Of mice and Mendel

The predicted rise in the use of knock-out and transgenic mice should cause us to reflect on our justification for the use of animals in research

It is no accident of linguistics that the phrase 'to be a guinea pig' is used to mean 'to subject oneself to an untested product or procedure'. Indeed, guinea pigs were among the first animals to be used in medical research. They produce particularly strong immune responses to many antigenic agents; they are a favourite choice for hearing experiments because the outer ear, as in humans, is very accessible; and they are still used in the critical diagnosis of mycobacterial infections in humans—a procedure that leads to their death. But guinea pigs are just one of many vertebrates that are being used in increasing numbers, at least in some areas of biomedical research.

Over the last two decades, resistance to the use of animals in research and testing has been growing in the USA and Europe. One of the major animal protection groups, People for the Ethical Treatment of Animals, PETA, is dedicated to establishing and protecting the rights of animals, claiming that animals are not to be eaten, worn, experimented on, or used for entertainment. Other, more fundamentalist groups in the USA and the UK, such as the Animal Liberation Front, pursue this goal by rescuing animals and causing financial loss to animal exploiters, usually through the damage and destruction of property, as can be read on their web sites. In 1990, a researcher in Bristol, UK, barely escaped death when a bomb, planted by antivivisectionists, exploded under his car. More recently, animal rights activists have posted threatening letters filled with razor blades to university researchers across the USA, calling for an end to their experiments on animals. In April this year, a 26-year-old activist from Cheshire, UK, faced 15 charges of sending explosive devices to homes and businesses targeted by animal rights campaigners.

Activist groups routinely publish names and addresses of researchers who perform experiments with animals on their websites, and picket their laboratories, and criminal vandalism has occasionally resulted in the destruction of these laboratories. Relations between activists and

to exterminate them. However, when kept as laboratory animals in what would be considered 5-star hotels with room service and jacuzzis, we accord them rights. What rights does a cow have not to be killed for human purposes? Is killing a cow to make a steak better than killing a

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researchers took an interesting turn earlier this year with the publication by the UK Bioindustry Association of a manifesto calling for restrictions on the right of activists to protest, and legislation that would make it an offence to 'organise a campaign purely to attempt to cause the demise of a legitimate business.'

Essentially for the foreseeable future we must resign ourselves to a quirk of human value judgement, and try to improve the

mouse for diabetes research? Groups such as PETA claim that many of the experiments are not necessary since alternative non-animal research methods exist. Many researchers, however, maintain that animal experiments are a vital part of medical research. There seems to be no compromise in sight.

This should be bad news for Nadia Rosenthal, the recently appointed Coordinator of the EMBL Mouse Biology Programme at Monterotondo, Italy, who is committed to ensuring good practise and comfortable living conditions for her mice. If anyone knows what makes a mouse tick it is Rosenthal, who explained that mice 'are very picky when it comes to partners and living conditions.' Exercise and diversions are also important, because mice are very active and 'insatiably curious' as Rosenthal put it. She is already developing 'toys' for the Monterotondo mice, because a happier mouse is also a healthier, metabolically more normal mouse, and that is important for a scientific experiment. Rosenthal predicts that the use of mice in gene knock-out and transgenic experiments will 'sky-rocket' in years to come. Since the mouse is 98% genetically identical to a human, it proves an excellent model for many human

way we treat animals: when mice do little more than nibble our food, we justify using some of the most abominably painful mechanical and chemical means

physiological processes and diseases. With the help of more refined pre-screening techniques, clinical trials of human medications are jumping the gulf from mice to men without going through primates; good news for our close relatives.

This increase in the use of mice is a cause for concern for organisations like the RSPCA (Royal Society for the Prevention of Cruelty to Animals). Penny Hawkins, Senior Scientific Officer in the Research Animals Department thinks that some researchers 'may be regarding animals as expendable research tools', and has doubts that the large number of mutant mouse phenotypes are subjected to a proper review of their scientific usefulness. Furthermore, she noted that, whereas experimental dogs and cats routinely receive post-operative analgesics, founder mice operated on to remove pups, often do not. Mice should be treated as equally sentient animals according to Hawkins. But the RSPCA has certainly never considered terrorist activities to assert their views. Rather they take an ethical stance to encourage people to examine their own ethical boundaries and campaign to foster a greater understanding of animals and their needs.

While animal welfare groups are relatively new, research using animals dates

experiments on animals. The functioning of the cardiovascular and nervous systems were among the first discoveries. In the

briefing from the European Science Foundation—are used in research, including safety testing of biological and chemical products. Of these, around 81% are rodents—overwhelmingly mice—and rabbits, 13% fish and other cold-blooded vertebrates, 4% birds, and a mere 0.09% non-human primates. Though there has been a decrease of up to 50% over the last 20 years, mainly in chemical and pharmaceutical testing, the numbers seems likely to be on the rise again because of the proliferation of knock-out and transgenic mouse models of human diseases. Since their development in the late 1980s, approximately 1500 transgenic mice variants have been produced. How do we reconcile this with Replacement, Reduction and Refinement, the guiding principles behind reducing

animal use and suffering in research? The extent to which the three Rs are feasible depends on the type of experiment, and its relevance to human health.

As Andrew Blake, director of SIMR (Seriously Ill for Medical Research) remarked 'Almost every medicine we have has been developed through animal research', and he certainly doesn't take for granted the use of animals in medical research. Blake is a sufferer of Friedrichs Ataxia, a disease caused by an accumulation of iron in neurones. Though he may well not be saved by the research that in 1996, using transgenic mice, discovered the gene responsible for his condition, he is convinced that animal research will produce results that unequivocally justify this approach. 'We are now at a stage where medical benefits aren't yet coming through, but when they do, the whole public mood will change,' thinks Blake.

As for the usefulness of alternatives to animal experiments, Blake remains convinced: 'If there were a replacement, do you think we would still be doing

early 20th century animal experiments proved Koch's postulates of the infectivity of germs. From the early 1920s the chemical, and later the pharmaceutical industry employed rodents, pigs and dogs in toxicity testing, an application that would explode in subsequent years. Vaccines, and many of the treatments for serious ailments, which we take for granted today, owe their development to animal experiments. Much newer medical advances were also made feasible by the use of animals, for exam-

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back millennia. Aristotle (384–322 BC), may have been the first to scientifically dissect animals for anatomical study, and later his compatriot, Galen (129–199 AD), the first to perform experiments on living pigs. However, it was not until the mid-1800s that medical research started to make massive strides as a result of

ple organ transplantation, stroke rehabilitation and diabetes treatment.

In the USA the use of animals in biomedical research, testing of household products and cosmetics contributes to an annual total of between 17 and 22 million animals. In the EU 11.8 million vertebrates a year—according to a recent policy

animal research?' Blake, the owner of many domesticated animals, including a Vietnamese pot-bellied pig emphasises that he does not celebrate what he sees as the inevitable increase in the use of

animal experimentation'. There are, indeed, cases of animal models being of 'no assistance' to medical research: for example the testing of penicillin on ruminants, which produced severe, even

animals used. Cultured tissues can even be genetically engineered by mutating the p53 tumour suppressor gene to act as faster tests for carcinogens. Some European countries, notably Germany, the Netherlands and UK have national centres for the development and evaluation of alternatives, (see Table I). That said, in the majority of cases, 'to replace an animal by another system is not feasible; it's too complex,' noted van Zutphen.

Indeed there is certainly no replacement in sight for the unfortunate 'oncomouse' (a transgenic patented in 1988, which contains the human *c-myc* gene), and its genetically modified friends. As Wilkins noted, 'we have seen a dramatic increase in [experimental animal use] the last 2 years, especially in transgenic animals—10% of the animals used in research are transgenic mice. 'It certainly appears that there is a need to use animals to study human disease', Wilkins conceded. However, improvements will come with a greater awareness of the arguments on both sides of the debate. Most progress has been made via dialogue between organisations like the Eurogroup and research scientists in order to improve their knowledge of the alternatives, and refine their experimental design. In many cases the more careful planning and statistical analysis of an

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animals in research. However he is clearly pleased that this also marks a concerted effort to tackle the multitude of genetically determined disorders. 'About 6000 genes are involved in human disorders, and very few have been researched. With the help of genetically engineered mice, we can start to look at these,' Blake noted.

In a laboratory at the Institute for Molecular Pathology in Vienna, the Austrian researcher Erwin is studying the early gene products Fos and Jun using mutant mice. His work is making a significant contribution to our understanding of bone development, myeloid cell development and liver regeneration, which indicate their importance in human cancers, osteoporosis and normal bone development. In these respects, a mouse is almost as good as a human, but to step further down the ladder to a lower vertebrate? 'I don't think that's acceptable,' Wagner said. And to move higher up the ladder? Though he does not condone the use of whole primates in research, he 'would be in favour if there were more studies in primate cells.'

Clearly, the closer the animal is to humans, the better it models the human condition. And for modelling physiology, there appears to be no better substitute than animals. Though, statements such as '...animal models are useless for curing and preventing human disease...', as published on the website of Americans for Medical Advancement, do not seem to be in the repertoire of the more conservative European animal welfare organisations, the latter are without doubt campaigning for an animal-free future in biomedical research.

As David Wilkins, head of the Eurogroup for Animal Welfare, and a veterinarian himself remarked, '[the Eurogroup] is opposed to all experimentation and scientific procedures that cause pain or distress to animals... and was founded to push for the eventual halting of all

fatal side-effects that are not seen in humans. The carcinogenicity of cigarette smoke was also a fact which, according to Wilkins, was already entirely supported by epidemiological studies, without the need to involve animal testing. And in monoclonal antibody production the use of the 'ascites' method—in which essentially tumours are grown in the peritoneal cavity of a mouse—could be replaced by *in vitro* cell cultures. Some would go even further: 'If we had not used animals for scientific purposes from the start (but used other scientific methods/type of information instead) we would possibly have had an even better improvement of human health and treatment of diseases', asserted Roman Kolar, and ethicist at the deutscher Tierschutzbund Akademie für Tierschutz.

There are certainly examples of where we can do better, but is there genuinely

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hope for the development of alternatives to animals? 'Of course there are alternatives' noted a spokesman from the Institute for Health and Consumer Protection, which incorporates ECVAM, the European Centre for the Validation of Alternative Methods, 'but scientific validation needs to be done, and that can take years'. Bert van Zutphen, head of the Department of Laboratory Animal Science at Utrecht University, agrees; real replacement possibilities are not yet very numerous because it is a gradual process that involves pan-European harmonisation of legislation—something that the Eurogroup on Animal Welfare is pushing hard for—regulations and standardisation of procedures. Pre-screening of substances using cell cultures, tissues or organs, some of which can be derived from human operations, is a step towards reducing the number of

experiment to reduce the numbers of animals used also results in a scientifically superior experiment. In transgenics, scientific advances will also help. Wagner pointed out that it is now possible to produce 100% mutant mice by growing the ES cells with tetraploid cells instead of the usual diploid somatic cells. This avoids the intermediate stage of chimera production and selection, hence reducing the total number of mice involved in making a particular mutant.

In education and pharmacology, computer modelling can frequently substitute for animals. Furthermore regulations on animal use will become stricter with continually changing ethical stances. As to whether regulations hamper biomedical research, van Zutphen is ambivalent: 'yes, because one can't proceed as fast as one would like; and no, because the quality of research in

Table I. Alternatives to animal use in research and safety testing

Procedure/test	Alternatives/refinements under study	Comments
Monoclonal antibody production	<i>In vitro</i> cultures as opposed to ascites tumours grown <i>in vivo</i>	Problem: insufficient integration of regulations and practices throughout Europe, hence no united stance and standards
Skin testing (general)	Improved Quantitative Structure Activity Relationship studies (QSAR) and cell culture systems	Animal experiments still necessary to assess skin sensitising potential of new chemicals; the skin is too complex to be modelled as yet
Skin irritants	Use of human keratinocytes and human skin models. Analytical techniques: squamometry corneofametry transepidermal water loss electrical methods for skin hydration microrelief laser-Doppler flowmetry colorimetry narrow band spectroscopy ultrasound image analysis clinical assessment	Human keratinocytes can be useful for demonstrating <i>lack</i> of irritancy potential of water-soluble, non-cytotoxic materials, and for studying mechanisms of irritation
Respiratory toxicity	Human fetal tissue could be useful for particular studies	Use of cells isolated from laboratory animals e.g. alveolar macrophages, Type II cells and Clara cells, still necessary at present
Systematic toxicity testing	Closer study of blood and liver variables could indicate biomarkers that predict the effect of a substance, and shorten the endpoint of an experiment	In general, at present insufficient understanding of mechanisms of toxicity to allow identification of meaningful biomarkers
Carcinogen testing	<i>In vitro</i> cell transformation assays can be used for identifying animal carcinogens. Cells used: Syrian hamster embryo cells (SHE cell assay), and mouse fibroblast cell lines, Balb/c 3T3 and C3H/10T 1/2; more recently human cells	Mutation of the p53 tumour suppressor gene can accelerate carcinogenesis, and therefore improve the assay
Production of polyclonal antibodies in laboratory animals	Improved conjugation technology and the multiple antigen peptides (MAP). In contrast to traditional approaches, where the carrier is a large immunogenic protein, a relatively nonimmunogenic core matrix, consisting of trifunctional amino acids (such as lysine), is used as the 'carrier'. Use of more refined/more specific adjuvants	
Eye irritation testing (Draize test)	Improved experimental design, using smaller quantities, pre-screening using <i>in vitro</i> cytotoxicity tests, and improved application of statistical analysis	Replacement not yet feasible because of the complexity of the eye's response to irritants
Potency testing of vaccines	Serological assays and <i>in vitro</i> tests (ELISA)	<i>In vitro</i> antigenicity models generally considered invalid replacements for <i>in vivo</i> assays: they quantify the amount of antigen and do not reflect the immunogenicity of an antigen; do not reflect the complex cascade of responses <i>in vivo</i>
Toxicity of solid xenobiotics (e.g. natural minerals and industrial fibres and aerosols)	<i>In vitro</i> carcinogenicity on cultured cells. Possible surrogate markers: cytokine release and inflammation for potential carcinogens; molecular markers of responses to DNA damage (e.g. increased expression of p53)	Mammalian cellular assays for genotoxicity and transformation have low sensitivity and reproducibility, but cells can be genetically engineered (p53 mutation) to show increased susceptibility to the genotoxic substances
Transgenic animals	When an alternative, non-transgenic method at late stage of development and likely to be reasonably and practicably available in the near future, it should be considered as a replacement for the transgenic animal e.g. appropriate cell culture methods could be used before, or instead of, using whole transgenic animals	
Use of animals in higher education	Computer and audio-visual models; animals observed in natural setting or during brief periods of captivity; animals obtained from an ethical source, e.g. dissection of animals that have died naturally/were humanely killed for other reasons; learning in clinical setting, where only animals in need of veterinary intervention are studied	

Information collated and adapted from the Proceedings of ECVAM workshops.

those countries in which the regulations are implemented has risen'. Wagner believes that another important component in reducing animal use is the sharing of results and animals between scientists. This will help to reduce duplication, but it is, perhaps, a little utopian given the commercial concerns—patent and intellectual property rights—often associated with such research.

So animals in biomedical research are here to stay. Their justification, however, hangs in the balance of ethical arguments, the education of the younger generation of scientists, and better formulation of scientific and medical aims. As Wagner concluded: 'I'm not in principle against scientists thinking a bit more before they do an experiment.'

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Proteomics: biology in the post-genomic era

Companies all over the world rush to lead the way in the new post-genomics race

In June 2001, when the Human Genome Project and Celera completed the first maps of the human genome, Francis Collins, head of the government-sponsored HGP, warned that only then would the real race begin. This was a prophetic insight indeed. No sooner was the human genome decoded than we found ourselves in the 'post-genomic era'—where the name of the game is proteomics. Proteomics is not only the systematic separation, cataloguing and study of all of the proteins produced in an organism, it is also the study of how proteins change structure, interact with other proteins, and ultimately give rise to disease or health in an organism. Since its application in drug discovery promises huge economic returns, it comes as no surprise that biotechnology, computer and software companies around the world are rushing to pour capital and resources into this new research field.

Proteomics is more complex by several orders of magnitude than genomics, with no one company, laboratory or consortium

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remotely able to run the race alone, acknowledged Brian D. Chait, head of the mass spectrometry laboratory at Rockefeller University in New York City. Moreover, no one technology will be able to fulfil proteomics' numerous tasks, and new

developments are sorely needed, added Roy Whitfield, CEO of Incyte Genomics (Palo Alto, CA).

With the publication of the draft of the human genome in the February 16, 2001 issues of *Science* and *Nature* came what many had already suspected: instead of the earlier estimate of about 100 000 human genes, the actual count reduced this figure by 75%. If humans have only 10 000–20 000 more genes than the fruitfly and the roundworm, then the big question according to Eric Lander, head of the Whitehead-MIT Genome Center in Cambridge, MA, is how do we manage to be so complex? The answer: proteins—not genes—are responsible for an organism's complexity. The interaction of proteins in a complex network adds up to how an organism functions, according to Denis Hochstrasser, Professor of Medical Biochemistry at the University of Geneva's (Switzerland) Faculty of Medicine. The key to understanding health and disease within an organism is, therefore, to understand how its proteins function. 'In a multicellular organism, one needs to be able to look at the entire system in an integrated way. Proteomics is the study of where each protein is located in a cell, when the protein is present and for how long, and with which other proteins it is interacting', said Brian Chait. 'Proteomics means looking at many events at the same time and connecting them,' he added. New tools are necessary to enable the study of this web of events—to create a 'movie', rather than a static snapshot of the activities taking place.

A further dimension was added to this complex picture when scientists from the University of Pennsylvania School of Medicine reported this May in *Nature* that proteins are more active and dynamic than they had imagined. The researchers

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used nuclear magnetic resonance imaging to track the activity of a calmodulin-peptide complex across 13 different temperature settings ranging from 15 to 73°C. The data showed that there is a much larger range of internal motion in calmodulin than crystallographic studies can show. 'The interior of a protein is much more liquid-like than scientists originally anticipated,' A. Joshua Wand, Professor of Biochemistry and Biophysics and principal author of the study, said. 'Everything is moving, and it's moving all the time, very fast. They [proteins] move so much that potentially it dramatically influences how they work,' he said. 'This is the beginning of a long new story that will have a lot to do with understanding protein function.' The concept of proteins as dynamic entities may ultimately help scientists target more accessible sites for drug development, said Wand.

Currently, drug developers are working with only about 400–500 targets, many of which are receptors. With the shift from