

Media Briefing

April 2004

Replacing and reducing animals used in research

An introduction to BBSRC-funded research that offers potential alternatives to the use of animals in bio-medical science.

By law in the UK, animals can be used in research **only** when there is no alternative, and then the use must involve the minimum number of animals possible, consistent with valid experimentation. It is not surprising therefore that there is no simple single approach for attempting to reduce the numbers of animals used in experimental research.

Many promising avenues envisage a reduction through partial replacement. For example, replacing animals with alternatives, such as isolated cells, in the early stages of investigations into the behaviour of a potential new drug, could mean that animals are used only later after successful completion of this stage and when full "testing" of the putative drug is required.

In other situations, where genetic influences on specific molecular or cellular responses are being studied, it might be possible to replace traditional laboratory species with the simpler "model" species that have been the focus of modern molecular genetics. Many genes and their products are extremely similar in different species, so it might be possible to use a comparative genomics approach in which some studies on rodents might be replaced by similar work in simpler organisms such as chicken embryos (fertilised eggs) or fruit flies.

Nationally, deploying virtual centres of research that can network and share materials and data, for example, microarrays, comparative maps, bioinformatics, models for laboratory species such as



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mice, helps to reduce the overall number of animals needed to generate experimental material, makes better use of information and resources and avoids duplication.

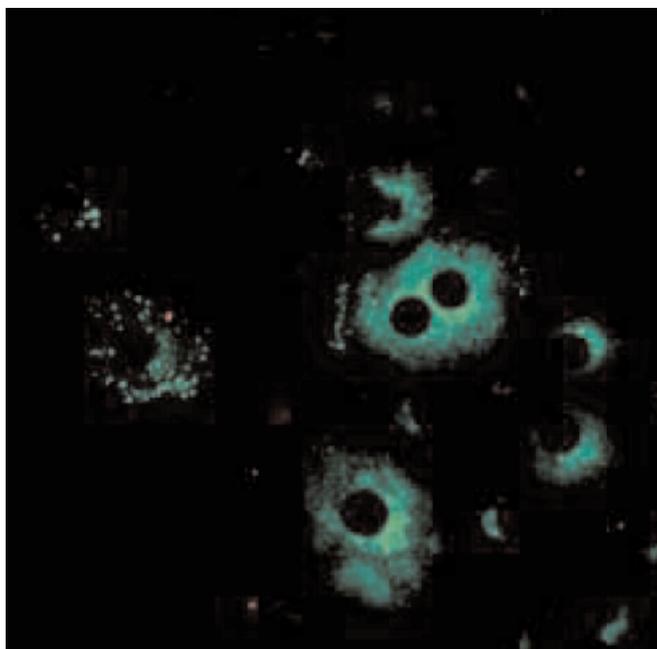
BBSRC has recently invested over £1m in a new priority area on developing alternative methods to reduce and replace animal experiments. This includes: in vitro methods, computational methods and non-animal methods. In addition, several areas of BBSRC research also have potential to generate alternatives to some animal experimentation, although this is not the primary goal of the research.

RESEARCH FROM THE NEW PRIORITY AREA

Testing the value of novel liver cells

The liver has a multitude of functions including the regulation of carbohydrate and fat metabolism, the production of bile and the detoxification of potentially harmful substances. In theory, some studies of liver functions could be carried out on isolated liver cells (hepatocytes) rather than on live animals. Past attempts to establish long-term cultures of liver cells have not been very successful, mainly because the cells rapidly lose their distinctive liver-like properties. Alternative approaches include the use of permanent cell lines but these are not always useful since they do not possess the full panoply of liver functions.

Now, however, scientists at the University of Bath have come up with a potential solution. They are exploring the developmental switches that drive embryonic endoderm tissue to specialise into different cell types including liver and pancreas. They have found a way of converting, or "transdifferentiating", existing cultures of rat pancreatic cells, into liver cells. The process is very simple and involves the addition of a synthetic hormone called dexamethasone. After addition of dexamethasone, the pancreatic cells change shape (they become larger and flatter) and lose their ability to synthesise and secrete the digestive hormones and then begin to express functions characteristic of normal liver including the synthesis of transferrin and phenol sulphotransferase. Intriguingly, the newly-formed liver cells appear to express many functions of cells in a whole liver and are stable long-term. Significantly, they can be manipulated in the laboratory to enhance particular liver functions so that these might be studied singly.



Transdifferentiated pancreatic cells synthesising transferrin (green)

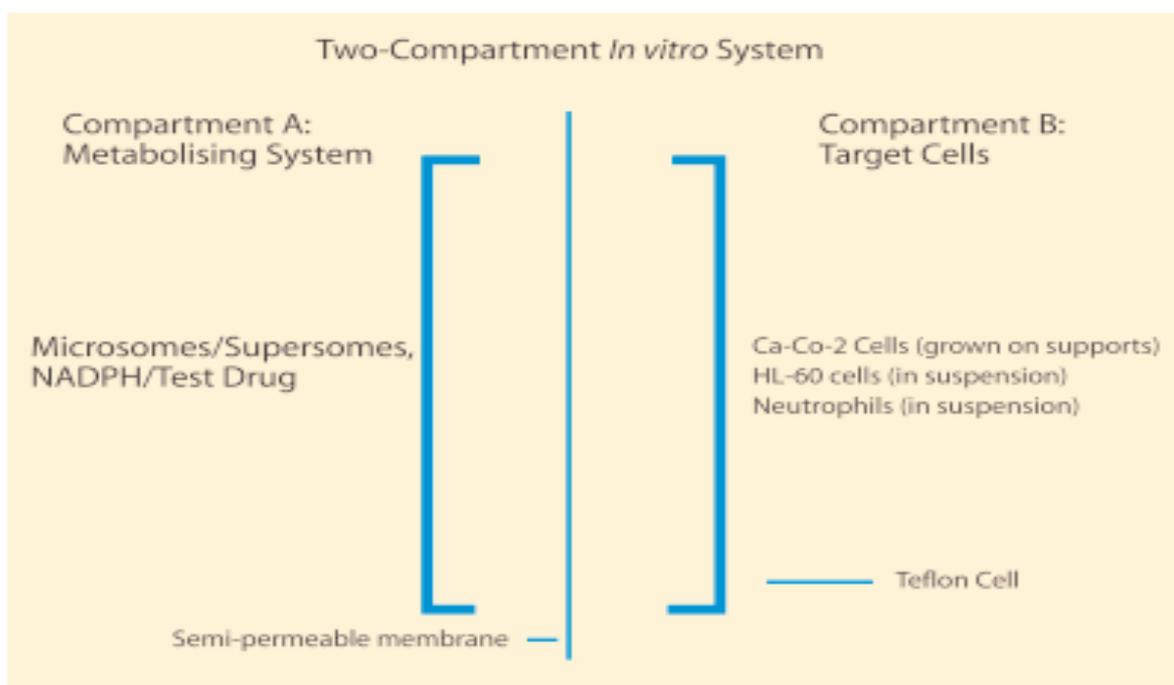
The researchers are now ascertaining just how reliable transdifferentiated cells are, as models of liver function. Their ultimate goal is to develop "pure" lines of highly functional cells in which metabolic regulation and function of key liver cell proteins can be analysed.

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Human cells for toxicity tests

Pharmaceutical companies need to be able to test whether potential new drugs will be safe, or whether they will be broken down into toxic products that could damage patients. Researchers at the University of Aston are identifying and measuring the metabolic



***In vitro* model aimed at mimicking liver metabolism of drugs/toxins**

changes that occur in cultures of immortalised human cells that are exposed to toxic breakdown products. Their aim is to see whether these metabolic changes might act as accurate indicators of toxicity levels. If so, the cells could potentially replace some animal testing, particularly in the early stages of screening to weed out unsuitable candidate drugs.

The researchers take suspensions of different types of human cells, including white blood cells, and expose them to toxic metabolites that have been produced either by enzymes isolated from human liver cells, or from the activity of isolated human liver cells that are attached onto a bead matrix.

The study is looking, not for "all or nothing" indicators, i.e. whether or not the cells survive, but for sophisticated changes in cell behaviour that reveal how the metabolites are affecting the cells' ability to function normally. These include an impaired ability to transport the amino acid proline – an indicator of changes in the cells infrastructure- and lower than normal levels of glutathione – a compound that cells use to protect against stressful "wear and tear". A key aim is to identify reliable and reproducible indicators that would help to "standardise" such in vitro testing across laboratories. An added benefit in this approach would be that the human cells could provide early warning of rare human-specific effects that might not otherwise arise until very late on in the testing programme when the putative drug is tested in human volunteers.

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In-vitromodels of infection

When researchers want to develop ways to prevent or treat serious bacterial and viral diseases in livestock, the traditional approach is to study infected animals and/or to characterise the diseasecausing organisms. However, while research in the target species remains clinically important, some basic and underpinning studies might be conducted in model species or alternatives thus reducing the numbers of experimental animals required in later studies.

Researchers at Cambridge Veterinary School have pioneered the use of an in-vitromodel of infection. This is an air interface organ culture that keeps respiratory tissues functioning under nearly normal body conditions for several days. The model allows researchers to compare the infectivity of different genetic variants of bacteria and viruses, and so identify genes that influence the establishment of infection, and which, therefore, might be targeted by novel vaccines.

One application has been in the study of

Streptococcus suis infection of the epithelial lining of the respiratory tract of pigs. This organism causes sepsis, meningitis, and other serious infections in piglets, and can cause meningitis in humans.

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Latex beads (mimicking a bacterial inoculum) on an organ culture. Clearance of the beads over a number of days is used as a measurement of tissue viability.

Delivery of artificial chromosome in virus vectors as alternative to generation of transgenic lines for analysis of gene function.

Transgenic laboratory animals are important models of some human genetic disorders. They are also used extensively in basic research into how genes influence cell and tissue function, and to study the impact of genetic variation (mutation and polymorphism). The number of transgenic animals is increasing as scientists develop and maintain different lines of animals with different genetic modifications, and different lines expressing the same modification in different tissues.

A research group at the University of Liverpool is exploring the potential for using herpes simplex viruses, from which virtually all of their own DNA has been replaced with an artificial chromosome fragment containing not only the gene of interest but (as it is large enough) also all the regulatory DNA sequences to drive correct expression of the gene, once delivered into laboratory animals. Because the virus genome they use is relatively large, this should be equivalent to adding extra alleles to the animal or complementation for a specific loss of function. The idea is that once the virus has entered the animal, the gene is expressed with all the characteristics of the normal cellular gene at the site of infection. This approach allows one to address protein function in vivo whilst dramatically reducing the number of animals used, as no special strains have to be generated or maintained prior to the experiment. There is also avoidance of generating strains with no

phenotype that can occur for a variety of reasons. The model is potentially more relevant to models of human disease as therapeutic targeting of the human protein and its relevant regulatory sequences (including polymorphisms correlated with disease) can be addressed.

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OTHER RELEVANT BBSRC-FUNDED RESEARCH

Genomics

One of the amazing findings of modern biology is the similarity of corresponding genes in different species, and the fact that the organisation of genes on the chromosomes is also remarkably similar in very different species. As a result, DNA sequences (genome data) that provide the location and function of genes in relatively simple organisms, say a yeast cell or a fruit fly, can provide clues to the whereabouts and function of gene counterparts in humans and other animals.



Technician visually checks the health of a Sprague Dawley rat. © RDS/Wellcome Trust Photographic Library

BBSRC is funding research that could see the use of cultured chicken cells and fertilised eggs as alternatives to some experiments involving mice embryos. Scientists at the University of Nottingham, Dundee and UMIST and the Roslin Institute (Edinburgh) have compiled a catalogue of ~30000 chicken genes (www.chick.umist.ac.uk) that will allow researchers to study their function in cells and fertilised eggs. These new resources are being used by the Roslin Institute (www.ark-genomics.org) to create whole genome gene expression chips. In collaboration with colleagues in Germany and the USA, a set of 12000 full-length cDNA clones is being sequenced at the Sanger Centre (Hinxton). In

addition, the Roslin Institute, UMIST and EBI (Hinxton) are working together with colleagues in the USA to integrate this catalogue with the chicken genome sequence itself. These new tools will allow researchers to test the function of genes in chicken cells and fertilised eggs. For example, the DT40 cell line derived from lymphoblasts can be used to examine the role of genes in metabolism and cell division using an efficient gene knockout system. Recent progress in the use of RNAi for gene knockdowns in chicken embryos also holds much promise for alternatives in whole animal experiments.

Research at the University of Glasgow has revealed genes in the fruit fly (*Drosophila*) that govern functioning of its renal tubule, which have direct functional counterparts in rats and humans. Indeed, a gene from rat kidney can work in place of its corresponding gene in the fly's tubule. The implication is that some genomics work could be performed on simpler organisms that hitherto imagined, thus reducing the number of higher animals used in experiments.

Researchers at the University of Manchester believe that for some research, yeast can be used as the model species for human and animal disorders. They point out that many of the 6000 genes of yeast have corresponding human versions, and an estimated 40% of genes implicated in heritable diseases in humans have counterparts in yeast cells. For instance, the protein specified by the gene that can mutate to produce the neurodegenerative disease Friedreich's Ataxia can substitute for a yeast protein that moves ferrous ions from one cellular compartment to another. It is a failure to transport iron properly that causes paralysis in Friedreich's patients.

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Stem Cells

It is possible that in the future many different cell types might be derived in the laboratory from cultures of stem cells. These cells may be isolated from embryonic or adult tissues and have different abilities for differentiation. ReInnervate is a spin-out company from the University of Durham where the BBSRC funds neural stem cell research. One of the company's goals is to develop novel technologies to produce human neural tissues from cultures of stem cells that could be used as an alternative to animal testing for drug screening purposes and toxicological tests. Recent work by the company includes the development of methods to increase the efficiency of producing human neurons in culture and the validation of such cells to ensure that they possess the molecular and physiological characteristics typical of functional neurons.

Moreover, ReInnervate's scientists are employing such cells to produce defined neural networks that can be readily used for more informative assessment of drug action on neural function.

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Improving the usefulness of cell cultures

In theory, cells maintained in sterile culture conditions for extended periods of time, could offer an attractive alternative to cells isolated from human or animals sources. There are, indeed, many examples where cell cultures offer an excellent test-bed for research, for example into drug effects on cell behaviour. In practice, cell cultures can be problematic.

Chemical engineers at the University of Birmingham are working with bioscientists to understand how to improve the physical and chemical control of animal cell cultures so that they last longer and are more consistent. The Birmingham group, for example, has previously identified a protein that can help cells to overcome some of the "stresses" that they are subjected to under conditions of large-scale culture. This helps to keep the cells in a stable state and increases their longevity.

Neural networks and other monitoring systems are being used to elucidate how factors such as pH, temperatures and hydrodynamic forces influence the metabolism of cells in culture and how this in turn affects the usefulness and lifespan of the culture.

Similarly, Oxford scientists have developed ways to direct growth of cultured cells according to pre-defined patterns. This allows cells to develop more natural shapes and internal structures, and aids their interaction with neighbouring ones – a crucial facet of cell activity in the body.

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Computer modeling

Analytical computer models of individual heart cells are being developed by a research group at the University of Oxford to provide simple, reproducible and representative models of cardiac tissue.

These models are used to conduct 'dry screening' of experimental interventions to select the most promising strategies and targets for 'wet' research. In this context, models benefit from their theoretically unlimited spatial and temporal resolution, which confers to them the ability to dissect individual mechanisms in complex responses, repeat interventions, stop chain reactions, etc. Models are therefore important at the outset of experimental research, and any scientist uses them – even if not in the shape of formal quantitative simulations.

The same models are used for experimental data integration and analysis, thereby aiding the interpretation of research. This is important, as all the various mechanisms that give rise to normal or disturbed cell function are interlinked, so that identification of causal chains is often very difficult with a less formal approach.

Modelling, therefore, forms an integral part of experimental research. It allows scientists to conduct fewer experiments of potentially higher quality – but it cannot fully replace experimental research in the life sciences, as all modeling predictions need to be evaluated in the real world.

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CPBAR

BBSRC is a co-funder of the Centre for Best Practice for Animals in Research (CPBAR) at the Medical Research Council, which is dedicated to ensuring high standards in all aspects of laboratory animal use.

The formal terms of reference of CPBAR are: to provide independent advice and guidance on laboratory animal use and welfare, including issues related to reduction, refinement and replacement, to: the scientific community, research funding organisations, policy makers; and the public to be a focus for coordination and collaboration between organisations engaged in research and training related to the use of animals in research.

More information is available at:

www.mrc.ac.uk/prn/index/public-interest/publicethics_and_best_practice/public-cbpar.htm

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