary infection and is associated with resolution of clinical signs if the animal was clinically affected. Following initial viral replication, the virus is transported to and maintained in the trigeminal nerve and trigeminal nerve ganglia where a latent infection is established for the life of the animal (Ott-Joslin, 1993). Recrudescence can occur. However, factors leading to this are poorly understood. Stress, fever, ultraviolet light, tissue or nerve damage, and immunosuppression are factors which can result in reactivation of herpes simplex lesions in humans and may play a similar role in B virus recrudescence in macaques (Mansfield and King, 1998). If recrudescence occurs, virus is shed in oral and genital secretions. Virus has also been isolated from saliva, blood, faeces and urine. Virus shedding frequency at any given time is very low (2-3%) even in colonies 100% seropositive for herpes B (Mansfield and King, 1998; Centers for Disease Control and Prevention, 1987; Montali, 1995; Southers and Ford, 1995). Many colonies of macaques have endemic B virus infection with no apparent impact on the general health of the animals.

Clinical signs

Clinical disease in macaques associated with herpes B virus infection is usually mild, self-limiting, and characterised by the presence of vesicular lesions on the oral and genital mucosa. Vesicles usually progress to ulceration within a period of 10-14 days. Occasionally these lesions are accompanied by conjunctivitis. Dissemin-ated infection occurs rarely in macaques, and is characterised by either peracute or slowly progressive debility, respiratory distress and, often, death (Mansfield and King, 1998; Southers and Ford, 1995).

Zoonotic infection

The greatest concern over B virus infection in macaques is the zoonotic risk it presents to humans (Holmes *et al.,* 1995). Twenty-four human cases of B virus infection have been documented. This is a low incidence considering the common use of macaques for display, circuses, pets and research. The reasons for such an apparently low rate of infection in humans may be the infrequent B virus shedding by macaques, cross-reactive immunity against B virus stimulated by herpes simplex virus infection, and rarely, undetected asymptomatic infection. The virus is also very labile, and human infection usually requires direct inoculation by a scratch or bite, or exposure of conjunctiva or broken skin to secretions from macaques shedding the virus. Most human cases have developed following macaque-induced injury, although other methods of transmission e.g., human to human contact, needle stick injury, laboratory exposure (handling infected macaque tissues, especially brain), aerosolisation or unknown exposure have been reported (Mansfield and King, 1998; Centers for Disease Control and Prevention, 1987; Montali, 1995; Holmes *et al.,* 1995). Even though herpes B positive animals exist in zoos and wildlife parks, human clinical cases have never been documented in these settings (Montali, 1995; Holmes *et al.,* 1995).

In humans, vesicular dermatitis at the site of inocula-

tion develops one to five days post-exposure to herpes B virus. Pruritus at the inoculation site may be intense. These localised changes are followed by lymphangitis and lymphadenopathy. Fever, paraesthesia, muscle weakness and conjunctivitis may also occur. An ascending myelitis develops after three to seven days. Death due to severe encephalitis occurs 10-14 days post exposure in 70% of herpes B virus infected humans. Early recognition of the disease is critical as the use of acyclovir and gancyclovir in the initial stages of infection may be beneficial.

Conclusion

Prevention of human exposure to herpes B virus is dependant upon the education of personnel, the use of appropriate barrier safety equipment, and primate facility design that allows the use of restraint devices, such as squeeze cages which prevent the need to capture animals in nets, thus reducing the stress and need for close contact. Barrier safety equipment, such as safety glasses, latex gloves, face masks, coveralls and boots should be worn whenever macaques or macaque tissues are handled. High standards of husbandry and infection control must be implemented when caring for macaques. Herpes B virus exposure first aid kits and exposure protocols should be available wherever macaques are housed or handled. It is essential these health and safety programs regarding the care of macaques are carefully documented within the facility's standard operating procedures.

The confirmation of the presence of herpes B virus in macaques in Australia should serve as a reminder to veterinarians, medical practitioners, researchers, primate facility managers, laboratory personnel, and private owners of macaques of the significant zoonotic risk this virus may pose. It is important that we become more aware of the health status of captive primates through the use of specific diagnostic tests, and that protocols, equipment, and education programs are in place to prevent human exposure to potential pathogens.

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References and further reading

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Editor's Note

For further information on herpes B virus in zoo macaques, see the article, *Reality bites* by Debora MacKenzie in *New Scientist*, 22 April, 2000, pp16-17.

Innovation, ethics and animal wel - fare: public confidence in science and agriculture

Proceedings of this conference, which was mounted jointly by the New Zealand Animal Welfare Advisory Committee and ANZCCART in Wellington, New Zealand in November 1999 have recently been published.

These Proceedings will be valuable to anyone interested in the place of science and agriculture in the new millennium. They will be of special interest to those involved in the agricultural, scientific and veterinary professions, and to those interested in livestock production, animal welfare, the developing biotechnologies and their social regulation.

They are available now, for $NZ35, from the ANZCCART (New Zealand) office -

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and an order form is inserted in this issue of *ANZCCART News*.

Microbranding: a low impact permanent marking technique for small reptiles and frogs as an alternative to toe clipping

Introduction

Toe clipping is probably the most widely used permanent marking technique for small reptiles, mammals and frogs because it is easy to do and requires only a pair of surgical scissors. Its major disadvantage is that amongst the first 100 marked individuals, 11 lose one toe, 33 lose two toes, 40 lose three toes and 16 lose four toes. It is likely that the impact on climbing and burrowing species is significant when over half (56%) of 100 marked individuals lose 15 or 20% of their toes. With greater numbers marked the toe losses increase further.

Heat branding is one of several marking techniques used in field studies of reptiles and frogs (Ferner, 1979). This communication outlines a modified branding and numbering technique which is as easy to apply as toe clipping and is particularly suited to smaller lizards, frogs and snakes. Microbranding is fast and probably causes less immediate stress and probably has a lower impact on fitness and survival than other permanent marking techniques.

The preferred and chosen marking technique should be the one that has the least overall impact on the study animals.

Other workers have suggested or found that toe clipping has a negative impact on some species of lizards and amphibians (Woodbury, 1956; Clark, 1971; Clarke, 1972). My field research on the arid zone nocturnal burrowing Pernatty Knob-tail Gecko (*Nephrurus deleani*) includes mark / recapture studies. Toe clipping was considered unsuitable for my study because the geckos depend heavily on their ability to dig efficiently. I sought to reduce the impact of marking on the lizards and adapted aspects of several published marking techniques and refined the application of marks with equipment developed for the purpose.

Marking techniques for small reptiles and frogs

The table below provides a comparison of the most commonly used permanent marking techniques.

The most frequently used branding methods have involved the use of relatively large branding tools shaped as symbols. Thin nichrome wires shaped into numerals have been flame-heated and used to brand numbers onto lizards, toads, frogs and salamanders (Clark, 1971; Taber *et al.*, 1975). Weary (1969) used a modified woodworker's pyrographic needle to mark the ventral scales of snakes and Clark (1971) used flame-heated nichrome wire in a similar way. Weary (1969) also suggested using a small 12- volt soldering pencil powered by a rechargeable battery.

Small animals marked by branding usually need to be picked up and examined to read the marks, so the size of the brand can be reduced to a practical minimum, which will also reduce overall stress and impact. If the brands are small dots placed at coded positions then large numbers of individuals can be sequentially numbered for subsequent recapture recognition.

I have adapted Jenssen's (1970) marking scheme (1, 2, 4, 7) which by addition allows each number from 1 to 9 to be marked (e.g., 3 = 1 + 2, 9 =2 + 7), similarly for 10's and 100's. I apply microbrand spots according to the system shown in Figure 1 and write the actual number onto the belly with a fine waterproof overhead projector pen to aid rapid recognition of a recently microbranded recapture.

Microbranding and marking schemes

For permanent marking of many lizards the tail is not a suitable site, due to the capacity for voluntary and involuntary autotomy. I have therefore assigned the microbrand sites so that those which are most frequently used (units, tens and hundreds) are placed on more robust (larger) parts of the animal. This has the benefit of making the branding process easier.

I did consider the branding of a mark on the mid-back for rapid determination whether an animal was a recapture but decided against it because the little time it would save did not justify the impact of the microbrand on the animal. From number ten onwards there is always at least one microbrand mark on one of the hind limbs and they are quite easy to check. The marks are permanent, although without an understanding of how the branded skin heals they could be overlooked.

I have not been able to assess the long-term impact of microbranding on fitness and survival in my study. I routinely recapture individuals that were marked as hatchlings weighing about 1.5 g for up to four years (to date) by which time they can weigh as much as 28 g. I have not found any evidence of infection at recent microbrands (1, 2, 4, 6, 12, 18, 21+ days). Between two and 12 days the scars have sand particles adhering to them and presumably they adhere to the small amount of fluid that seeps from the wound. After 18 days the lizards had moulted (pen number on belly absent) and the microbrand scars had narrow frills of epidermis. After seven weeks the spots had a covering of small flat sub-equal scales, part of the entire local scalation, which is more heterogenous in size and profile.

Microbranding by its very nature minimises pain and stress as there is no blood loss due to its cauterising action, the area of scarring is minimised and position of brands can be chosen to minimise their impact and optimise examination. Microbrands

Comparison of the most commonly used permanent marking techniques

Marking method	Speed, complexity and cost	Immediate and short term stress	Impact on fitness
Toe clipping	fast, low, low	brief pain, bleeding risk	toes permanently lost
Ventral scale clipping	slow, low, low	long pain, bleeding and infection risk	scar tissue
Tattooing	slow, high, medium	long pain, infection risk	
Pit tags	medium, high, high	brief pain, infection risk	possible obstruction
Freeze branding	slow, high, medium	long pain (~10 secs / mark)	scar tissue
Heat branding	fast, medium, low	brief pain (~1 sec / mark)	scar tissue

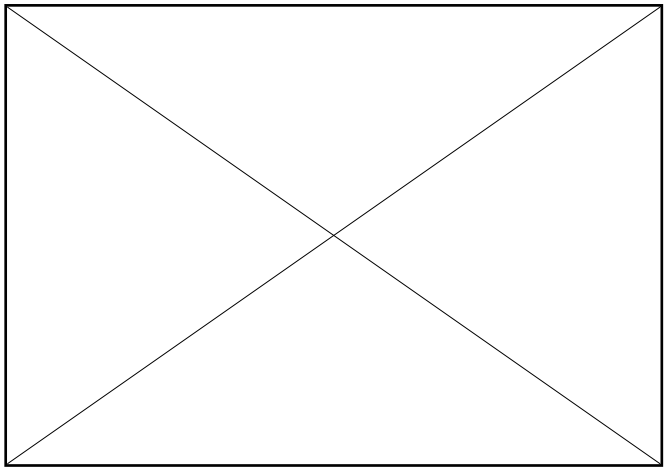

Figure 1. Unit numbers are placed well to the left of the vertebral line, tens along the left leg, hundreds along the right leg, etc. The parallel sided areas indicate the general area for each number. The actual microbrand spots may be larger than shown.

are so small that they do not compromise the colour and pattern of the animal towards predator detection.

Equipment and its use

Portability, speed and convenience in the field are important considerations for equipment and techniques, particularly at night. For the first two years of my study I used a small soldering iron (12-volt scope iron) connected to a vehicle battery or cigarette lighter. This required walking up to three km to the vehicle with all animals captured which had to be marked and then returning them to their points of capture.

To overcome the disruption to the geckos' activity time and my time loss I carried a 12-volt rechargeable gelcell which allowed me to process and mark animals at their points of capture. However, the power drain in getting the iron up to temperature at each capture point frequently resulted in a flat battery before the night's work was done. I then used a propane gas miniature soldering pencil iron for almost a season, until one day, about 30 seconds after turning the flame off, the plastic fuel tank (handle) exploded with enormous force. Working in remote bushland sites at night in midsummer made this unexpected safety hazard absolutely unacceptable.

I then developed a microbrand tool which has an electrically heated nichrome wire tip and is based on the same principle as medical diathermy units. This design significantly reduces the power needs to reach branding temperature. Furthermore, the tip cools rapidly due to its small mass, thus reducing the risk of personal skin burns and bushfire.

I use electric jug element wire to wind a three loop tip coil (2.3 mm outside diameter x 1.8 mm long) with two tails of the wire (18 mm long) which are attached into a small two pin plug. A matching socket is wired to a momentary switch and these two items are combined into a handy sized and custom shaped hand tool using a resin based putty. Power is supplied to the hand tool via a 1 m long twin flex (as used for 240-volt lighting) from a DC socket on my six volt rechargeable torch. The lizard is variously held in the fingers of one hand so as to prevent its body, limb and tail from moving towards the tip coil during branding. I take particular care to immobilise the limb to be branded and the tail. The hand tool is held in the other hand. Due to the small size of the animal and the equipment and the torch light conditions I find that this is best done as a one person procedure.

The tip coil is energised with the switch momentarily until it just starts to glow and then I wait just until the glow is gone (less than a second) before applying the tip to the epidermis and dermis at the brand site. This ensures that the tip coil is not too hot. The application is light and only as long as is needed to cauterise the dermis at the contact spot (less than a second). This ensures that the microbrand is not too deep or severe. I wind the three loop tip with the loops separated by 0.2mm so that the fresh microbrand has two bridges of intact dermis running through the brand spot. I believe that these help the healing process by occluding the full area of the microbrand and possibly by acting as skin grafts.

Microbrands to the distal parts of the hind legs (40, 70, 400, and 700 points) need to be placed precisely and without a need to re-apply to avoid damaging the blood vessel that passes down the trailing side of the leg. This vessel is visible through the skin in many geckos. Dissection of several preserved specimens (if other species are to be marked) will provide precise microbrand sites. To ensure that I place the mark precisely I first go through the full brand procedure without heating the tip, lift the tip about 0.2 mm, heat the tip and then microbrand. The tip cools rapidly and sufficiently between brands on the one animal due to its small mass.

Application to other vertebrates and field conditions

Before using this marking technique on animals in wetter habitats it would be advisable to do a small sample. Prior to 1990 I did microbrand skinks, geckos, snakes and frogs on a small scale as a personal preference alternative to toe clipping while doing research and teaching work in wetter habitats in and around Sydney. No attempt was made to rigorously evaluate the impact of those brands, but small skinks in a long-term backyard study appeared unaffected and survived for at least three years. It may be possible to use microbranding as an alternative to toe clipping with other amphibians such as salamanders and newts. In the case of small mammals (especially possums and *Antechinus*), microbranding may possibly replace both toe clipping and scissored ear notches by applying the brands in the same sites used for ear notching. The ear would need to be restrained in a suitable way.

Acknowledgements

I thank Sue Carthew, Hans Osley and Gerhard Kubach for stimulating discussions and ideas, without which this equipment and refinement of my earlier technique would not have been developed.

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Book reviews

Health and Safety in Laboratory Animal Facilities

ISBN 1-85315-421-0 249 pp.

Eds. Wood, M and Smith M
The Royal Society of
Medicine Press Ltd
London, 1999

Price: £37.00 (incl. postage)

Available from: Hoddle,
Doyle, Meadows Ltd.,
Station Road, Linton, Cambs.
CB1 60X, UK

Fax: 44 1223 893855

This publication of contemporary health and safety issues as they relate to animal facilities replaces the 1981 Laboratory Animal Handbook Five - *Safety in the Animal House*. The editors believe that, although the orientation is principally towards conditions in the UK, this work reflects best practice and they recommend it to those working with laboratory animals regardless of their location.

Malcolm Gamble's introductory chapter provides a broad and informative outline to health and safety in laboratory animal facilities. There is little new in Gamble's list although it is presented in a logical sequence and sets the scene for a deeper elaboration of the contemporary health and safety issues. Gordon and Tee's contribution (chapter two) on Allergenic Hazards includes over 140 references and provides an extremely useful resource for anyone wishing to research Laboratory Animal Allergy (LAA) in greater detail. This is arguably the most researched and written about occupational hazard animal workers are exposed to and the chapter covers well-worn ground but it is presented at an academic level more commonly found in scientific papers. If molecular weights of allergens are of interest or relevance to the reader this information is included. At a more practical level the comparisons between common laboratory animals and where the majority of allergens congregate should be helpful when designing LAA management strategies. The five-page section on *Management and prevention of allergy* brings together the main LAA issues very effectively.

Michael Dennis, from the Centre for Applied Microbiology and Research (Wiltshire), contributes 40 pages (chapter three) on all you need to know about infectious hazards in animal facilities. This is a particularly well structured chapter and includes a detailed contents list which allows the reader to efficiently locate a specific point of interest. More than half the discussion on zoonotic infections is dedicated to non-human primates although other common laboratory animal species are included. This chapter achieves both breadth and depth in its discussion on infectious hazards. Anyone responsible for designing and reviewing operational procedures for pathogen containment and exclusion facilities will benefit from its contents.

The inclusion of a chapter (four) dedicated to health and safety issues arising from genetic manipulation of laboratory animals, by M. Smith (MS Management Services), highlights the increasing relevance of this discipline in biomedical research. The chapter focuses on aspects of the production and use of transgenic animals in the research environment and avoids the controversies surrounding the implications of genetically modified animals on the environment in general.

There appears to be little relevance to health and safety in the discussion and even less relevance to anyone outside the UK as most of the chapter is dedicated to referencing (UK) government regulations and legislation. Smith may have been better advised to focus on the health and safety issues only and save the general legislative details for a separate *Managing a Transgenic Laboratory* publication. Readers outside the UK would be better served referencing their relevant government and institutional regulations to ensure compliance when establishing a transgenic laboratory.

Palotai's chapter (five) on *Chemical hazards* refocuses on health and safety and although there are frequent references to the *Control of Substances Hazardous to Health Regulation 1999*, it also provides many recommendations on practical management strategies. Taylor's chapter (six) narrows the focus considerably and provides a thorough account of radiation safety both in general terms and in the context of animal house activity. The benefits from reading through the first few sections (aspects of radiation; radiation units; philosophy of radiation protection; principles of radiation work) include a far greater knowledge of radiation and more importantly a significantly improved understanding of the implications of working with radioactive material in the animal house. The sections on radiation protection in the animal house and protection against ultraviolet and microwave radiation provide many practical recommendations for the facility manager in developing policies and procedures.

Safety Management (chapter seven) by John Ryder continues the practical approach to health and safety management although very much directed at the departmental Safety Officer. Ryder explores many safety management initiatives which would assist in lifting the awareness of health and safety in an animal facility.

Kevin Dolan's (chapter eight) overview of the legal requirements, as they relate to animal facility operations, makes an effort to provide an international perspective with a brief outline of the European requirements, but it remains essentially a very comprehensive list of UK Acts, Codes and Regulations. Readers may choose to use the naming of a UK regulation as a starting point in their research on the existence of an equivalent regulation in their own country.

Overall the book achieves its objective, although it is more successful in outlining the principles and legal obligations involved in occupational hazards than it is in the more practical application of hazard management. The relevance of this book beyond the UK increases significantly if the facility supports primate research (infectious hazards) and/or radiation research. At £35 (approximately $80.00AUS) there is enough new material to complement existing occupational safety material, including the United States National Research Council's 1997 publication, *Occupational Health and Safety in the Care and Use of Research Animals.*

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The UFAW handbook on the care and management of laboratory animals, 7th edition

(ed. T. Poole)

ISBN 0-632-05133-7

Price:
$A576 (two volume set)
or $A450 (volume 1) and
$A179 (volume 2)

Available from:
Blackwell Science
54 University Street
Carlton South
Victoria 3053

Tel: 03 - 9347 0300
Fax: 03 - 9347 5001

email: finance@blackscia-sia.com.au

The UFAW Handbook (the abbreviated title by which I have known this publication for many years) was first published in 1947 and has long been an essential reference text for anyone working with traditional laboratory animals of all shapes and sizes, as well as for zoo curators and others with a special interest in the husbandry of captive animals.

The scope of the previous (sixth) edition, published in 1987, was already very wide and this has been further extended by the inclusion of new sections on the chimpanzee, on higher invertebrates and on the tropical animal house.

The seventh edition (1999) is again edited by Trevor Poole, with the assistance of Pauline English and is now in two volumes. The first volume (840 pages) covers terrestrial vertebrates, while the second volume (190 pages) covers amphibians and aquatic vertebrates and advanced invertebrates. It is unclear why the seventh edition was published in two volumes , other than to provide the option of buying only one if the information in the other volume is not required, as it is only about 100 pages longer than the sixth edition.

While the price is substantial, particularly for Australia and New Zealand, it remains the classic reference text in its field.

The seventh edition for the first time includes a foreword by Professor William Russell, co-author with the late Mr Rex Burch of UFAW's 1959 classic publication *The Principles of Humane Experimental Technique.*

Volume One comprises three parts. Part One defines laboratory animals and considers issues such as environmental enrichment, animal production and breeding methods, nutrition and feeding and an introduction to laboratory animal genetics, all of which is useful and valuable background information. Part Two is titled *Animal Units* and covers animal house design, equipment and environmental control, safety and hygiene, transporting animals and the tropical animal house.

Part Three comprises the majority of the volume and covers species kept in the laboratory, classified in 37 chapters under mammals, birds and reptiles.

In addition to traditional laboratory mammals, there is a chapter on Australian marsupials (which includes much useful data on housing, breeding, feeding and laboratory procedures (including physiological data) on three commonly used species - the tammar wallaby, fat-tailed dunnart and brush-tailed possum. Other interesting mammals covered include the laboratory (or grey) opossum of Central and South America, pigs and minipigs, sheep and goats, cattle and the horse. Nine chapters are given to non-human primates, including a chapter by Poole and other UFAW colleagues on marmosets and tamarins. Species of birds included are the domestic fowl, Japanese quail, doves and pigeons, the zebra finch and European wild birds.

Reptiles are covered in one chapter.

Volume Two is in three parts - *The Captive Environment* (a new chapter on life support systems for aquatic research centres), *Vertebrates* (five chapters, on fish, amphibians and aquatic reptiles) and *Invertebrates* (chapters on cephalopods and decapod crustaceans). References are appended at the end of each volume, rather than after each chapter and each volume is indexed.

This edition no longer includes a chapter on the laws relating to the use, care and management of laboratory animals. This is reasonable, as the Handbook is very much an international publication and a focus on UK laws is therefore not helpful to a large number of readers. Nevertheless, some attention to the ethics and welfare of laboratory animals would have been valuable and would have reinforced the foreword by Professor Russell.

The UFAW Handbook has been for many years the outstanding general publication in the management of laboratory animals. The seventh edition is well printed and illustrated and maintains its high standard. It will continue to be an essential reference text for all who work with experimental animals.

Robert Baker
ANZCCART
Adelaide

The use of wildlife for research

The Proceedings of the ANZCCART conference held in Dubbo, NSW in May, 1999

The conference papers cover a wide range of topics from basic scientific curiosity, to conservation biology, to vertebrate pest management.

The proceedings were edited by Professor David Mellor and Dr Vaughan Monamy and are available from ANZCCART's Adelaide office for $A25.30 (which includes 10% GST).

anzccart@waite.adelaide.edu.au

All ANZCCART publications may be paid for by cash, cheque or credit card.

Newly Published

Three new publications on ethical and welfare aspects of transgenic animals

1. The production and use of transgenic animals

This is a special supplement (number 1) to volume 27 (1999) of the journal *ATLA* (Alternatives to Laboratory Animals), published by FRAME in the UK.

It includes the full text of ECVAM (the European Centre for the Validation of Alternative Methods) Workshop Report 28: *The use of transgenic animals in the European Union* (ed. T. Mepham *et al.*), as well as seven other papers and an introduction by Professor Michael Balls, who examines whether the use of transgenic animals raises particular welfare and ethical concerns. He argues that they do and that this is reflected in the new guidance notes from the UK Home Office for project licences involving the generation and maintenance of genetically modified animals. These notes are published in full in this ATLA Supplement.

Contributed papers include comments from the Canadian Council on Animal Care (CCAC) on whether CCAC guidelines, which were published in 1997, meet the ECVAM recommendations on transgenic animals (Griffin and Gauthier), who end with the reminder that it is imperative that investigators intending to embark on research programs involving transgenic animals consult institutional animal care staff as early as possible and that such programs do not proceed until the AEC is assured that the institution has provided adequate resources to meet the special needs of transgenic animals. The special ethical issues that are inherent in the production, monitoring and use of transgenic animals must continue to be examined to ensure that scientific gain is always balanced by appropriate animal welfare considerations.

ANZCCART has three free copies of this Supplement at its Adelaide office.

2. Genetic engineering: animal welfare and ethics

This is a discussion paper from the Boyd Group in the UK and explores issues associated with the production and use of genetically manipulated animals, including:

- fundamental ethical objections;
- consequences for animal welfare;
- strategies for improving understanding of welfare ethics; and
- benefits sought from genetic modification and the need to use transgenic animals.

The Boyd group is a forum for the exchange of views on issues of concern related to the use of animals in science, with members drawn from diverse backgrounds. Its objectives are to promote dialogue and, where there is consensus, to recommend practical steps towards achieving common goals.

For further information contact the Boyd Group
PO Box 423
Southsea PO51TJ, UK

www.boyd-group.demon.co.uk

3. Genetic engineering and animal welfare: preparing for the 21st century

This 127-page monograph, edited by Janet Gonder, Ernest Prentice and Lilly-Marlene Russow, was published in October 1999 and is the proceedings of the conference held by the Scientists Center for Animal Welfare (SCAW) in Chicago in September, 1996. The proceedings cover three topic areas:

- an overview of genetic engineering and the well-being of animals (two papers);
- animal well-being and xenotransplantation (four papers); and
- ethical considerations related to animal use in genetic engineering (four papers).

Copies can be obtained for $US40.00, from SCAW, 7833 Walker Drive, Suite 410, Greenbelt MD 20770, USA.
Email: info@scaw.com
Website: www.scaw.com

Reducing the use of laboratory animals in biomedical research: problems and possible solutions

This is number 29 in a series of Reports and Recommendations from workshops held by ECVAM. Papers from all of these were published in the journal *ATLA* (see *ATLA* **26,** 283-291, 1998 for this paper) and are available free of charge as offprints from FRAME.

This workshop was held in January 1998 and chaired by Dr Michael Festing, of the MRC Toxicology Unit at the University of Leicester (see Dr Festing's Facts Sheet which will be included in the next issue of *ANZCCART News*). Its aims were to find ways of reducing the number of animals used in biomedical research without reducing research output, and to make recommendations for practical ways in which this might be achieved. The Report considered the following topics:

- improving research strategy;
- experimental design;
- statistical analysis;
- interpretation and communication;
- legislation and internal review; and
- education and information.

It ends with a series of recommendations and possible methods for their implementation, including data from other published papers.

Other recent ECVAM Reports

Workshop Report 36 (1999)
The potential use of non-invasive methods in the safety assessment of cosmetic products (Rogiers *et al.*, *ATLA* **27,** 515-539).

Workshop Report 37 (1999)
The principles of good laboratory practice: application to in vitro toxicology studies (Cooper-Hannan *et al.*, *ATLA* ***27,*** *539-577).*

Workshop Report 38 (1999)
The use of keratinocytes and human skin models for predicting skin irritation. (van de Sandt *et al.*, *ATLA* **27,** 723-743).

Workshop Report 39 (1999)
Cell transformation assays as predictors of human carcinogenicity. (Combes *et al.*, *ATLA* **27,** 745-767).

Taskforce Report 1 (1999)
The integrated use of alternative methods of toxicological risk evaluation. (Blaauboer *et al.*, *ATLA* **27,** 229-237).

Letter

New curriculum in veterinary science at the University of Sydney to include ethics and animal welfare

There are exciting changes afoot in the Faculty of Veterinary Science at Sydney University. The current first-year students are the lucky recipients of a new curriculum in veterinary science, the culmination of a long process of revision involving academics, members of the veterinary profession and experts in the field of education. Students now have a new learning experience before them with a shift in emphasis from delivering information as discreet disciplines to a more integrated approach to knowledge. Learning contextually is the aim. While there have been some welcomed casualties to the old curriculum, such as the subject of physics, there have also been some new additions.

One in particular is a unit of study called Professional Practice. This will run for the first three years of the course and aims to introduce students to a wide range of generic skills which have previously been difficult to locate in traditional units of study, although of high vocational importance. Communication and business skills, stress management skills, computer and study skills are just a few. First-year students will also receive their first taste of practice as they are assigned to Education Support Practices for a semester. An important element of this unit of study is keeping a portfolio which contains both examinable material, in the form of essays and assignments, as well as a self-reflection component. By encouraging students to record their interests, thoughts and reactions to aspects of the course and undergraduate life they will learn to assess their own progress and identify personal and professional strengths and weaknesses. It is also a wonderful way for academic staff to get to know their students, whose previous accomplishments have proven quite staggering! There are graduates in science, arts, philosophy, medicine and engineering, mature age students and students from as far away as Norway and Botswana.

Animal welfare will also feature as a formal component of the new curriculum. First-year students have the opportunity to discuss animal welfare issues in both Professional Practice and Animal Husbandry units. Dr Paul McGreevy, who is a veterinarian and ethologist, explains how an understanding of an animal's behaviour can lead to better management and better welfare of animals. He outlines how the behavioural needs of a species can be studied and what relevance "environmental stress" can have for animal welfare. In third year he will expand on these areas in a unit of study called Animal Behaviour and Welfare Science.

Dr Cathy Schuller is another lecturer in animal welfare, who has a degree in veterinary science and a doctorate in applied ethics. Students are introduced to animal welfare by being asked to consider the role the veterinary profession should play and what they think their own role should be when they graduate. Veterinary students understand that they can achieve more than just "front-line" care for animals. Individually, or as a profession, they can influence change through research, extension activities in the community and by lobbying for legislative reforms. Students regard the veterinary involvement in the debate over tail docking dogs as setting a precedent for the profession's moral voice on issues where animal welfare is compromised.

Discussing the ethical dimension of animal use is an area Dr Schuller is keen for students to become involved in. Ethics is a widely used, perhaps overused term, and her aim is to clarify the difference between normative and professional ethics and how both are important in professional decision-making. The topic of euthanasia of healthy companion animals has provided a good entry to understanding the various ethical dilemmas that a veterinarian can confront in practice, particularly where client, patient and veterinary interests conflict. Students will also be introduced to a variety of topics which are relevant to ethical discourse: theoretical frameworks for discussing the moral status of animals; current research on different attitudes to animals within the community; and the legislation and codes of practice relating to animal use.

The new curriculum will challenge veterinary students to think beyond traditional boundaries of veterinary science. If the future intake of students continues to reflect such a diversity of backgrounds and additional tertiary qualifications it is certain that the veterinary graduate of tomorrow will be annexing some exciting, new territory for the profession.

Cathy Schuller
Faculty of Veterinary Science
University of Sydney

Coming up

International Conference on Animal Science and Veterinary Medicine towards the 21st century
(ICA SVM 2000)
12 - 15 August, 2000
Beijing, China
Contact: Ms Xu Jinhua
Fax: 86 10 6289 5351
email:
xmskyczy@public3.bta.net.cn

Australian Koala Foundation Conference
23 - 25 October, 2000
Noosa, Queensland
Status of the koala in 2000
Contact: Jane Mathers
email:
jane@savethekoala.com

INVITOX 2000
European Society of Toxicology *in vitro*
25 - 28 October, 2000
Alicante, Spain
Contact: Dr Jose Castell
Fax: 34 96 3868 718
email: invitox2000@gva.es

ANZSLAS Conference
13-17 November 2000
Hong Kong
Contact: Dr Tony James
Tel: 852 2609 6036
email:
tonyjames@cuhk.edu.hk

ANZCCART 2000 Conference
Farm animals in research — can we meet the demands of ethics, welfare, science and industry?
30 November - 1 December, 2000 Adelaide
Contact: ANZCCART's Adelaide office for details.
tel: 08-8303 7393
email: anzccart@waite.adelaide.edu.au

News

ANZCCART STUDENT AWARD

The Board of ANZCCART Australia is again offering its ANZCCART Student Award, in conjunction with the 2000 Conference in Adelaide on November 30 and December 1, whose theme is *Farm Animals in Research - can we meet the demands of ethics, welfare, science and industry?*

The purpose is to encourage attendance and involvement at the Conference by honours and postgraduate students.

The award is open to all disciplines and is worth $1,000 inclusive of travel costs.

Students are required to submit an abstract on an animal welfare theme relevant to the conference and be prepared to give a 10 minute talk at the conference.

Applications should be submitted by 31 August to:

Dr R M Baker
Director
ANZCCART
PO Box 19
GLEN OSMOND
SA 5064

Telephone:
(08) 8303 7393

Facsimile:
(08) 8303 7113

Email: anzccart@waite.adelaide.edu.au

HSUS Awards

The website of the Humane Society of the United States (HSUS) includes an annotated listing of about 90 courses currently being taught at universities in North America which address animal ethics issues.

The HSUS awards for academic excellence in the design and instruction of courses addressing issues of animal ethics, rights, and/or welfare are intended to help foster the availability of high-quality curricula and instruction in relevant academic fields such as biology, law, environmental studies, philosophy, psychology and animal science.

Each year, the HSUS recognises two college-level courses:

- an established course currently taught at an institution; and
- a new course scheduled for instruction.

The awards are $US1,500 each, and are given to the institutional department in which the course is taught. No special forms are necessary

Nominations must include:

- a letter of recommendation from the relevant department chairperson;
- a course outline or syllabus;
- a brief (one paragraph) description of how the award will be used; and
- student evaluations for the previous two years the course was offered (for established courses only).

Send nominations by September 1, 2000 to:

Dr Jonathan Balcombe
HSUS

Fax: 1 301 258 7760

email: jbalcombe@hsus.org

The website address is: http://www.hsus.org/programs/research/courses.html

Using animals in science online

http://anzccart.rsnz.govt.nz

This exciting new resource has been prepared jointly by ANZCCART New Zealand and the Animal Welfare Science and Bioethics Centre at Massey University in New Zealand. It includes a useful list of articles, books and videos related to animal welfare and the use of animals in research and teaching. There is also a list of web addresses to other relevant sites.

Click on the headings in the main menu to explore each section of this resource. Another way is to click on particular keywords.

Topics covered in the resource are:

- Why study animals?
- Minimising the harm done to animals used in science;
- Benefits of animal-based science;
- Balancing harm and benefit;
- The three dimensions of science;
- Ethics and animal use in science; and
- Control of animal use in science.

This excellent teacher and student-friendly site is designed as:

- a school project for school teachers;
- a teaching resource for school teachers;
- interesting and helpful information for parents;
- important information for tertiary students in any animal-based science;
- useful general information for tertiary students in other disciplines;
- useful background information for researchers and tertiary teachers; and
- a valuable resource for independent members of Animal Ethics Committees.

* A slightly modified version for Australian readers will soon be added to ANZCCART's Australian website.

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:

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