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ANZCCART Conference 2011 Report

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From June 26 to June 28 I attended my first ANZCCART conference. It was held in Rotorua (also a first for me) with the theme "Science with feeling – animals and people". I would like to say at the outset that I found the conference stimulating, interesting and enjoyable. I met many people involved in animal research: researchers; members of animal ethics committees; and government officers. Everyone was welcoming and I enjoyed many discussions on a range of animal-related topics (and other topics as well of course).

The New Zealand organising committee outdid themselves with an excellent venue and conference. Unfortunately, due to recalcitrant volcanoes and the resultant ash cloud a number of Australian delegates could not make it, or arrived late. Not being aware my flight had been cancelled I rocked up at Brisbane airport with my car safely parked a distance away and my animals organised with a friend. When I walked in to the terminal I was met with the departure board covered with 'cancelled' announcements. Not to be so easily defeated I went to the Qantas information desk and was told that if I was willing to accept the risk they could get me on an Emirates flight which was still flying. Since hundreds of people were already accepting this risk, I assumed, I agreed. The flight was uneventful and I arrived safely at Rotorua.

The opening address was via a video link with David Bayvel and Judy MacArthur Clark in London. This was a great start to the conference with their identification of two challenges to animal science: complacency and communication. They also pointed to several trends in today's world: changes in disease demographics (for example, an increase in chronic diseases and diseases associated with aging) and that animal models are less relevant to these types of disease; a general lowering of the risk threshold leading to increased safety testing; and increases in genetic modifications.

One of the themes of the conference, pain and pain control, was picked up in the first session. We heard that any definition of pain must include both sensory and emotional aspects. The session continued with a look at pain in specific groups: fish and invertebrates; birds; ruminants and foetuses. Assessing pain in certain species can be difficult as hiding pain can be beneficial to survival. For example, wild birds and ruminants may suppress normal pain responses. Assessing pain in fish, invertebrates and foetuses is difficult for other reasons. We also heard that species' responses to analgesics and non-steroidal anti-inflammatories differ and specialist knowledge is required to ensure pain control in animals used for research purposes.

Session 2 looked at the dilemmas inherent in serving on an animal ethics committee. These include the difficulty a committee faces when assessing research proposals: Does the proposal meet all the required ethical guidelines? Does the committee have the necessary expertise and knowledge to make this assessment? Have sufficient efforts been made to address the 3Rs? And so on. These sessions led to many useful discussions during lunch.

The other theme of the conference, people, was picked up in Session 3. The training and support of staff with respect to euthanasia was examined. This must be recognised as an important and on-going issue while animal research continues. The people who are the best carers of animals are the most likely to suffer trauma from being involved in the euthanasia of those same animals. The attitude of young people towards animal research was also addressed and how a student animal welfare group can be formed to help raise the awareness in young people about a range of animal welfare issues.

The final session aimed to bring the two themes together. Animal welfare is often measured by looking for the absence of negative states. It is difficult to measure positive emotional welfare particularly due to its subjective nature. One issue raised was that of anticipation: Do experimental animals anticipate pain or discomfort? Can they possibly be aware of the intention of the researcher? On occasion what we see is arousal in the animals and it may not mean distress. For example, one can see arousal before a positive experience such as the arrival of food. All these issues require further thought, research and consideration.

I came home after the conference with many things to think about and the knowledge that I had met many like-minded people. I am certainly planning to attend next year's conference in Perth, Australia.

Developing Alternatives

Geoff Dandie, CEO, ANZCCART

It has been a goal of a number of researchers for many years now, to develop viable alternatives to the use of animals in research. This has been a particular goal for those working in areas like toxicity testing and the pharmaceutical industry who have traditionally been among the biggest users of laboratory animals for the various screening assays that are legally required as part of the certification process or for the determination of potentially bioactive products. Follow-up work during the drug development process also includes completing the safety testing studies required to demonstrate both efficacy and safety prior to trialling new drug in patients. As all of these different processes and stages have traditionally required the use of large numbers of animals, they are very time-consuming and incredibly expensive so the incentives to develop and validate alternatives to the use of animals are genuine.

The concept of being able to undertake both screening assays and preliminary safety testing *in vitro* holds enormous appeal as it would not only streamline the process of drug development but also potentially save many millions of dollars in research and development costs. So the obvious question arises, why isn't there more work being done to develop viable alternatives to the use of animals in these areas? The short answer is the paucity of funds available to support this kind of work. However, the news is not all bad. In spite of the barriers that

exist, work is being done to develop alternatives to the use of animals and the really good news is that some of this effort is now coming to fruition. Interestingly, some of the most interesting developments have come out of the biotechnology and pharmaceutical industries, which might indicate an increasing awareness of the value of developing alternatives.

While progress in this area has been much slower than many would like, it is encouraging to see that it continues and seems to also be picking up pace in some areas. Again, one such area is the Biotech industry where, ethical concerns aside, the use of animals is seen as both expensive and slow, so in recent years we have seen the development of high throughput *in vitro* screening assays capable of detecting bioactivity with high sensitivity from within hundreds to literally millions of fractions a week (Young, Curry et al. 2004; Crouch and Osmond 2008; Zhu, Zhang et al. 2010). The ability to screen for bioactivity in this way allows researchers to identify compounds or fractions that have potential and in some cases, even rank their potential value on the basis of the level of activity detected during the screening assays. The fact that human cell lines are commonly used in such assays also brings potential benefits as it reduces any risks that may be associated with interspecies variation. While the potential and value of screening assay systems of this kind are fairly clear, it has to be acknowledged that these assays are generally still only indicative and the need to conduct further tests on positive samples will frequently involve at least some use of animal models. Of course, the process of identifying samples with the desired activity and being able to separate these from the potentially large number of samples that do not warrant further testing, will mean that there is a potential here to reduce the number of animals used enormously. Realistically, the use of this kind of technology could mean up to a 99% reduction in the number of animals used for screening and testing of potentially bioactive fractions when compared with more traditional screening techniques.

When it comes to the development of alternatives to the use of animals, a lot of work has also gone into developing *in vitro* models of human skin that can be used for both efficacy and toxicity testing among other things. Because the skin is so commonly exposed to a vast array of chemicals, cosmetics, creams, etc., it is clearly an important organ to focus on for this kind of work. The development of a number of *in vitro* skin models – generically referred to as 3D human reconstructed skin models, has seen the increasing acceptance of these systems and we are now seeing them being promoted internationally as viable alternatives to the use of animals (Cotovio, Grandidier et al. 2005) by organizations such as the European Centre for the Validation of Alternative Methods (ECVAM). When these *in vitro* models of skin started to seriously emerge in the late 1990s there were some issues with both false negative and false positive test results limiting confidence in these systems even through the reproducibility of data between laboratories was generally good (Roguet, Cohen et al. 1998). Similarly, the inter-batch variability was also found to be good (Roguet 1999) which was obviously seen as a very important consideration. From the researcher's perspective, the reconstructed skin cultures were histologically very similar to skin and quickly developed to the stage where the 'epidermal layers' were exposed to air (rather than being submerged in cell culture medium) which allowed a vast array of substances to be tested by painting them onto the external surface in a way that more closely resembles natural exposure. Continual improvement in the culture and assay systems has seen their endorsement by ECVAM for a number of toxicological studies in recent years and increasing acceptance (Tornier, Roquet et al. 2010). There are of course still some limitations to these systems as the reconstructed skin cultures still lack functional innervations or a viable circulation and these factors limit their applicability in some situations.

Importantly, the development of *in vitro* models for different organ systems has also continued to progress. A recent article in the journal *Nature* has highlighted an example of the progress being made in developing alternatives to the use of animals in research models. The Technology Feature article "A Living System on a Chip" (Baker 2011) describes a system that has been developed by researchers at Harvard University in the US that allows miniature versions of organs like lungs or the spleen to be used for therapeutic drug testing and toxicity testing studies among other things. The principle aim of these studies has been to develop a miniaturized system that is part *in vitro*, part *in silico* testing for potential toxicity of candidate drugs.

To describe the system as a refinement of the tried and generally failed model of scratching channels into a silicon chip and then growing cells in those channels would be an understatement. The chips used in this study contained a pair of microchannels that were separated by a flexible, porous 10 micrometer thick membrane. One set of microchannels was set up to mimic the lung (i.e. air-filled with a layer of epithelial cells), while the other set of microchannels was designed to mimic the blood vessels that are integral to lung function. These microchannels were filled with the same kind of cells that line blood vessels and a liquid that simulates blood, flowed through these 'vessels'. The air-filled microchannels could even be connected up to vacuum chambers to simulate the mechanical forces / effects of breathing. The chip itself could then be used to monitor changes in the behaviour of cells as they were stretched in a way that mimics breathing. The system was then used to monitor the effects of volatile substances, nanoparticles and potential pollutants or inhaled agents on the most delicate cells in the lungs. By adding white blood cells to the blood-like solution, it has also proven possible to simulate the immune effects of inhaling agents likely to cause inflammation of the lungs.

In spite of these inspiring developments, when it comes to the idea of organs on chips, it is still a case of 'watch this space' as there is still a very long way to go before we have a full repertoire of such systems available. It will still require a lot of additional work to develop these systems to the point where we can effectively replace the use of animals altogether.

While these and other similar model systems may still not be ready to completely replace the use of animals for work of this kind, they are clearly making in-roads that mean the number of animals required to complete the mandatory efficacy and toxicity testing studies that are part of drug development, can already be greatly reduced. Of course, the hope is that with continued effort, technological advances like these may, one day, replace animal use altogether. So it is most encouraging to not only see the continued progress in the development of genuine alternatives that will allow a lot of the routine testing of compounds to be undertaken without using animals, but also to see real progress being made in terms of the acceptance of these alternatives at the regulatory level.

Another area where significant progress is being made in the development of alternatives is the broader discipline of toxicology. This is a discipline where, like drug development, there are strict regulatory requirements that currently involve the mandated use of animals for safety testing. This is also an area where substantial efforts are being made to develop alternatives that will not only reduce the number of animals that must be used, but also satisfy the strict regulatory requirements associated with safety testing. One institution that is playing a leading role in the development and ratification of alternative testing methods is The Johns Hopkins Center for Alternatives to Animal Testing (CAAT). This month, CAAT announced that it has received a $6 million grant from the National Institutes of Health (NIH) to pioneer potentially revolutionary new methods for toxicological testing to improve human health and reduce animal testing.

CAAT Director Thomas Hartung, MD, PhD, and his team at the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, along with partner Agilent Technologies and noted scientists from government and industry, received the funding for a consortium to develop a new technological methodology for mapping the molecular pathways of toxicity within cells. Funding for the project comes from the Common Fund's NIH Director's Transformative Research Projects Program (R01), which is designed to support exceptionally innovative, high-risk, original and/or unconventional research that has the potential to create or overturn fundamental scientific paradigms.

Current toxicological testing relies on a patchwork of 40+-year-old animal tests that are expensive (more than $3 billion per year), time-consuming, and often provide results of limited predictive value for human health. The low-throughput of current toxicity testing approaches (which are largely the same for industrial chemicals, pesticides, and drugs) has led to a backlog of more than 80,000 chemicals for which potential toxicity remains largely unknown.

Scientific understanding of how genes, proteins, and small molecules interact to form molecular pathways that maintain cell function has evolved rapidly, thanks to advances in molecular and computational tools.

Pathways that lead to adverse health effects when perturbed are referred to as pathways of toxicity (PoT). Thomas Hartung explained one of the major areas of research by saying “Mapping the entirety of these pathways – which I’ve termed the ‘Human Toxome’– will be a large-scale effort, perhaps on the order of the Human Genome Project.”

As a first step to mapping the Human Toxome, Hartung and his collaborators have proposed comprehensively mapping the pathways of endocrine disruption, a perturbation of the hormonal system that can cause tumors, birth defects, and developmental disorders. The physiological pathways of the endocrine system are relatively well understood, making PoT identification simpler than for other potential targets. The team will develop a common, community-accessible framework that will enable the toxicology community at large to comprehensively and cooperatively map the human toxome using integrated testing strategies that combine “omics” (transcriptomics and metabolomics) data with computational models. The consortium will also create a public database of PoT, enabling full access to researchers around the world. While this work is still very much in its infancy, the prospect of such a significant project receiving funding at this level is certainly encouraging and bodes well for future developments.

When we stop and objectively look at the current status of research using animals and the development of viable alternatives, it is clear that we are nowhere near a position where we can become complacent, but nor are we still solely reliant on the use of animals for biomedical research – including safety and toxicological testing. It is more than clear to those who want to work in this area that we are still severely hampered by a lack of funding for research into alternatives in Australia and yet some Australian researchers are still making valuable contributions to the international effort. So perhaps the best way to objectively assess the progress made in this area is that we have come a long way since Russell and Birch published their treatise *“The Principles of Humane Experimental Techniques”* in 1959 and the first version of *the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* was published in 1969, but we still have a long way to go yet.

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Reduction of Animal Use in Teaching Despite Increasing Class Size

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As a Department, Physiology at the University of Otago recognises the need to use animals for research and teaching, and strongly supports the legislative framework that permits the use of animals for these purposes. The Department runs a suite of second-year B.Sc. Physiology projects that include a small number of laboratory classes that use animal tissue. We want our students to appreciate not just the methodology used to generate new information in health sciences, but also the ethical issues surrounding the use of animals for this purpose. Integral to our teaching, we expect students to actively engage in laboratory work and we make every effort to ensure that the best learning outcome is achieved for each student through their participation in laboratory classes.

As class sizes have tracked upward in recent years, staff have had reduced opportunity to engage deeply with students on a one-to-one basis. Recognising this, in 2009, we produced a series of short videos to improve student understanding of the use of animals in their laboratory classes. Broadly, these videos fall into three classes: an introduction to the ethics of animal use in research and teaching; demonstration of the dissection of the animal tissue used by the students in the laboratory; and instructions on how to properly set up the tissue that the students use in the laboratory.

The introduction to animal ethics is designed to encourage students to think about ethical issues associated with the use of animals in research and teaching, and to gain some knowledge of the legal processes required to support such work. The dissection videos are designed to illustrate where the tissue comes from in the experimental animal, what has to be done to extract that tissue in good condition, and to show that the tissue came from a real animal that was killed to afford them their valuable practical learning opportunity. This reinforces the message that each student has an ethical and legal obligation to make the best possible use of their tissue. The set-up videos are intended to minimise the incidence of rough handling of tissue by students, and so reduce the need for extra animals to be killed to prepare spare tissue.

Our experience is that the use of these short videos has increased student awareness of the ethics of animal use for research and teaching and has reduced the failure rate when setting up tissue. In the feedback for our first semester course in 2010, only 6% of a class of over 300 students indicated that the laboratory introductions had not prepared them for the ethical issues of work on animals and only 1% indicated that they believed the use of animal tissues in the course was unethical. We have calculated that refining our laboratory classes in this way has reduced our animal usage for each of these laboratory classes in 2010 by 15 – 20% on the numbers that were used in 2009.

Public consultation on proposed revisions to the

"Australian code of practice for the care and use of animals for scientific purposes"

The proposed revision of the Australian Code of Practice for the Care and Use of Animals Used for Scientific Purposes has now been released in draft form for comment. The process of public consultation will close on 2nd December, 2011 and ANZCCART strongly encourages everyone who is interested in The Code to participate.

All the relevant information about the public consultation process, including a draft version of the revised Code can be downloaded from the NHMRC website at the following address:

http://consultations.nhmrc.gov.au/open_public_consultations/australian-code-of-practice/

ANZCCART members will also be given the option of submitting comments via ANZCCART. Details will be circulated shortly.

Recent Articles of Interest

Regulate research at the animal-human interface
Transgenic rodents, inter-species cell lines and animals grafted with human tissue have been created worldwide helping with the investigation of health and disease and the development and testing of therapies. The following article discusses how the governance of this research can be improved.
http://www.nature.com/nature/journal/v475/n7357/full/475448a.html?WT.ec_id=NATURE-20110728/

Editorial: Animals in Research: "Can they suffer?"
This article appeared as a one page editorial in the July edition of The Lancet and refers to a report published in the UK that month. The report which looked into the welfare of animals containing human material was produced by the UK Academy of Medical Sciences and (along with a synopsis report) can be accessed via the Academy's web site at:
http://www.acmedsci.ac.uk/index.php?pid=99&puid=222/
Both the article and the report raise some interesting questions about the potential welfare implications and risks that may arise from genetic modifications in animals.
http://www.sciencedirect.com/science/article/pii/S0140673611611495/

http://www.thelancet.com/ Vol 378 July 23, 2011

Why animal research needs to improve
Many medical discoveries have made real differences to the lives of a great number of people, but could the research be done better? Questions can be asked about the design of many experiments, including the number of animals used and the way in which they are assigned to experimental groups. One of the changes suggested is for a more flexible system whereby all experiments can be registered, so that investigators can receive credit for work done and those seeking to summarize what is known, have access to all relevant data.
http://www.nature.com/news/2011/110928/full/477511a.html?WT.ec_id=NATURE-20110929/

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