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Chytridiomycosis - a new disease of amphibians

Chytrid fungi and amphibian declines

Many amphibian population declines are obviously due to habitat degradation, but in the last twenty years there have been mysterious population crashes in protected high altitude areas where no habitat problems have been detected. This pattern is particularly evident in the montane rainforests of Queensland and Central and South America (Laurance *et al.*, 1996, 1997; Lips, 1998). In some cases, these crashes have resulted in extinction of stream-dwelling rainforest species.

A new species of chytrid fungus has been found infecting the skin of frogs dying during mass mortality events in forests in Queensland and Panama, and may be the cause of these precipitous population declines (Berger *et al.*, 1998). Chytrids are small spherical fungi that produce motile infective stages called zoospores. Some species are found free-living in soil and water where they degrade organic matter such as chitin or keratin, and others are parasites of algae, plants, nematodes or insects (Barr, 1990). Before the discovery of the amphibian chytrid, none had been found to cause disease in vertebrates. The epidemiological data support the hypothesis that this fungus has been introduced to these rainforest areas and is the cause of the population crashes.

In Queensland, seven rainforest frog species disappeared during the past twenty years (Richards *et al.*, 1993; Mahony, 1996). The first extinctions occurred in the D'Aguilar and Conondale mountains near Brisbane in the late seventies/early eighties. The amazing southern gastric brooding frog was last seen in 1979. This was an incredible species whose tadpoles developed in the stomach of the female and the newly metamorphosed froglets emerged through the mouth. In the mid eighties, frog populations in central eastern Queensland declined, and the northern gastric breeding frog (the only other gastric brooding frog in the world) has not been seen since. By then it was clear that our frogs were in trouble, and so remaining high-altitude populations were intensively monitored. In the early nineties, populations in north Queensland suffered similar sudden declines, but this time zoologists were present to witness ill, dying and dead frogs as mass mortalities occurred (Laurance *et al.*, 1996).

In many of these episodes of declines, tadpoles were seen for months after adult frogs had disappeared.

A range of causes have been proposed to explain the declines, but introduction of a waterborne infectious disease fatal to adult frogs appears the most reasonable explanation. Abnormal levels of water pollutants were not detected, water pH was stable and population changes were not associated with habitat disturbance or unusual weather (Richards *et al.*, 1993; Laurance *et al.*, 1996). Increased ultra-violet radiation can be discounted as ground-level solar ultraviolet radiation has not increased significantly at tropical latitudes, and most of these frogs are nocturnal an live in dense rainforest. The asynchronous timing of the declines and apparent spread of the declines from south to north is consistent with a new epidemic agent progressing through a naïve population. All species of frogs that suffered significant declines are stream-breeding and stream-dwelling, suggesting the problem is waterborne (Williams and Hero, 1998).

During the mass mortality in north Queensland in 1993, about twenty dying frogs were collected for diagnostic investigations (Berger et al., 1998). The species found dying included the sharp-snouted day frog, waterfall frog and common mist frog. Pathology revealed the presence of chytrid fungi in the keratinised layer of the skin, and acute, non-specific degenerative lesions in some internal organs. Bacteriological and virological studies did not identify any causative agent. The chytrid fungi appeared to be associated with minor local changes in the skin, and the reasons why frogs died was not apparent. This fungus had never been seen before, and as there was no background information available on diseases in healthy populations of these frogs, it was difficult to determine the significance of its occurrence.

Between 1995 and 1998, a network was set up around Australia to collect any sick wild or captive frogs found by zoologists, and to investigate their diseases pathologically. A range of new diseases was detected, but importantly, the

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chytrid fungus was found to be widespread and associated with mass mortality in a range of species where no alternative causes of the deaths were detected (Berger *et al.*, 1998). Infections were found in frogs from a wide range of habitats in both unpopulated and populated regions, including cities such as Brisbane and Adelaide.

In two instances where tadpoles were being raised in captivity, almost all frogs died with chytridiomycosis in the weeks after metamorphosis. When healthy tadpoles were euthanased and examined, the fungus was only found in the mouthparts, which is the only keratinised area on tadpoles. As the fungus appears to be keratinophilic, this could explain why tadpoles survive while adults die. We suspect that the chytrid spreads from the mouthparts to the skin on the body when it becomes keratinised after metamorphosis.

As the chytrid fungus is difficult to culture, a preliminary experiment was conducted using skin scrapings collected from a dead great barred frog and added to the water of six captive bred frogs (Berger *et al.*, 1998). Four controls were given filtered skin scrapings with the fungus removed, and four controls were untreated. Between 10 and 18 days later, all six frogs given skin scrapings became lethargic and were shown to be infected with the fungus. One died and the rest were euthanased with MS 222. The eight controls remained uninfected and healthy.

In 1997, a mass mortality event in rainforest frogs and toads in Panama was detected (Lips, unpublished) and an apparently identical chytrid fungus was present in the skin (Berger et al., 1998). As the pattern of declines in Central America and Queensland was remarkably similar, it seems the chytrid may be one of the most significant causes of global amphibian population decines, particularly in isolated frog populations in pristine environments.

Many questions remain to be answered, and investigations into the origins, distribution, and spread of the amphibian chytrid are ongoing.

Diagnosis and management in captive amphibians

Chytridiomycosis can cause high mortlity in captive amphibian collections. A group at the Washington Zoo working independently of our group also identified the amphibian chytrid as the cause of death in several species of frogs (Pessier *et al.*, in press).

Clinical signs of chytridiomycosis vary between species, and include lethargy, reddening of ventral skin, convulsions with extension of hindlimbs, accumulations of sloughed skin over the body and occasional ulcers. Death usually occurs a few days after the on-set of signs of disease. Chytridiomycosis should be suspected if frogs develop any of these signs. A high mortality rate in two to three-week-old metamorphs is also suggestive of this disease. None of these signs are pathognomonic for chytridiomycosis and diagnostic tests are required to confirm an outbreak.

Examination of unstained pieces of the excess skin slough from fresh, fixed or frozen specimens, under a light microscope, is a quick diagnostic method. The round or oval fungal bodies (8-20 mm) have a distinct refractile wall, and may be empty or contain zoospores (Figure 1). Occasional empty sporangia are divided into two, four or more internal compartments by thin membranes.

The fungal sporangia are easily seen in histological section with haematoxylin and eosin (H and E) or PAS stains (Figure 2). Various stages of the lifecycle are present in the keratinised epidermis. On H and E stained sections sporangia appear as solid basophilic bodies, contain numerous dark zoospores, or are empty after the zoospores have discharged. The discharge tubes project just above the skin surface and are occasionally seen in a section. Skin of the ventral body, limbs and feet are most consistently infected, and should be examined histologically. Hyperkeratosis or erosions of the epidermis occur in heavily infected areas. Tadpoles can be screened by examining sagittal histology sections that include the mouthparts, as sporangia are found under the surface of the dark brown keratinised "teeth".

No drugs have yet been tested for treatment, but antifungals currently used for amphibians or fish may prove useful in treating chytridiomycosis.

Benzalkonium chloride is a disinfectant that has been used at 2 mg/l to successfully treat a similar superficial mycotic dermatitis in amphibians caused by *Basidiobolus ranarum* (Groff et al., 1991). The regime used experimentally was 30 minutes of bath treatment, on three alternate days. This was repeated in 8 days (i.e. 6 treatments in total).

Oral itraconazole has also been used to treat *Basidiobolus ranarum* infections (Taylor, 1999).

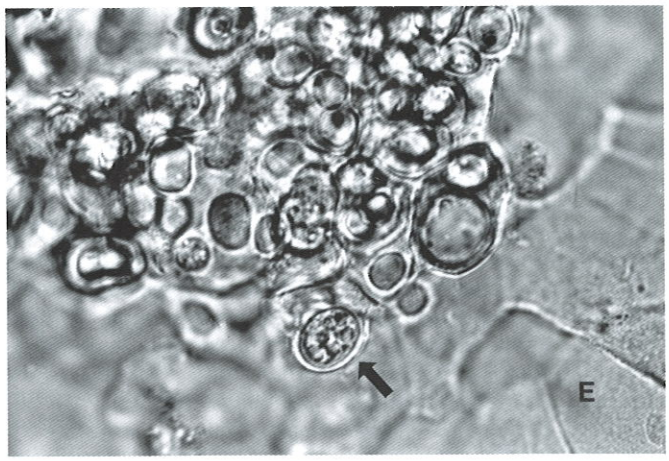

Figure 1:
Unstained skin slough from a green tree frog examined by light microscopy. Note refractile, round and oval fungi. Most are empty, but one contains developing zoospores (arrow). E = epidermal cell.

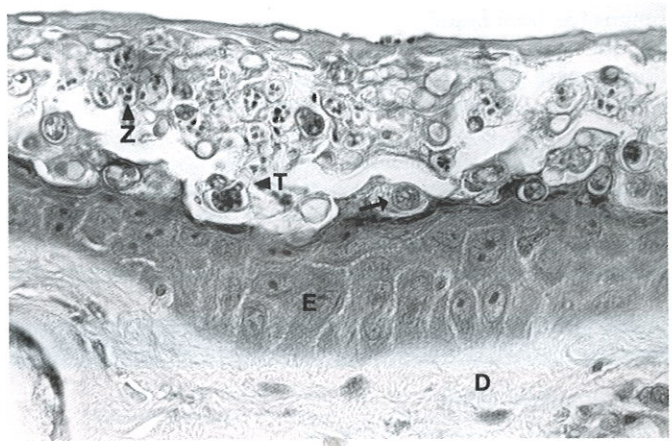

Figure 2:
Histological section of skin from a heavily infected green tree frog. The fungus does not invade through the epidermis, but occurs in the superficial keratinised layer which becomes thickened in severe infections. Solid, immature sporangia are present (arrow) as well as mature sporangia containing zoospores (Z). Zoospores swim out through a discharge tube (T), and the empty sporangia remain. The amphibian chytrid does not grow hyphae. E = epidermis. D = dermis.

Other possible antifungal treatments include oral ketoconazole (10mg/kg once a day) or copper sulphate baths (500mg/l dip 2 minutes daily to effect) (Raphael, 1993).

Routine quarantine procedures have been adequate in restricting outbreaks to certain tanks, and no airborne transmission has been observed (G. Marantelli, unpublished). Every group of frogs should be kept completely separate to ensure no water-borne transmission of disease can occur. By changing and discarding gloves between every tank, avoiding splashing water between tanks, and disinfection of tanks and implements using 2% hypochlorite before reusing, many frogs have been housed in close proximity without transmission of disease.

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NB: We have made available an amphibian disease site on the world wide web - http://www.jcu.edu.au/dept/PHTM/frogs/ampdis.htm. This contains instructions on how to collect skin and toes to test for the chytrid, how to collect frogs for pathology, how to prevent transmission of amphibian pathogens between locations, a bibliography of amphibian declines and disease, and a list of people with expertise in frog disease.

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Facts Sheet on Amphibians

The next issue of *ANZCCART News* will include a facts sheet by Associate Professor Michael Tyler of the University of Adelaide on husbandry and handling of amphibians and their use as laboratory animals.

Immuno-Adjuvants Monograph now available

This 35 page monograph was published by ANZCCART in December 1998 and an order form is enclosed with this issue.

It includes three contributed papers as well as guidelines for dose rates and routes of administration for Freund's adjuvants in nine species of animal and should be of interest to researchers, technical staff and animal ethics committees.

Xenotransplantation

Introduction

Xenotransplantation is the transplanting of tissue from one species to another. In this article we will be concentrating on the transplanting of tissue from animals to humans.

Limited supplies of human organs from cadavers results in long waiting periods for those in need of organs and, in some cases, demise of these people before such organs are available. In Australia last year there were 1783 potential recipients waiting for allografts, but only 841 organs were provided from 190 donors (Australian Co-ordinating Committee on Organ Registries and Donation, 1998)

In an attempt to overcome this lack of supply consideration is being given by the community to use organs and tissues obtained from animals, especially pigs. Pigs are chosen because of their biological similarity to humans (Hannon *et al.*, 1990, Miller and Ullrey, 1987) The use of other animals such as non-human primates is possible but unlikely.

Recent trials

Xenotransplantation of animal tissue into humans commenced at the beginning of the century in an era when methods of preventing rejection were not understood. There have been some organs xenotransplanted over the past decade but it has been only in the last few years that the trend for carrying out this sort of procedure has been on the increase throughout the world. All recent trials have utilised cells rather than organs for this purpose because rejection appears to be less of a problem with cells (see below). The most recent trials have been:

- 1991-4: 10 diabetic patients in Sweden received insulin-producing fetal pig cells. The grafts functioned for several months with histological confirmation of survival of the graft at three weeks (Groth *et al.*, 1994).
- 1994-7: 6 diabetic patients in New Zealand received insulin-producing neonatal pig cells. These grafts, which were placed inside microcapsules to protect them from immune destruction, also functioned.
- 1995-7: 24 patients with advanced Parkinson's Disease in Boston received fetal pig neuronal cells. Clinical improvement has been reported in these patients and histological survival of the grafts was demonstrated in one patient who died for unrelated reasons (Deacon *et al.*,1997).
- 1996-8: Patients with fulminant liver failure were treated by *ex vivo* perfusion utilising pig liver cells.

The basis for these human xenotransplant trials has been rodent studies where porcine tissue was xenotransplanted with successful reversal of degenerative neuronal disease (Galpern *et al.*, 1996) and diabetes (Korsgren *et al.*, 1991)

Risks of xenotransplantation

The risks are that it will not work because the graft is rejected. Rejection of pig tissue can occur acutely by means of pre-formed antibodies reacting with galactose ∝(1,4) galactose epitopes, especially on blood vessels, less acutely by antibodies reacting also with endothelial cells, and chronically via cells of the immune system, especially the lymphocytes. Antibody mediated rejection occurs either within minutes to hours (acute), or over days (less acute); cellular mediated rejection occurs over days and weeks. Xenografted cells in contrast to organs may not undergo significant antibody mediated rejection (Mandel *et al.*, 1997) because of the lack of donor blood vessels, but will certainly be destroyed by cellular rejection. In contrast to the two forms of rejection of xenotransplanted pig tissue, rejection of transplanted human tissue occurs only by cellular means.

In an attempt to prevent antibody-mediated destruction of pig organs, transgenic animals are being bred (Cozzi and White, 1995) These animals usually are engineered by the insertion of DNA from the human clotting system into a fertilised egg. Expression in the adult of the protein from this DNA prevents complement activation and hence antibody mediated cell lysis. Other strategies, such as the use of anti-rejection drugs are needed to prevent cellular rejection.

The recipient will develop an infection, either as a side effect of chronic immunosuppressive therapy, or by direct transmission from the graft. This is called a *xenozoonosis*, as distinct from *zoonosis*, which is the infection on non-immunosuppressed humans by a micro-organism from an animal.

There are concerns that pathogenic viruses, especially a retrovirus akin to the human immunodeficiency virus (which most likely came from simians) will create a public health problem. Prions causing neurological disease have been transferred from sheep to cattle (resulting in bovine spongiform encephalopathy or BSE), and possibly to humans, the effects of which paralysed the beef and dairy industry of Britain (Sternbach *et al.*, 1997). During 1997-1998, H5N1 influenza virus was transmitted from pigs to chickens and then to humans, resulting in 19 documented cases and three deaths (MMWR Report, 1998)). Transmission of porcine paramyxovirus to humans has been postulated on the basis of serological testing, although direct linkage with human disease remains unproven (Chant *et al.*, 1998). In these examples, the organism was transmitted by oral (BSE), respiratory (H5N1 influenza) or unknown (probably parenteral) mechanisms. Parenteral transmission would be the only likely method of transmitting any agent from xenotransplanted tissue.

The focus of our current work is on identifying and preventing transmission of porcine endogenous retroviruses (PERVs) that are present in pig tissue. What is the evidence for the existence of a retrovirus in pigs that could create a public health problem? In Britain, two PERVs have been described that are capable of infecting human cells in the laboratory (LeTissier *et al.*, 1997; Patience *et al.*, 1997). Whether such retroviruses survive or are pathogenic in immunosuppressed recipients of porcine tissue in unknown. To address this possibility, we have looked for the presence of these viruses in humans xenotransplanted with insulin-producing fetal pig cells. While the retrovirus was present in untransplanted porcine tissue, it was absent from all five recipients of pig cells (Deng *et al.*, 1998).

Guidelines for xenotransplantation of pig tissue into humans

In the interests of public safety, guidelines are being developed around the world (Anon, 1996a, 1996b) to allow further xenotransplanation of pig tissue into humans. The NHMRC in Australia should shortly release a report on xenotransplantation from an *ad hoc* working party for public comment. If Australia's guidelines follow those from

the U.S. Food and Drug Administration (Anon, 1996b) they will include:

- donor pigs should be reared in closed colonies and be serologically defined
- donor pig tissues and organs must be free of microbiological contamination
- blood and tissue from the donor and blood from the recipient are to be archived
- permission will be required from a central regulatory authority as well as from the local ethics committee.
- the xenotransplant team is to include an infectious disease physician, a veterinarian, transplant immunologist, hospital infection control specialist, the director of the hospital clinical microbiology laboratory, and a transplant surgeon.

The most economical way of implementing these guidelines in Australia is to establish a single site for breeding pigs that will be free of human pathogens. It is time to crease this colony since it does not exist in Australia, but does in other countries, including the USA.

Risk benefit analysis

Ultimately decisions about the transplantation of porcine tissue into humans will be based on a risk-benefit analysis. The benefits are potentially enormous to the 80,000 Australians with type 1 diabetes and the 30,000 with Parkinson's disease. This is provided both cellular rejection can be overcome. Currently the risk that there will be a public health problem is theoretical. We and others are attempting to quantitate this, and respond to the theoretical risks with practical answers. However, it will require a small number of pilot trials, for example, with insulin-producing pig cells transplanted into immunosuppressed diabetic recipients, as well as the ongoing monitoring of previously transplanted recipients, to fully assess the risk benefit ratio.

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ANZCCART's Guidelines for Animal Ethics Committees

Readers are reminded that this package is available from our Adelaide office for $35.00. It includes notes and a set of references on the functions of the AEC, focussing on the different categories of membership, as well as a copy of Dr Vaughan Monamy's monograph *Animal Experimentation: A Student Guide to Balancing the Issues*, the 1997 edition of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and the relevant State or Territory legislation.

Two Massey University initiatives to promote animal welfare

New Professor of Animal Welfare Science

David Mellor has been appointed AGM-ARDT Professor of Animal Welfare Science at Massey University in New Zealand following the departure of Neville Gregory, its first incumbent, who is now Science Director of the Meat Industry Research Institute of New Zealand (MIRINZ).

The Chair provides leadership in animal welfare sciences and related bioethical developments at Massey, through research, education and consultancy. It improves understanding of what good animal welfare is and the ways high animal welfare standards are achieved. These include educating students and the wider community about how the needs of animals can be met. Professor Mellor has led the Massey University animal welfare science research group since its establishment in 1989, and introduced animal welfare science and animal ethics teaching at the university. He also set up and led an interdisciplinary bioethics discussion group from 1989 to 1994.

Professor Mellor is a consultant in New Zealand for the Animal Welfare Advisory Committee and Ministry of Agriculture and Forestry and for the U.K. Farm Animal Welfare Council and has been a member of the National Animal Ethics Advisory Committee for the past six years. He is Vice-Chairman of the New Zealand Board of (ANZCCART) and is also the Royal Society of New Zealand representative on the Australian Board of ANZCCART.

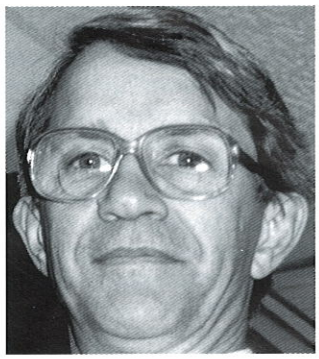
New Animal Welfare Science and Bio-ethics Centre

The welfare of animals in New Zealand will be enhanced by the establishment of the Animal Welfare Science and Bioethics Centre (AWSBC) at Massey University.

The new Centre brings together expertise in animal welfare science and the increasingly important field of bioethics, a unique combination in the animal welfare arena. The Director of the Centre is Professor David Mellor.

Associate Professor Kevin Stafford (Institute of Veterinary, Animal and Biomedical Sciences) leads the veterinary aspects of the Centre's animal welfare activities and Mr James Battye (School of History, Philosophy and Politics) facilitates bioethical developments. Other Massey University staff from the College of Sciences and the College of Humanities and Social Sciences are Centre members.

The AWSBC is an example of how multi-disciplinary teams can provide advice and leadership in areas which are of strategic importance to livestock industries and of significant social concern.



Professor David Mellor

The scientific expertise it can provide includes animal welfare science, applied animal behaviour science, applied physiology, animal husbandry, livestock systems, and veterinary and wildlife sciences, as well as devising "alternatives" - computerised and other substitutes for animal use in teaching.

Two Animal Welfare bills currently before the New Zealand Parliament reflect a change in animal welfare thinking in New Zealand, moving away from pursuing those who are cruel to animals to encouraging the concept of a "duty of care" towards animals, emphasising personal responsibility for meeting animals' needs.

Members of the AWSBC work closely with other animal welfare research groups in New Zealand and internationally. Cooperation helps to ensure that research in New Zealand achieves the maximum benefit for animal welfare. The Centre has close ties with New Zealand organisations, including Crown Research Institutes, MIRINZ, the Ministry of Agriculture and Forestry, the NZ Veterinary Association, and the Royal (NZ) Society for the Prevention of Cruelty to Animals.

A recent agreement among members of animal welfare research and advisory groups in Australia, Canada, the UK and the USA to participate with the Centre in an e-mail network will ensure international exchange of information on the promotion of animal well-being.

For further Information on animal welfare in New Zealand .....

see the article by Dr David Bayvel in ANZCCART News 10 (4): 5-6, December 1997.

This issue also featured an article about the new Animal Welfare Centre in Melbourne.

Book Reviews

Principles of Animal Design. The Optimization and Symmorphosis Debate

Edited by E. R. Wiebel, C. R. Taylor and L.. Bolis, (1998) Cambridge University Press, ISBN 0521 583704, 314 pages, $A59.95 (soft cover)

This is a fascinating collection of short papers which focus on an intriguing question- are animals built economically? The authors follow a diverse array of experimental approaches to determine whether animals are optimized for the functions they perform. To address the highly integrative and cross-disciplinary nature of this field, the 47 contributors are drawn from zoology, anatomy, physiology, biochemistry, evolution, biomechanics, neuroscience and nutrition, among other fields. The authors participated in the 12th International Conference on Comparative Physiology in July, 1995.

Some critics reject the concept of optimisation in animal design as unlikely and consider that evolution will lead to development of adequate, rather than optimal structures. The optimisation concept is particularly hard to test where one body component performs multiple functions. However, the authors have devised some creative approaches to overcoming this obstacle.

The book succeeds superbly in its stated aim of presenting the arguments for symmorphosis in an accessible but challenging form suitable for a wide scientific readership. The papers are clear and well written, providing sufficient background for a general scientific reader to be able to understand each of the examples presented.

The book begins with a clear definition of the hypothesis of symmorphosis, that animals are designed so their capacity is matched to their functional needs. Three aspects of symmorphosis are considered in detail; functional performance, functional capacity and structural economy in design. Each major chapter of the book begins with an overview of the evidence for optimal design. Each chapter considers one body system or function. These include the skeleton, skeletal muscle, energy metabolism, respiration through lungs and gills, digestive system and nutrient supply, vision and neural function.

The importance of safety factors in the locomotory system is considered in chapters on muscle and bone. The evolutionary cost of developing and maintaining excessive structure is real. The way that bones respond to stress by constant macroscopic and microscopic remodelling is used by P. Dullemeijer to reinforce the concept that built-in systems exist to enable animals to adapt their structures to be appropriate to their environment/experience. Loss of bone density occurs rapidly when load is removed, such as in the weightlessness of space. A. W. Biewener compares the bone-muscle-tendon design appropriate for animals with different patterns of locomotion, such as the horse and kangaroo. It is clear that optimal biomechanics in muscle-tendon design provide elastic energy savings, although these come at the expense of some loss of directional control of movement.

This leads naturally into reviews by L. C. Rome and T. J. Roberts of the aspects of muscle and tendon function which are important for locomotion on land, in water and in air. The plasticity of muscle fibre type in response to the typical pattern of movement performed is used by D. Pette and R. S. Staron as a compelling example of symmorphosis in response to functional demand. A series of theoretical predictions of the optimal speed for movement is compared to the actual speed of free-living animal movement. From this analysis biomechanical models are developed to explain the usual patterns of movement observed.

One of the particular strengths of this series of reviews is the use of comparative studies to identify similarities and differences in ways which different animals have resolved various physiological problems. For example, J. N. Maina presents an illuminating tale of the adaptations seen in the cardiorespiratory system of bats which enable them to meet the high metabolic demand for oxygen during flight. It is clear that birds have a much broader structural base to support flight, through their specialised lung-air sac system which occupies a very high volume of the total body with more intimate association of air and blood capillaries, compared to mammals. In contrast bats succeed by having very finely tuned respiratory systems, which are most similar to those of elite athletic species like the horse. They have high haematocrits, high red blood cell counts and a very thin blood-gas barrier, so that they achieve maximal efficiency in gas exchange across a mammalian lung. However there are limits to this specialisation, so it is highly unlikely that bats could fly if they were as large as some birds (up to 18 kg).

These examples support the concept of symmorphosis, but reinforce the idea that there are often multiple ways in which optimal functional design can be achieved.

Adaptations of the digestive and intestinal transport capacity for nutrients to meet the needs of lactation are discussed by K. A. Hammond. It is clear that during periods of peak lactation, particularly where there are multiple young placing high demands on milk production, the mother's nutrient uptake rate may reach a maximum level, so any further metabolic demands, for example from cold stress, exercise or disease will rapidly exceed the overall capacity of the system. I. D. Hume, the sole Australian contributor to this book, presents a variety of interesting models for fermentative digestion in herbivores and concludes that function is optimised by use of continuous-flow, stirred tank reactors (typical of the rumen) and modified plug-flow reactors (such as the equine hindgut). The adaptations of the rumen microflora to dietary changes are used by R. R. Hofman to highlight the range of change which can be achieved. It is clear however, that through evolution the concentrate selectors (e.g., deer) have become sufficiently different in structure from the grazing ruminants (e.g., cattle) that they can no longer occupy identical nutritional niches. In contrast the opportunistic mixed feeders (e.g., goats) are able to utilise a much wider range of plant feeds, although they are selective feeders which avoid fibre where possible.

Overall this is a fascinating "read" in comparative physiology, which provokes the reader by providing illuminating insights into the questions of "how" and "why" animals work as they do. It is accessible to a very wide scientific audience and may also provide some provocative focal points for teaching students. Recommended (scientific) reading for the holidays!

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EBRA and FELASA: the ethics of animal experimentation

Proceedings of the European Congress, 17 and 18 December 1996
ISBN 0 9533049 0 6.
(ed. P. N .O'Donoghue)
Available from EBRA
58 Great Marlborough Street, London, WIV, IDD, UK

In introducing the Proceedings of the 1996 Congress, the co-chairman indicated that one of the things being attempted at that time, was the pursuit of the middle ground, being the points of agreement between those opposing and those supporting animal experimentation. When such a highly diverse area as animal experimentation is considered, it is probable that facts can be found that will give some support even to conflicting points of view. It is not surprising that few real signs of consensus are obvious.

The basic philosophy, encapsulated as the Three Rs, continues to be extremely useful, but it is quite reasonable, at this time, to ask, are we building on the Three Rs and where to now? There are hints in the Proceedings that we are progressing, even though there are examples where mere adherence to the Three Rs seems to be practically sufficient.

Science may have reached the stage when the philosophy of animal use is becoming settled. The ethics of specific cases will begin to be the main point of discussion and replace the general principles of using live animals in experiments. The broadly descriptive categorisation of transgenic animals by Velpire *et al.*, is useful for example, but serves to illustrate the range of specific issues that emerge from the use of a single type of manipulation. Naquet raises, in general terms, the acceptability factor in research, particularly in relation to the study of epilepsy. What does emerge is the relation of models to the disorder being modelled.

Clearly, the use of cultured cells to study the manifestation of an *in vivo* network may be potentially complex, but a summary of the extent to which this has been achieved in immunology and haemopoiesis would be generally helpful in discussions on Replacement. In the last 25 years or so, a large amount of information has accumulated on how such cells differentiate and function in culture and the various permissive culture conditions that allow cells to behave as they do *in vivo*. The Proceedings contain a number of short contributions of a specialised nature that readers will find rewarding to browse.

It is interesting that the field of testing is beginning to be divided into the areas of moral obligation, legal requirement and scientific necessity (Balls). The adherence to the Pharmacopoeia is mentioned by several authors (e.g., Spielman), particularly in relation to the harmonisation of guidelines.

Both de Cock-Buning and Spielman suggest that good science could be the criterion for the level of ethical practice to which we should aspire. The internal norms of the scientific community are neither consistent throughout nor an adequate criterion for good science. Maybe our current perceptions of good science can be one of the major themes for the next European Congress.

The real meaning of a reduction in the number of animals used is still a controversial topic, although the number of animals necessary to set up a simplied toxicity protocol for a given new medicine in 1995, compared to 1991 standards is an interesting analysis (van Cauteren). What emerges is the need for scientific debate on animal use statistics before they are collected, if nothing else, to avoid setting up such spurious targets as the 50 per cent reduction by the year 2000 which was withdrawn by the European Union (O'Donoghue and Purchase).

The Proceedings summarise nicely how things were in 1996, albeit with an understandable Eurocentric flavour. As a document for persons not attending the meeting, the Proceedings could be of greater value if the Chairman had found an appropriate person to prepare a final section on the overall impressions of the Congress, possibly with a flavour of which things should be picked up in future meetings.

Readers will gain the impression that animal experimenters seem to be getting it right, but still have to keep promoting the principles that are emerging and let the public see what is happening. We may not have to go back as far as Ryder does to determine that things are not what they used to be!

John Marbrook,
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University of Auckland

Workshops to be held on the Three R s

The Queensland Department of Primary Industries and the University of Queensland will be holding three workshops in 1999 on the Three R s. Each will be at the University of Queensland.

The cost is $20.00 per workshop and the dates and topics are:

`Replacement' - Monday 22nd February, 1999

`Reduction' - Monday, 10th May, 1999

`Refinement' - Monday, 19th July, 1999

Details, including the program for the first workshop, are available from the University of Queensland website at -

http://www.uq.edu.au/oraps/oraps-home/eth&aw.htm

or contact

Dr Julie Reilly
Office of Research and Postgraduate Studies
University of Queensland

Tel: 07 3365 2713 or 07 3365 2925
Fax: 07 3365 4455

Email:
j.reilly@research.uq.edu.au

Letters

Management of animal ethics at a multi-campus regional university

Charles Sturt University (CSU) is a regional university with campuses at several locations, situated several hundreds of kilometres apart. Research and teaching activities using animals, including ecological fieldwork activities, occur at the Albury-Wodonga, Bathurst and Wagga Wagga campuses. Applications for the use of animals for research are also received from the Murray Darling Freshwater Research Centre at Albury and infrequently from other establishments. The CSU Animal Care and Ethics Committee (ACEC) meets 11 times a year to consider applications.

The ACEC has twelve members, three of whom are not employed by the University. It comprises two veterinarians (Category A), six members with recent research experience (Category B), two members representing animal welfare interests (Category C), and two independent members (Category D). The right of audience and debate is extended to two additional University staff members: an animal technician (from the University's Animal House) and the Head of the School of Biomedical Sciences. The ACEC is chaired by a Category B member, a Senior Lecturer with several years research experience. The large membership ensures that each Category is represented at meetings, as required under the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes,* and that there is representation from each of the three major campuses of the University. These are important considerations as a number of the ACEC members have heavy teaching commitments and research responsibilities which may prevent them from attending all meetings. The ACEC is also serviced by a Secretary, who is responsible for preparation and distribution of meeting agendas and minutes, and an Executive Officer who retains the ACEC's records and prepares necessary reports, both internal and external.

The ACEC considers over 50 new applications for the use of animals in teaching, research or fieldwork activities each year. Applications for research approval are received mainly from: the School of Environmental and Information Sciences (Albury-Wodonga) and the Environmental Studies Unit (Bathurst) for ecological studies; the School of Agriculture (Wagga Wagga) for animal science research; and from the School of Biomedical Sciences (Wagga Wagga) for medical science/biotechnology research. Applications to use animals for teaching purposes are received from these Schools as well as from the Schools of Nursing and Health Sciences (Bathurst), Health and Human Services (Wagga Wagga), Community Services (Albury-Wodonga), Science and Technology (Wagga Wagga) and Medical Radiation Science (Wagga Wagga).

Meetings of two hours duration are held every month, except January, by videoconference. This facility enables members from each campus to attend with the frequency required to provide researchers and teachers with a prompt response to their applications. The ACEC has a number of other functions, including:

- maintenance of a website address which provides researchers and teachers with access to application forms and information about animal ethics issues and links to various reference sites available on the internet;
- periodic inspection of animal research and teaching facilities on the three major campuses and the animal house at the Wagga Wagga campus;
- maintenance of a resource library for use by researchers, teachers and ACEC members;
- provision of general and specific advice to applicants (on an individual basis and via seminars) on acceptable methods to be used by researchers and teachers; and
- debate about contemporary animal welfare issues as a basis for ground rules for dealing with specifiic areas of concern, such as the Three Rs.

The ACEC's most recent activity has been to revise its application forms for the use of animals for teaching and research purposes. Six forms have been developed - four application forms and two review forms:

The research application forms are based on the NSW Department of Agriculture Animal Welfare Unit models. All forms and guides for completion can be downloaded by applicants from the ACEC website. This has two significant benefits: all submitted applications and review forms must now be word-processed, removing the problem of illegible handwriting; and any minor changes made to the forms will be apparent to the applicant when they download the source file.

In conjunction with the launch of the new forms, the ACEC conducted a series of information seminars on each campus. Attendees were pro vided with an extensive package of reference material including several *ANZCCART* articles, excerpts from the *Code of Practice* and NSW Agriculture *Guidelines for Wildlife Research.* The seminars provided teachers and researchers with the opportunity to gain insight into current animal welfare issues and new requirements of the Code, as well as providing a forum for discussing difficulties individuals may have with the ACEC approval process.

The CSU-ACEC's website address is:
http://www.csu.edu.au/research/resgrad/ethics/animal/front.htm

The CSU-ACEC is willing to liaise with other ACECs to share information and ideas. Inquiries can be directed to: Mrs Kaye Lander, Executive Officer, Animal Care and Ethics Committee, Charles Sturt University, Locked Bag 588, Wagga Wagga NSW 2678.

P.A. Towers and K. Lander
Charles Sturt University
Wagga Wagga NSW

Obtaining small volumes of blood from conscious rats

There are few references in the literature which provide detailed practical information on how to collect blood from the conscious rat. We therefore wish to describe the method that is used in our laboratory (modified and expanded from the "Duffy Rat Roll", as described by Marshall *et al.,* (1994)).

Place a rectangular towel (53.5 x 107 cm) on the bench and fold the lower end up to one-third length-wise. Put the rat parallel to the left edge of the towel but with its tail hanging out over the lower margin.

Fold the left hand edge of the towel over the rat swiftly Hold the rat firmly through

the towel, and roll it into a firm tube. On its cranial end, fold the towel over to form a blind end and secure it with a rubber band. Immobilise the rat completely by placing another rubber band around the distal one-third of the tube. This rubber band should be firmer than the one placed cranially.

Put the rat/tube on the bench and allow the tail to hang over the edge. Place the tail into a polystyrene cup containing luke-warm water for about 30 seconds. Identify a clearly dilated vein. With bevel pointed up, insert an insulin needle (e.g., Terumo® 29G, 100 or 50-U) in line with the vein at an angle of 10 degrees. Insertion is easier if the needle is placed between the scales of the tail.

Advance the needle whilst withdrawing the plunger slightly (avoid excessive withdrawal as this will collapse the vein). Successful puncture is indicated by the "flash-back"of blood and can also be "felt" as the needle slides into the vein. Place the collected blood into a standard eppendorff tube. After removal of the needle control the bleeding with an alcohol swab.

Up to 500 ul of blood can be collected with this technique and surprisingly, most rats remain relatively still. This entire procedure takes less than 5 to 10 minutes per rat, is easily performed by a single person and most of the time we have a 100 per cent success rate. It is important to be confident, gentle and patient when handling the animal and identification of a dilated tail vein is paramount before attempting needle insertion. We find that the latter is easily achieved, possibly because of the increased body heat generated by the rat being within the towel; application of the rubber band; and/or the tail warming with water.

Commercial perspex devices are available for physical restraint, but in our experience rats become physically distressed and refused to enter the container on a second occasion. Moreover, they are expensive and different containers are necessary for rats of different ages, whereas we have used the above technique quite easily for rats weighing between 200 and 900 g.

Gopala K Rangan
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Westmead Hospital, Sydney

Reference

Marshall, S., Milligan, A. and Yates, R. 1994. Experimental techniques and anaesthesia in the rat and mouse. *ANZCCART News*, **7** insert. pp. 1-4

ANZCCART/ AWAC CONFERENCE, NEW ZEALAND

Innovation, Ethics and Animal Welfare: Public Confidence in Science and Agriculture

18 - 19 November 1999
Wellington
New Zealand

A brochure and registration form is inserted in this issue.

For further information please contact:

Mrs Gill Sutherland
PO Box 598
Wellington NZ

Tel: 64 4 472 7421
Fax: 64 4 473 1841

Email:
sutherland.g@rsnz.govt.nz

ANZCCART's 1999 Australian Conference

The Use of Wildlife for Research

This conference will be held at the Western Plains Zoo, Dubbo, NSW on 26 and 27 May 1999. The topic is particularly interesting and a very stimulating group of speakers will address the subject.

The conference comprises six sessions:

- Opening session. The keynote speakers will be Professor Angus Martin, from the University of Melbourne, on "The view from the far side: attitudes to animals" and Dr Vaughan Monamy, from the University of New South Wales, on "The ethics of zoology: wildlife research as a case study".

- The second session examines wildlife research in the field, in the laboratory and the role of the public in caring for injured or orphaned native animals. It includes reference to the section on wildlife in the 1997 edition of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.*

- Session three goes to the zoo, with Dr Ian Denney, General Manager of the Western Plains Zoo, discussing zoo-based research in Australia. This is followed by a paper on zoos as education resources and the session concludes with a tour of the Zoo, including demonstrations of current research activities.

- Four case studies are presented in session four. These examine problems with over-population of koalas, the conservation program for the kakapo, wildlife as reservoirs of human and animal disease and marine mammal disease.

- The ethical and scientific issues associated with vertebrate pest management in Australia and New Zealand are addressed by two speakers in session five, together with a paper from Animals Australia on the importance of reducing animal suffering in vertebrate pest control.

- The final session is in the style of point-counterpoint, with two philosophers, Professor Andrew Brennan (University of WA) and Dr Glen Albrecht (University of Newcastle) giving their views on the question 'Ethics versus science in experimentation on native animals'.

The proceedings of the conference will be published.

This promises to be one of ANZCCART's most interesting conferences and is set in a fascinating venue. Dubbo is one hour's flight from Sydney and is serviced frequently by Qantas and Ansett.

A registration form is inserted in this issue.

Animal Health Monitoring in the Research Environment

Held in Melbourne on 21 September 1998, this was the third ANZCCART workshop this year and was jointly sponsored by ANZSLAS and AVERT (Australian Veterinarians in Ethics, Research and Teaching – a Special Interest Group of the AVA). Forty-six people attended and participated in a very interesting and worthwhile program.

There were four keynote speakers:

* Dr Bill White (Charles River Laboratories, Maine, USA)
* Dr Earl Steffen (University of Missouri, USA)
* Dr Patrick Hardy (Charles River Pharmservices, Lyons, France)
* Dr Kevin Doyle (AVA, Canberra).

The overseas speakers also made major contributions to the ANZSLAS meeting, held over the following three days. The speakers were experts in their fields and drew on their experience in presenting interesting and informative papers.

The workshop was in four sessions, each featuring one of these keynote speakers, followed by a discussion.

In Session One, Dr Bill White discussed "Animal health monitoring that is cost-effective and produces meaningful results – the balance between too much and too little". His very practical paper was enlivened with delightful slides and frequent references to "White's rules of life". He emphasised that all risks are relative (a metaphor for life) as well as the need to be realistic and objective when undertaking risk assessment, which must be cost-effective.

He addressed the importance of the questions asked by a health monitoring program and the precision required by the answers. What are the constraints? The need for results to be relevant for researchers was emphasised (false positive results are not helpful). When using sentinel animals, their health status must be known before their use. He ended with an excellent coverage of the use of sampling statistics.

In Session Two, Dr Patrick Hardy discussed "Large scale containment, health management and monitoring of multiple transgenic rodent colonies". He reviewed the history of transgenic animals from the first publication in 1982 to the present, where there are over 5000 publications per year.

He discussed the Charles River facility in Lyons and how it is managed, particularly with regard to biosecurity, risk assessment and risk management. The health monitoring program was discussed (it is very similar to that recommended by FELASA), as was the concept of SOPF animals (specific and opportunistic pathogen-free). A filter-top cage system was described, in which all conditions are as for a barrier unit, except that the staff are not required to shower. Rederivation was described, using either embryo transfer or caesarean section.

The concept of risk analysis was further developed in the third session by Dr Kevin Doyle, formerly of the Australian Quarantine and Inspection Service (AQIS) and now with the AVA. In his paper, "Solving the complexity of importation, quarantine and monitoring of laboratory animals, particularly rodents for issue in Australia", he defined quarantine in Australia, from the first Quarantine Act in 1908 through to the present. He defined risk analysis in the context of quarantine decisions about importation of animals, as the problem of an event occurring versus the consequences if it occurs. Probability is important only with regard to the consequences. The objective of a proposed import (e.g., of diabetic dogs for research) must be weighed against the risk (e.g., of the dogs introducing rabies to Australia).

He emphasised the need for governments to receive expert advice (from organisations such as ANZSLAS), to ensure they made the best policy decisions. Professional bodies need to lobby government and to seek representation on government advisory panels (e.g., with AQIS).

In the last session, Dr Earl Steffen comprehensively covered the topic "New infectious agents of rodents". He stressed the need to know whether a new infectious agent – "the agent du jour" is truly new or a known organism which has only recently been recognised. What is the significance to researchers and animal breeders and what are its morbidity and mortality rates in various species?

The majority of this paper related to specific new agents, such as the discovery of *Helicobacter* species, rat respiratory virus and scaly-skin condition in nude mice (whose aetiology is uncertain but is probably viral).

He concluded by observing that new agents are often found simply by persistent good observation, combined with serology and histology.

Each of the four papers will be expanded and published as facts sheets in future issues of *ANZCCART News*.

Coming up

Australian Veterinary Association Conference
16-21 May 1999, Hobart

Contact: Ms Doreen Culliver
AVACOS, 7 Phipps Place
Deakin, ACT 2600
Tel: 02 6285 3600
Fax: 02 6285 3913
Email: avacos@ava.com.au

The Use of Wildlife for Research
26-27 May 1999
ANZCCART's 1999 Australian Conference
Western Plains Zoo, Dubbo, NSW
Contact: ANZCCART's Adelaide office for information.

A registration form is inserted in this issue

Third World Congress on Alternatives and Animal Use in the Life Sciences
29 August - 2 September 1999
Bologna,Italy
Contact: JRC Public Relations and Publications Unit
Tel: 39 33 278 9889
Fax: 39 33 278 2435
Email: prp@jrc.it
hhtp://www.ei.jrc.itecvam/bologna/testbol.html

Innovation, Ethics and Animal Welfare: Public Confidence in Science and Agriculture
18-19 November 1999
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Contact: Mrs Gill Sutherland,
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Wellington NZ
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Fax: 64 4 473 1841
Email: sutherland.g@rsnz.govt.nz

A registration form is inserted in this issue.

News

US conference on Evaluation of Animal Research

The Kennedy Institute of Ethics at Georgetown University and the College of Health Professions in cooperation with the College of Veterinary Medicine at the University of Florida will hold a conference entitled 'The Balanced Evaluation of Animal Research: Fulfilling the Obligations of Science and Society" at the University of Florida, Gainesville, Florida on 6 - 8 February, 1999.

The program will consider issues of decision-making in a variety of animal-use contexts with a particular focus on the Institutional Animal Care and Use Committee (IACUC). The conference is intended for biological, biomedical, behavioural, and social scientists, clinicians, students, scholars of the humanities and philosophy, and members of the concerned public. The seminar is particularly important to those individuals concerned with the education of researchers and those involved in making decisions that directly impact the welfare of animals (e.g., researchers, veterinarians, members of animal care committees).

For additional information, please see the conference website at www.hp.ufl.edu. If you do not have access to the web or have additional questions, contact:

Robbie Eller, Office of the Dean, College of Health Professions, PO Box 100185, University of Florida, Gainesville, Florida 32610-0185. Phone (352) 392 4215, fax: (352) 392 6529, email: reller@hp.ufl.edu.

Ethical dilemmas

The weekly magazine *New Scientist* publishes a series of inserts entitled 'Inside Science'. Issue number 114, which was included in the 17 October 1998 *New Scientist* addressed ethical dilemmas in the life sciences in a four page colour facts sheet. These included how to decide between animal experimentation and animal rights, cloning, genetic manipulation and assisted reproduction in humans. It included an explanation of ethics and the bases for ethical argument. ANZCCART has obtained reprints of the insert from *New Scientist*, which are available for $A3.00 from the Adelaide Office.

International course on Laboratory Animal Science

A two-week intensive course on Laboratory Animal Science will be held at the Department of Laboratory Animal Science,Utrecht, The Netherlands from 7 - 18 June, 1999. This course has been offered each year since 1993.

The objective is to present basic facts and principles that are essential for the humane use of animals and for quality research. The contents of the course are in line with recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) regarding the training of 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 in other countries. For this purpose, during the course the acquisition of teaching materials can be discussed with the course committee.

For information and application forms please contact: Prof. 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@las.vet.uu.nl.

European College of Laboratory Animal Medicine

Veterinary specialisation in Europe is conducted by a range of European Veterinary Speciality Colleges, who are overseen by a European Board of Veterinary Specialisation (EBVS). An informal meeting was held between EBVS and Laboratory Animal Medicine Specialists in October 1998 which concluded that the time is right for the formation of a European Speciality College of Laboratory Animal Medicine (ECLAM).

Excellent liaison between the existing laboratory animal medicine general organisations such as the European Society of Laboratory Animal Veterinarians (ESLAV), and laboratory animal science organisations, such as the Federation of European Laboratory Animal Science Associations (FELASA) is essential.

Formation of ECLAM will be proposed at the ESLAV meeting to be held as a part of the 1999 FELASA meeting in Mallorca. The role of ECLAM should focus on training and certification in Laboratory Animal Medicine.

A website, established with funding from Laboratory Animals Ltd, has been established and contains more information: -
www.eclam.org
Email: eclam@ltk.unih.ch

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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