

MEDICAL ADVANCES AND ANIMAL RESEARCH

The contribution of animal science to the medical revolution: some case histories



MEDICAL ADVANCES AND ANIMAL RESEARCH THE CONTRIBUTION OF ANIMAL SCIENCE TO THE MEDICAL REVOLUTION: SOME CASE HISTORIES



RDS

Coalition for
medical
progress

PUBLISHED BY

RDS: Understanding Animal Research in Medicine *and* Coalition for Medical Progress
© 2007

RDS represents scientists in the public and political debate about the use of animals in medical research. info@rds-net.org.uk, www.rds-net.org.uk

Coalition for Medical Progress, an alliance of organisations with the common aim of ensuring the UK continues to lead advances in human and animal medicine, explains why animals are used in medical research. info@medicalprogress.org, www.medicalprogress.org

CMP MEMBERS

Academy of Medical Royal Colleges
Academy of Medical Sciences
Amicus
Animal Health Trust
Association of the British Pharmaceutical Industry
Association of Medical Research Charities
AstraZeneca
B&K Universal
BioIndustry Association
Biotechnology & Biological Sciences Research Council
Biosciences Federation
British Pharmacological Society
Covance
Eisai
Genetic Interest Group
GlaxoSmithKline
Huntingdon Life Sciences
Institute of Animal Technology
Laboratory Animal Science Association
Laboratory Animal Veterinary Association
Lilly
Medical Research Council
Merck Sharp and Dohme
Novartis
Patients' Voice for Medical Advance
Pfizer
Physiological Society
Prospect
RDS
Royal College of Physicians
The Russell Group
Sanofi Aventis
Sequani
Society of Endocrinology
Wellcome Trust
Wyeth

PICTURE CREDITS

Cover: All images: *Wellcome Photo Library* except mouse, dog and monkeys *Institute of Animal Technology* and sheep *RDS*. **Inside pages:** All images: *Wellcome Photo Library* except p7 dog, p9 mouse, p11 monkeys *Institute of Animal Technology* and stereotactic surgery *Sovereign, ISM/Science Photo Library*, p12 polio vaccine *World Health Organization*, p14 buffalo gnats *CDC Public Health Library*, p15 guinea pigs *RDS*, p16 rabbit *RDS*, p18 mouse *Professor Dominic Wells*, p20 heart valve *Steve Allen/Science Photo Library* and p21 sheep *Philippe Plailly/Eurelios/Science Photo Library*, p22 stem cells *Professor Geoffrey Raisman* and p23 mice *RDS*.

CONTENTS

PAGE 2
FOREWORD

PAGE 3
INTRODUCTION

PAGES 3
TEN MEDICAL ADVANCES THAT CHANGED THE WORLD

PAGES 4-5
TIMELINE

PAGES 6-7
THE THERAPEUTIC REVOLUTION – FROM ANTIBIOTICS TO
ANTI-REJECTION MEDICINES
[Helping premature babies](#)
[Medicines for high blood pressure](#)

PAGES 8-9
STATE OF THE ART CANCER THERAPY – NOVEL APPROACHES
[Advances in breast cancer therapy](#)
[Antibody therapy](#)

PAGES 10-11
HOLDING DISEASE IN CHECK
[HIV: anti-retroviral therapy](#)
[Brain implants for Parkinson's Disease](#)

PAGES 12-13
THE POWER OF VACCINATION
[Meningitis](#)
[Cervical cancer](#)

PAGES 14-15
FIGHTING DISEASE IN THE DEVELOPING WORLD
[River blindness](#)
[Tuberculosis](#)

PAGES 16-17
BEYOND MEDICINES AND SURGERY
[Blood transfusion](#)
[Anaesthetics](#)

PAGES 18-19
GENE THERAPY – A REVOLUTION IN THE MAKING
[Muscular dystrophy](#)
[Sickle cell disease](#)

PAGES 20-21
FROM OPEN HEART SURGERY TO A PHARMED FUTURE
FOR PROTEINS AND ORGANS
[Open heart surgery](#)
[A new clot buster](#)

PAGES 22-23
STEM CELLS – THE MASTER BUILDERS
[Spinal cord repair](#)
[New frontier for heart patients](#)
[Blindness](#)

FOREWORD



Scientific and medical research is a drawn-out process and the contribution of animal research is frequently overlooked by the time successful therapy reaches patients. We live longer and healthier lives than ever before. Whilst there have been remarkable improvements in the human environment, animal research has played a major part in developing improvements in human health. Animal research advanced the treatment of infections, helped with immunisation, improved cancer treatment and has had a major impact on managing heart disease, brain disorders, arthritis and transplantation. My own field, the prevention of genetic disorders in babies, was possible only because of humane work on animals.

2

Animal research contributed to 70% of the Nobel prizes for physiology or medicine. Many award-winning scientists affirm that they could not have made their discoveries without animals. Polio would still claim hundreds of lives annually in Britain without the animal research of the Nobel laureate Albert Sabin. "There could have been no oral polio vaccine without the use of innumerable animals," he once said. Animals are still needed to test every new batch of polio vaccine produced for today's children.

The work we do is performed with compassion, care, humanity and humility. All my rabbits, when I worked with them years ago, were stroked and petted every day. Their contribution changed the understanding of ectopic pregnancy – the commonest cause of maternal death worldwide. My genetically modified rodents breed happily, and their offspring are indistinguishable from those of other rats and mice. Medical researchers are compassionate people seeking to alleviate pain and suffering. They are unlikely to do anything that is unnecessary or cruel. Indeed, they are not allowed to, because of the rigour with which animal licences are granted by government.

Animal research is not done in isolation; it is one vital strand of medical research. Of course, there are differences between animals and people. But there are also striking similarities, meaning animals provide invaluable information – information that cannot be replaced by computer modelling, cell culture or human experimentation. Mice have virtually the same genes as humans, which is why they are so useful for understanding human physiology and disease. And it is important to remember that animal research plays a vital role in understanding animal ill-health, as well.

For honest, open debate we need to understand the role of research using animals in medical progress. Organisations such as RDS and CMP are crucial, and this report highlighting case studies of important medical advances is most welcome. Tracing the research process – from 'blue-sky' or chance observation, through careful studies which include the use of animals for the ultimate benefit of people who are ill – will increase understanding of this essential endeavour.

PROFESSOR ROBERT WINSTON

INTRODUCTION

TEN MEDICAL ADVANCES THAT CHANGED THE WORLD

Throughout the world people enjoy a better quality of life because of advances made possible through medical research, and the development of new medicines and treatments. A small but vital part of that work involves the use of animals.

Mainstream medical and scientific organisations all agree that animal research is essential for medical progress. For example, a Royal Society report¹ stated in 2006 that: "We have all benefited immensely from scientific research involving animals. From antibiotics and insulin to blood transfusions and treatments for cancer or HIV, virtually every medical achievement in the past century has depended directly or indirectly on research on animals."

The following are examples of these achievements:

PENICILLIN Florey and Chain first tested the effects of penicillin in mice in 1940. By 1941, penicillin was being used to treat dying soldiers. This research won the Nobel Prize in 1945. **BLOOD TRANSFUSION** Blood transfusion has saved the lives of countless people and animals. The technique was developed when citrated blood was shown to be safe for transfusion in dogs in 1914. **FIRST MEDICINE FOR TUBERCULOSIS** A hundred years ago, tuberculosis (TB) was one of the most common causes of death. Nobel Prize-winning research on guinea pigs in the 1940s led to the antibiotic streptomycin. **MENINGITIS VACCINE** Vaccines for meningitis were developed in mice and have resulted in a huge fall in the disease. Previously many victims died or had amputations or organ damage. **KIDNEY TRANSPLANTS** Of the 5,000 people who develop kidney failure every year in the UK, one in three would die without a kidney transplant. Transplantation techniques were developed using dogs and pigs. **BREAST CANCER** Breast cancer is the commonest cancer among women. Animal studies led to the development of tamoxifen, one of the most successful treatments, and more recently Herceptin and aromatase inhibitors. **ASTHMA INHALERS** Asthma is the commonest serious childhood illness and still causes about 2,000 deaths a year in the UK. Animal research was vital for the medicines in the inhalers seen in many schools today. **POLIO VACCINE** This advance alone has saved millions of lives. Forty years of research using monkeys and mice led to the introduction of the vaccine in the 1950s. **INSULIN FOR DIABETES** Banting and Best won the 1923 Nobel Prize for the discovery of insulin in dogs. This has saved millions of lives. **IMPLANTS FOR PARKINSON'S DISEASE** Research carried out on experimental animals, including primates, has led to an electrical implant (similar to a heart pacemaker) for the treatment of Parkinson's Disease.

Medical research with animals has helped to extend and improve the quality of life for millions of people. It has had an equally far-reaching effect on pets and farm animals. This is why I accepted the invitation to write this report. It is based on a small selection of case histories that highlight key animal research contributions to the scientific revolution which has transformed healthcare.

As a medical journalist for more than 30 years, I know about the critical role of animal research, and as an individual I have good reason for being grateful. I almost certainly owe my life to animal research. The emergency operation that saved my life in 1994 was based on techniques developed through animal studies. I am literally one of millions.

In an ideal world scientists would not use animals (mainly mice and rats) for medical research. They would use alternative techniques. In fact, they are doing so increasingly. Non-animal methods now account for about 90% of medical research and include mathematical and computer models, advanced tissue and cell cultures and scanning technology.

However, the scientific consensus is that many key questions can still only be answered by animal studies. These studies offer hope to millions of people with conditions such as Alzheimer's disease, cancer, sickle cell disease, stroke, spinal cord damage and tropical diseases like malaria.

Of course, animal models have limitations. They do not always adequately mimic human disease or responses to medicines. But they remain crucial and have made a major contribution to many of the biggest medical advances of our age. The timeline on the following pages underlines the massive scale of this achievement.

JOHN ILLMAN

TIMELINE

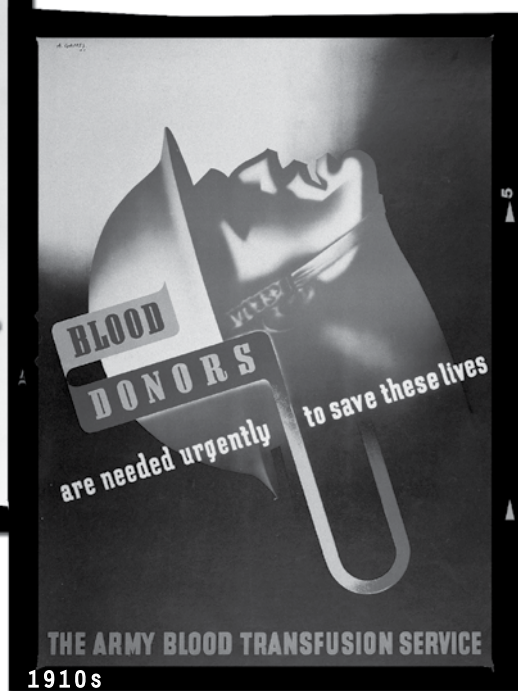
4



- Malaria parasite lifecycle (*cattle, birds*)
- Vaccine for smallpox (*cattle*)
- Vaccine for anthrax (*sheep*)
- Early anaesthetics (*cats, rabbits, dogs*)
- Rabies vaccine (*rabbits, dogs*)
- Typhoid, cholera and plague vaccines (*mice, rats*)
- Treatment for beriberi (*chickens*)



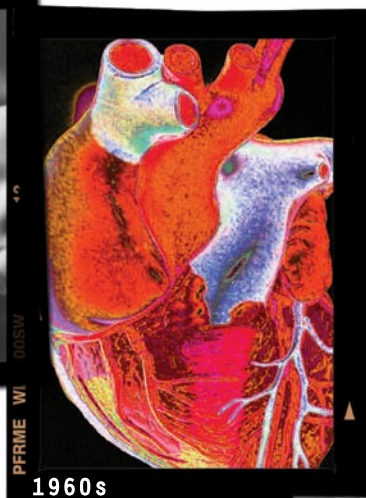
- Treatment for rickets (*dogs*)
- Corneal transplants (*rabbits*)
- Local anaesthetics (*rabbits, dogs*)
- Discovery of Vitamin C (*guinea pigs*)



Blood transfusions (*dogs, guinea pigs, rabbits*)



- Polio vaccine (*mice, monkeys*)
- Hip replacement surgery (*dogs, sheep, goats*)
- Kidney transplants (*dogs*)
- Cardiac pacemakers (*dogs*)
- Medicines for high blood pressure (*rats, mice, dogs*)
- Replacement heart valves (*dogs, calves, rabbits, guinea pigs, rats*)
- Chlorpromazine and other psychiatric medicines (*rats, rabbits, monkeys*)



- Heart transplants (*dogs*)
- Coronary bypass operations (*dogs*)
- German measles vaccine (*monkeys*)
- MMR vaccine (*monkeys*)
- Antidepressants and antipsychotics (*rats, guinea pigs, rabbits*)



- CT scanning for improved diagnosis (*pigs*)
- Chemotherapy for leukaemia (*mice*)
- Medicines to treat ulcers (*rats, dogs*)
- Inhaled asthma medication (*guinea pigs, rabbits*)

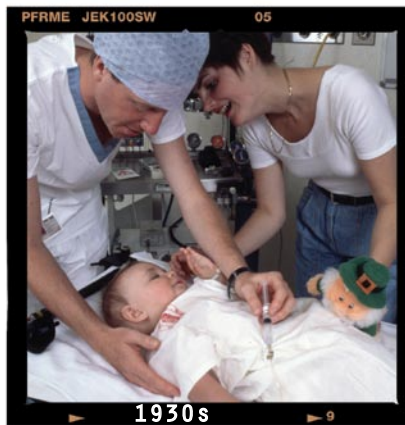


- MRI scanning for improved diagnosis (*rabbits, pigs*)
- Prenatal corticosteroids improving survival of premature babies (*sheep, rabbits, cattle*)
- Treatment for river blindness (*rodents, cattle*)
- Life support systems for premature babies (*monkeys*)
- Medicines to control transplant rejection (*mice, rabbits, dogs, monkeys*)
- Hepatitis B vaccines (*monkeys*)
- Medicines to treat viral diseases (*many species*)
- Treatment for leprosy (*armadillos, monkeys*)



1920s

Insulin (*dogs, rabbits, mice*)
Canine distemper vaccine (*dogs*)



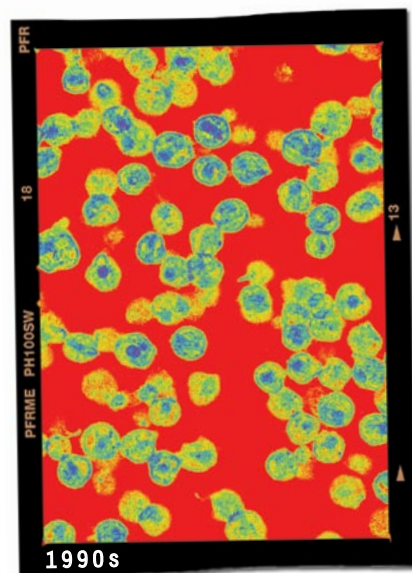
1930s

Modern anaesthetics (*rats, rabbits, dogs, cats, monkeys*)
Tetanus vaccine (*horses, guinea pigs*)
Diphtheria vaccine (*guinea pigs, rabbits, horses, monkeys*)
Anticoagulants (*rabbits, guinea pigs, mice, dogs*)



1940s

Penicillin and streptomycin (*mice*)
Discovery of rhesus factor (*monkeys*)
Kidney dialysis (*guinea pigs, rabbits, dogs, monkeys*)
Whooping cough vaccine (*mice, rabbits*)
Heart-lung machine for open heart surgery (*dogs*)



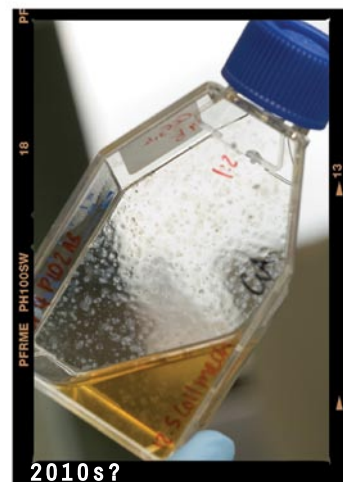
1990s

Combined therapy for HIV infection (*mice, monkeys*)
Meningitis vaccines (*mice*)
Better medicines for depression (*rats*)
Medicines for breast and prostate cancer (*mice, rats, dogs*)
Medicines for type 2 diabetes (*mice*)
New medicines for asthma (*guinea pigs, monkeys*)
Statins to lower cholesterol (*rabbits*)



2000s

Deep Brain Stimulation for Parkinson's Disease (*monkeys*)
Monoclonal antibodies for adult leukaemia, lymphoma (*mice*)
Cervical cancer vaccine (*rabbits, cattle*)
Clotting agent from milk (*goats*)
Bird flu vaccine (*chickens and ferrets*)



2010s?

Stem cells for spinal cord, heart repair (*mice, rats*)
Oral or inhaled insulin for type 1 diabetes (*mice*)
Angiogenesis inhibitors for cancer, blindness (*mice*)
Gene therapy for muscular dystrophy, cystic fibrosis, sickle cell disease (*mice*)
Alzheimer's vaccine (*mice*)
Malaria vaccine (*mice, monkeys*)

THE THERAPEUTIC REVOLUTION – FROM ANTIBIOTICS TO ANTI-REJECTION MEDICINES. IT IS HARD TO SEE HOW MANY OF TODAY'S LIFE-CHANGING TREATMENTS COULD HAVE BEEN DEVELOPED WITHOUT ANIMAL RESEARCH.

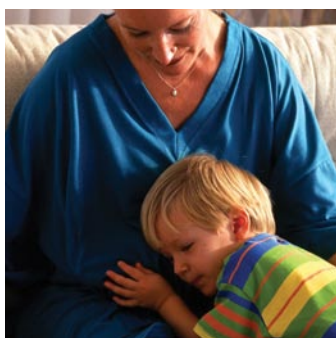
6

Major advances include asthma therapy; beta blockers for anxiety, high blood pressure and heart problems; chemotherapy for cancer; clot-busting medicines to prevent and treat heart attacks and strokes; ulcer medication which reduces the need for surgery; open heart surgery, joint replacements and organ transplants².

HELPING PREMATURE BABIES

Premature babies now routinely receive steroid therapy after a chance observation by a doctor 30 years ago that a premature lamb developed unexpectedly strong lungs after receiving a steroid injection. Benefits are still flowing from the discovery made in Auckland by Professor Sir Graham Liggins.

It led to research in sheep and rabbits showing that corticosteroids speed up lung development, stimulating synthesis of surfactants³. These soap-like substances lubricate the lungs, improve breathing and decrease the risk of severe respiratory problems. One study concluded that steroid therapy administered to babies at birth dramatically reduced respiratory distress in babies born at 26-32 weeks from 75% to just 8%⁴.



How did Sir Graham make the first vital observation? He had expected the lamb to die because it was so premature that "its lungs should have been just like liver and quite un-inflatable". However, when it did finally die, he found that its lungs were partly inflated.

Shortly afterwards Sir Graham met Mary Ellen Avery, who had discovered the role of surfactant in maintaining lung expansion. He recalled: "She couldn't get back to the US fast enough to set up experiments in rabbits – giving cortisol to fetal rabbits – and produced the definitive paper on the effects of corticosteroids on lung maturation. I thought 'Well, if it works in animals, why shouldn't it work in human babies?' As far as we knew, lungs in human babies had the same enzymes as animal lungs."⁵

Animal studies thus led to the development of synthetic surfactants for babies who were unable to develop their own⁶. Rabbit pups were the first preterm mammals successfully treated with replacement surfactants, derived from adult rabbit lungs. The first animal-based surfactants to be used in human babies, in the 1980s, came from cows, and improved the survival rates of babies born more than 12 weeks premature from 50% to 90%⁷.

Recent benefits from the initial Auckland⁴ work include refinements in treatment. For years, specialists speculated as to whether giving corticosteroids to pregnant mothers, rather than newborn babies, would produce even better overall results – or cause developmental problems. A study in *The Lancet* in 2006 concluded that babies born to mothers who had received repeat corticosteroids in pregnancy were less likely to have respiratory problems⁸. The study continues, to determine if there are also physiological benefits later in life: the trial babies' growth and development are to be monitored at the age of two and when they start school.

BORN TOO EARLY

- About one in ten of the 700,000 babies born in the UK each year is admitted to a special care baby unit. Over half these babies are born prematurely.
- Almost all premature babies have a low birth weight (less than 2,500g). The UK has Europe's highest rate of low birth weight babies.
- Babies born at 23 weeks have a 17% chance of survival; at 24 weeks, a 39% chance of survival; and at 25 weeks, a 50% chance of survival.

SOURCES: BLISS (premature baby charity) www.bliss.org; *Born Too Early*, Pete Moore, Thorsons (1995).



2



HIGH BLOOD PRESSURE: THE SILENT KILLER

It might seem bizarre that scientists should turn to deadly snakes to discover new medicines to treat life threatening conditions like hypertension (high blood pressure). But medicine and snake venom share a common aim – to change biological function. Venoms are extremely complex, each containing as many as 100 different substances, and scientists believe they can yield a wide range of medicines.

Research into the Brazilian pit viper venom produced the first in a new class of anti-hypertensive medicines – the angiotensin-converting enzyme (ACE) inhibitors⁹. It began because banana workers were known to collapse suddenly after being bitten by pit vipers due to a drastic fall in blood pressure.

ACE inhibitors expand constricted blood vessels by preventing the formation in the blood of a naturally-occurring substance, angiotensin, which raises blood pressure. The British Nobel Prize-winning scientist Sir John Vane played a key role in the development of the ACE inhibitors¹⁰, helping to isolate key components from the venom. This established how they reduced blood pressure in rats and led to development of similar synthetic compounds.

Another British Nobel prize-winner, Sir James Black, was at the forefront of animal research to develop beta blockers, initially to treat angina and cardiac arrhythmias¹¹. Hormone stimulation of beta receptors in the heart, one of the places where adrenaline has an effect, was known to increase heartbeat and demand for oxygen. Black worked out a way to block the beta receptor sites which stopped the hormones attaching to them. This reduced oxygen demand – and with it the accompanying pain of angina.

Use of beta blockers in hypertension began after observations in patients that the first beta blocker produced a small but persistent fall in blood pressure¹². Subsequent beta blockers with fewer side effects were developed in studies with rats.

It is widely accepted that a healthy lifestyle is important to prevent hypertension, but not all types of hypertension respond to, or can be prevented by, lifestyle changes. Before medicines became available, 'accelerated' hypertension could strike at virtually any age and was generally fatal within a year. Accelerated hypertension causes very high blood pressure and can result in organ damage in the eyes, brain and kidneys. Affecting about one per cent of hypertensive patients, it can now be controlled by medicines.

Although the most striking achievement in this field of research over the last 30 years is said to be controlling accelerated hypertension, treating less extreme forms of hypertension has been found to reduce the risk of stroke, heart attack and kidney disease¹³. These affect at least 10 million people in the UK alone. In most advanced industrial societies, age-adjusted death rates from coronary heart disease have fallen by 30-70% over the last two decades¹⁴.

Hypertension provides an interesting example of how animal studies can contribute, sometimes in a circuitous way, to the development of new medicines.

ONE IN FOUR

- About one person in every four in the UK has high blood pressure (hypertension).
- More than a third of these people are not being treated, putting their health at risk.
- Hypertension rarely makes people feel ill. Often the only way to find out if you have it is to have your blood pressure measured.
- Hypertension develops if the smaller blood vessels become narrow. Not doing enough physical activity, being overweight, having too much salt in your diet, drinking too much alcohol, a poor diet and having a family history of hypertension increase individual risk.

SOURCE: British Heart Foundation (www.bhf.org.uk)

1 Very premature babies with immature lungs can have difficulty breathing. Animal research has been critical in developing life support systems for these vulnerable babies. This includes treatments such as steroids, which can be given in the womb and help the newborn baby to breathe.

2 Treatments for high blood pressure, starting with snakes but developed using rats and dogs, have transformed and saved millions of lives. Medicines developed over the last 50 years have reduced the risk of stroke, heart attack and kidney disease for those suffering mild hypertension. These medicines also control the most serious form of hypertension which can kill within a year.

STATE OF THE ART CANCER THERAPY. THE STUDY OF CANCER IS ONE OF THE LARGEST FIELDS OF ANIMAL-BASED RESEARCH. THERE ARE MORE THAN 200 DIFFERENT TYPES OF CANCER AFFECTING OVER 60 ORGANS IN THE BODY. UNDERSTANDING THE DIFFERENT TRIGGERS, MECHANISMS AND DEVELOPMENT OF ALL THESE DISEASES IS THE KEY TO SUCCESSFUL TREATMENT.

8

Critics claim the rise in the number of people developing cancer shows that animal research is ineffective. Paradoxically, this trend highlights the success of medical research which has led to people living longer: cancer has most commonly been a disease of later years.

ADVANCES IN BREAST CANCER THERAPY

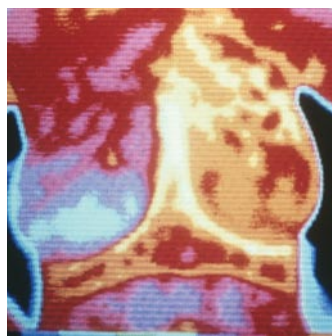
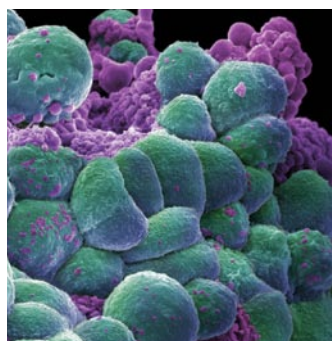
More than 42,000 cases of breast cancer are diagnosed each year in the UK, making it the most common cancer in women after non-melanoma skin cancer. Although the number of patients surviving for more than five years has risen to 80% over the past 20 years, breast cancer can still kill. New survival-boosting treatments include Herceptin (trastuzumab) and aromatase inhibitors.

Aromatase inhibitors block production of the female hormone oestrogen, 'starving' breast cancer cells of growth stimuli. Professor Angela Brodie of the University of Maryland School of Medicine developed the aromatase inhibitors¹⁵ and tested them in mice, comparing them with tamoxifen, the gold-standard treatment for women with common 'oestrogen-receptor-positive' cancers.

This research showed how animal models can predict patient response not just to a particular medicine, but to different combinations of therapy – a critical factor in cancer treatment. For example, animal studies with combinations of tamoxifen and aromatase inhibitors did not show any improvement over established treatments.

Professor Brodie described how aromatase inhibitor therapy alone had been shown to be the most effective¹⁶. Her preliminary research excited doctors who ran the first clinical trial. It was a success and aromatase inhibitors were approved for use in patients with oestrogen-fuelled breast cancer. Later studies with patients showed that sequential treatment with tamoxifen and then the aromatase inhibitor exemestane improved survival rates for this type of breast cancer, and could save a further 1,300 lives a year in the UK alone¹⁷.

The development of Herceptin is also a landmark. In 2005, researchers reported a study showing a 50% fall in the rate of breast cancer recurrence after one year of treatment¹⁸. This degree of benefit in early breast cancer was the largest reported since the introduction of tamoxifen, (see footnote) which was first used to treat breast cancer at Manchester's Christie Hospital in 1969.



Herceptin only seems to work when breast cancer cells produce too much of (over-express) a protein known as HER-2 which normally helps to regulate cell growth. Over-expression of HER-2 occurs in 20% to 30% of tumours in patients with breast cancer.

INCREASING UK SURVIVAL RATES

- One in nine women in the UK develops breast cancer.
- Four out of five cases occur in women over 50, but 3% of breast cancers occur in women under 35.
- Breast cancer accounts for more than 12,400 deaths each year in the UK.
- Breast cancer incidence among British women is increasing by more than one per cent each year. Between 1983 and 2002 it rose by 45%.
- Five-year survival rates have improved from 60% to 80% over the past 20 years.

SOURCES: Cancer Research UK (www.cruk.org) and Breast Cancer Care (www.breastcancercare.org.uk/)

FOOTNOTE

Tamoxifen has also been used to help develop an alternative to animals in breast cancer research. It has been used in studies to show that cultures of human tumour cells grown in the laboratory will respond to drugs that work in patients. Without animal work, it may not have been possible to show that the cell culture results were relevant and reliable.

3 Breast cancer is the commonest cancer among women. Animal studies led to the development of tamoxifen, one of the most successful treatments, and more recently Herceptin and aromatase inhibitors. Most of the animal studies involve rodents, but it is often necessary to test novel cancer treatments using monkeys.

4 Herceptin is an example of a monoclonal antibody (mAb), as are rituximab for treating lymphoma and arthritis, and Campath for adult leukaemia. About 40 years ago, Nobel prize-winning mouse research led to the development of these very specific antibodies. More recently, ways to produce pure human mAbs have been developed that do not involve mice or rats. mAbs are the basis of a third of all biotechnology medicines in development.

HER-2 was discovered in 1979 in neurological tumours of rats at the Massachusetts Institute of Technology¹⁹. It took nearly 20 years to develop Herceptin, a monoclonal antibody that targets HER-2. (See below for an explanation of monoclonal antibodies.)

Animal studies also highlight possible risks in specific groups of patients. For example, Herceptin is not routinely recommended for nursing mothers after studies showed that monkeys secrete it in milk²⁰.

ANTIBODY THERAPY

Cambridge researchers César Milstein and George Köhler won a Nobel Prize in 1984 for their work developing monoclonal antibodies (mAbs). Each mAb is a specific antibody, a 'magic bullet' protein that targets and attacks one particular substance or cell. Milstein and Köhler found a way to produce these magic bullets in unlimited quantities.

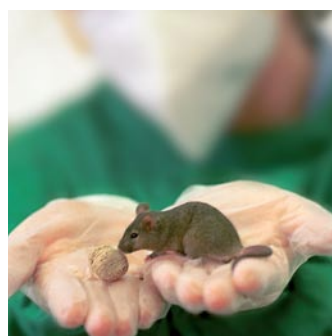
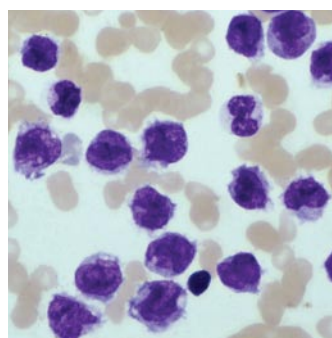
mAbs are proteins produced in the laboratory from the cells of the immune system that make antibodies. They act like natural antibodies, an important defence against disease, and attach themselves to specific proteins on the surface of cancer cells.

Using mice, Milstein and Köhler found a way to produce antibodies by fusing an antibody-secreting cell (B lymphocyte) with a non-immune cell, for example a cancer cell. mAbs have led to major advances in treatment and diagnosis.

Monoclonal antibody therapies include Herceptin (described left) and Rituxan (rituximab), which has had unparalleled success in treating lymphoma (cancer of the lymph nodes, part of the body's immune system).

Rituximab locks onto a protein called CD20, found on the surface of one of the main types of normal white blood cells (B lymphocytes)²¹. CD20 is also present on the surface of most abnormal B lymphocytes associated with most types of non-Hodgkin's lymphoma. Rituximab attacks both malignant and normal B lymphocytes, but the risk of side effects is very small because the body quickly replaces normal cells that have been damaged. Rituximab is also being used to treat rheumatoid arthritis.

Rituximab and Herceptin are the first of many antibodies for treating cancer. Campath (alemtuzumab), originally produced in Cambridge by Geoff Hale and Herman Waldemann²², is now successfully used in leukaemia where it has allowed bone marrow transplants in older patients, and from unrelated individuals. Unusually, this antibody comes from rats rather than mice.



Made from rodent antibodies, the first mAbs used in patients were rejected as foreign bodies. In the 1980s, Sir Greg Winter devised a way to 'humanise' mAbs, making them more acceptable to our immune systems. Genetic engineering provided part-mice, part-human antibodies. Researchers then created transgenic mice with human antibody genes instead of mouse ones, enabling efficient production of 100% pure human mAbs. Monoclonal antibodies today form the basis of a third of all biotechnology products in development.

MONOCLONAL ANTIBODIES

"It is not an exaggeration to describe César Milstein's contribution to science and medicine as the most important immunological advance of the century. His discovery of the method to produce monoclonal antibodies reinvented the field of immunology."

Dr Abraham Karpas, University of Cambridge, quoted in *The Times Higher Educational Supplement*, 29 March 2002.

It will take many years to realise the full potential of mAbs, but research has come a long way. Most mAbs are now made *in vitro* instead of in mice as scientists do not use animals in research once they have developed a viable alternative. Nevertheless, we probably would not have this powerful and targeted type of cancer therapy if it had not been for pioneering work with mice beginning in Cambridge in the 1960s.

HOLDING DISEASE IN CHECK. FIFTEEN YEARS AGO HIV INFECTION MEANT A DEATH SENTENCE FROM AIDS. BUT IN ONE OF SCIENCE'S MOST SPECTACULAR TRIUMPHS, NEW MEDICINES HAVE OPENED UP THE POSSIBILITY OF NORMAL LIFE EXPECTANCY FOR THE 40 MILLION PEOPLE LIVING WITH HIV.

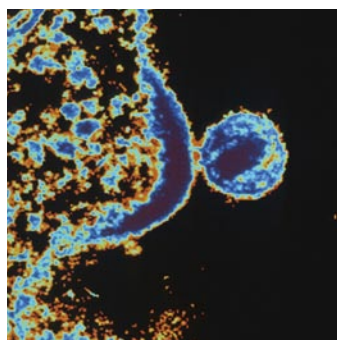
10

Animal studies played a key part in the development of HIV medication – transforming killer diseases into manageable conditions. A similar story unfolded in the last century with the development of insulin to treat people with diabetes²³. Before then a diagnosis of diabetes almost always meant wasting away to premature death.

HIV: ANTI-RETROVIRAL THERAPY

Acquired Immune Deficiency Syndrome (AIDS), caused by the human immunodeficiency virus (HIV), was identified in 1981. The story behind the medical world's response to HIV is one of intense discovery, and the "fastest in medical history", according to the late Jonathan Mann, then head of the World Health Organization AIDS Programme²⁴.

HIV is a retrovirus – one of the biggest challenges for research. A retrovirus integrates its genes inside the cells it invades. This enables it to bypass the immune system and quickly establish a lifelong foothold unless it is stopped at the time of initial exposure. What makes the speed of development of HIV medicine remarkable is that as late as the 1970s there was a mistaken scientific consensus that human retroviruses did not exist²⁵. The first human retrovirus, which causes a form of leukaemia, was discovered by Dr Robert Gallo in 1980.



AIDS took the world by surprise because it was a new disease in humans. No-one initially knew what caused it, but a team led by Dr Luc Montagnier in France first isolated the virus, HIV, in 1983. HIV was confirmed to be the cause of AIDS by several independent groups in 1984. By 1985, screening tests for blood donations were introduced.

The first clinical trial of an anti-retroviral medicine occurred in 1986. It involved a medicine identified from retroviral research in mice before the start of the AIDS epidemic. The virus quickly became resistant to it. However, by 1996, combination therapy with cocktails of anti-retroviral drugs had saved many thousands of human lives.

Animal studies were needed to study AIDS and to develop these successful treatments. Initially, hopes to use primates foundered because, while the virus infects chimpanzees (and indeed originally came from these animals), it does not cause disease in chimpanzees. However, macaque monkeys are susceptible to a simian (monkey) immunodeficiency virus (SIV) which is similar to HIV²⁶, and SIV is sensitive to the same drugs.

SIV causes an AIDS-like condition affecting the nervous system and behaviour in addition to destroying the immune system. HIV and SIV have similar genes and properties and both attack T helper (CD4) immune system cells.

There was initial concern that the macaque would prove to be an inappropriate model. Dr Ronald Veazey, of the Tulane National Primate Research Center in New Orleans, recalled: "What I heard from other investigators was: SIV in monkeys wasn't going to be like HIV in humans."

This speculation was based on studies showing an immediate rapid decline in the levels of T cells in the gut of infected macaques. After only a week these levels dropped to about half of those recorded in infection-free macaques²⁷. This was not thought to happen in people, suggesting that SIV was indeed significantly different from HIV. But similar findings then emerged from human studies. We now know that T cells in the guts of many patients all but disappear after HIV infection. So what had looked like a drawback was actually of huge benefit.

In the 1980s, time was of the essence in research into an unknown virus with the power to kill millions of young people. It only takes months for SIV infection to progress to simian AIDS, while it can take many years for HIV to progress to AIDS. Monkeys infected with SIV provide critical insights into what happens in the first weeks after infection in studies that cannot be carried out in HIV-infected people.

It is important to remember that HIV does not announce itself like the common cold or other viruses. In 2004, it was estimated that a third of people living with HIV in Britain did not know about their infection. This exposes unborn babies of HIV-infected mothers to an unnecessary risk of infection.

The success of anti-retroviral therapy can be measured not only in terms of millions of saved lives, but also in the way it is delivered. In 2000, it was not unusual for patients to be taking combinations of up to 24 pills per day, in as many as five different doses, with complex dietary restrictions. Many found it hard to follow prescribing instructions which were essential to obtain the optimum response and to avoid the serious threat of drug resistance.

Today, taking just two or three pills a day combining three different types of medication can stop HIV from reproducing, while helping the immune system to recover. The hope is for a once-daily pill – but the ultimate goal is a vaccine.

THE MONKEY MODEL – A POSSIBLE SOLUTION

Monkeys are used to develop and test HIV medicines because the virus does not infect small animals like mice and rats. Scientists have tried to bypass this problem by testing new antiviral medicines in mouse models into which human immune cells have been transplanted.

But HIV rodent models are notoriously difficult to make. There have been concerns that by the time researchers refine both an animal and the virus to produce an effective model, it will no longer bear resemblance to the actual disease.

However, after more than 20 years of HIV research, perseverance may finally have paid off. A team at the University of Heidelberg has evaluated the potential of a rat model for testing HIV medicines. In a study published in 2007, they found that antiviral activity of two established anti-HIV medicines in the rats was comparable to that observed in human patients²⁸. It will be necessary to test more compounds to validate the model, but these rats could reduce the need for non-human primates in HIV research.



6

BRAIN IMPLANTS FOR PARKINSON'S DISEASE

A treatment known as deep brain stimulation (DBS) has transformed the lives of more than 40,000 patients with the movement disorder Parkinson's Disease (PD). Introduced in the 1990s to treat drug-resistant disease, DBS is an illustration of why it is important to see research as a continuum.

In 1817 the British physician James Parkinson provided the first description of the disease that now bears his name in his famous essay on the 'shaking palsy'.

But effective treatment remained elusive for 150 years until the Nobel Prize-winning Swedish scientist Arvid Carlsson, in research using rats, discovered dopamine in the late 1950s; and the Czech neurologist Oleh Hornykiewicz showed that PD is due to a part of the brain called the *substantia nigra* degenerating and ceasing to produce dopamine²⁹. The distinguished Greek-born neurologist George Cotzias, working at Harvard, then found that giving levodopa, which the brain converts into dopamine, alleviates all the symptoms of PD³⁰ enabling millions of patients to live near normal lives for many years.

A bizarre discovery involving drug addicts who had been taking synthetic heroin gave researchers a better means of mimicking PD in animals, to study the disease and test new treatments. In 1976 Barry Kidston, a 23-year-old chemistry graduate in Maryland, developed PD symptoms within only three days of taking heroin contaminated with a drug called MPTP³¹. The US National Institute of Mental Health found traces of MPTP in his lab and discovered its effects by testing it on rats. These effects were confirmed by Dr William Langston who diagnosed PD in seven illicit drug takers in California³².

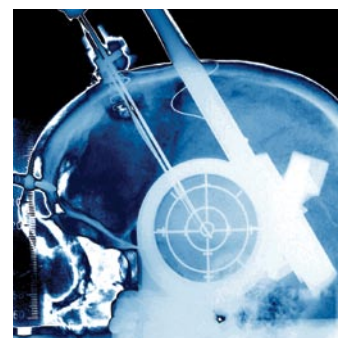
The discovery of the effects of MPTP led to the development of the most useful animal model of Parkinson's Disease; and this has led to major advances in our understanding of PD. But the need for new approaches is as great as ever because about half of all patients suffer a severe recurrence of their symptoms within five years of starting levodopa.

Using stereotactic techniques (see footnote) to study the monkey MPTP model of PD led to the identification of new targets within the brain for deep brain stimulation (DBS) – targets include a structure called the subthalamic nucleus (STN). Continuous stimulation is delivered by an electrode wire inserted into this target and driven by a battery stimulator implanted under the collarbone. For many patients it has transformed their lives – and, unlike earlier surgical techniques, it does not destroy brain tissue so it can be reversed if scientists develop better treatments in the future.

Professor John Stein, a neurophysiologist at the University of Oxford, says that many thousands of patients can lead normal lives again thanks to discoveries made with just a few monkeys³³. However, current treatments can fail to relieve the most disabling of all movement disorders. 'Locomotor akinesia' prevents patients getting up and walking or it may cause 'freezing' as they try to walk just a few feet, or it may result in them falling over when turning a corner.

The professor explained: "We found in our monkey experiments that this might be due to malfunction of yet another brain structure, the pedunculopontine nucleus (PPN). Within a few weeks of publishing this research, six patients with previously untreatable akinesia had DBS in their PPN and were able to give up their wheelchairs and walk around almost normally.

"This success has justified, a thousand-fold in our minds, our 10 years of monkey experiments. Like the monkey STN experiments, it involved only a few animals. Yet now thousands of previously untreatable patients can look forward to alleviation of their wheelchair-bound existence."



6

NOT JUST A DISEASE OF THE ELDERLY

- An estimated four million people worldwide have Parkinson's Disease.
- One in 500 people, about 120,000, in the UK has Parkinson's.
- About 10,000 people in the UK are diagnosed each year.
- Symptoms first appear, on average, when a patient is older than 50.
- One in 20 of those diagnosed each year is under 40.
- Statistically, men are slightly more likely to develop Parkinson's than women.
- The cause of Parkinson's is unknown. Areas of research include genetics and environmental factors. Different risks factors may interact to cause disease. Nine genes have been linked to Parkinson's. There may also be a link with herbicides and pesticides.

SOURCE: Parkinson's Disease Society (<http://www.parkinsons.org.uk/>)

5 Anti-retroviral therapy can now stop HIV reproducing, help the immune system to recover and delay or prevent the onset of AIDS. The ultimate goal is a vaccine.

6 Stereotactic surgery, used to guide electrodes deep into the brain, was developed 100 years ago. It was used in research on monkeys that led to identification of new brain targets and new treatments for Parkinson's Disease. It is still used to implant electrodes in the brains of patients today.

FOOTNOTE

Stereotactic surgery, the technique of guided brain operations, was developed in monkeys by Victor Horsley and Robert Clarke in the UK in 1906³⁴. They devised a frame that could be fixed to a monkey's head and used it to guide electrodes deep into the brain to study brain function. In 1947 Ernest Speigel and Henry Wycis³⁵ used this technique for psychosurgery in patients. It is still used today to implant electrodes in the brain.

THE POWER OF VACCINATION. VACCINATION HAS PROBABLY SAVED MORE LIVES THAN ANY OTHER PUBLIC HEALTH INTERVENTION APART FROM THE PROVISION OF CLEAN WATER SUPPLIES AND SANITATION. SUCCESS STORIES INCLUDE POLIO, MEASLES, MUMPS, RUBELLA, INFLUENZA, TETANUS, DIPHTHERIA AND PERTUSSIS (WHOOPIING COUGH).

12

Some parents are reluctant to have their children vaccinated for fear of vaccine damage. Arguably, vaccination has become a victim of its own success because new generations of parents have never seen the life threatening complications of illnesses like measles. The number of measles cases in the UK fell from between 50,000 and 100,000 cases a year to less than 10,000 after the introduction of the MMR vaccine in 1988. Many new vaccines are being developed.

MENINGITIS – A LIFE-THREATENING DISEASE

Meningitis, inflammation of the membranes covering the brain and spinal cord, is caused by various organisms including bacteria and viruses. Bacterial meningitis is the most dangerous – it can kill within hours. Meningococcal infection accounts for most cases of bacterial meningitis in the UK. Anyone can develop meningitis, but children, especially those under 18 months, are at greatest risk.

About 10% of the 3,000 people who develop meningococcal disease each year in the UK still die - despite advances in intensive care – with mortality rising to 40% in children who develop septicaemia.

Before antibiotics became available, overall mortality was more than 70%. In one outbreak, in the early 1900s, all 300 sufferers died. Thus, there is probably no better illustration than meningitis of the life-saving impact of antibiotics in the 20th century.

Animal studies were fundamental to the understanding of bacterial disease and development of anti-bacterial medicines. Howard Florey and Ernst Chain, who were working in Oxford to develop antibiotics at the start of World War Two, injected eight mice with a lethal suspension of bacteria. Four were also given penicillin. The latter lived and the rest died: definitive proof that penicillin worked against serious bacterial infection.

Of course, antibiotic therapy has its limitations. Its effectiveness depends on how quickly it is administered and the severity of the illness. Priority has been given to developing vaccines because of the devastating speed with which meningococcal meningitis can kill despite antibiotic therapy.

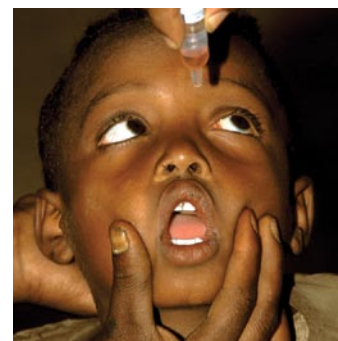
Vaccines are now generally available to prevent most types of meningococcal disease – namely groups A, C, W-135 and Y. The notable exception is meningococcal B, but supported by animal studies, doctors are testing a 'B vaccine' in babies. The ultimate aim is to produce a universal vaccine against all forms of meningitis.

Haemophilus influenzae type b (Hib) used to be the commonest cause of bacterial meningitis. The number of reported cases in the UK fell by 96% after the launch of the Hib vaccine in 1992³⁶. Previously there were 800 cases in the UK every year, mostly in children under one year of age. Each year about 30 children died and about 80 suffered long-term brain damage, deafness and other disabilities.

The characteristics of the antigen, the part of the bacterium which stimulates the immune system, stymied initial work to develop a Hib vaccine. The problem was a large molecule, known as PRP, on the surface of the bacterium. PRP vaccines could not produce immunity in children under a year – those at greatest risk. But studies in mice and rabbits showed that coupling PRP to a protein overcame this problem, producing a powerful immune response³⁷.

A major challenge today is to discover medicines which will prevent brain damage that may occur even after antibiotics have kicked in. Two natural chemicals have a key role in brain cell injury and could be targets for new therapies. One of these has been found to destroy parts of brain cells and to trigger activity resulting in inflammation and swelling in the brain – hallmarks of meningitis.

Blocking this activity was found to reduce brain cell injury in rats, but it can take years to translate a finding like this into life-saving therapy.



PATHWAYS TO MEDICAL ADVANCES

"Few realise that the revolution occurring during the last 17 years in the treatment of many diseases caused by bacteria could not have taken place but for carefully planned and executed animal experiments, and that the present safety in administering powerful antibacterial drugs depends upon their previous examination by so-called vivisection." Howard Florey, who shared the 1945 Nobel Prize for Medicine for his work on antibiotics³⁸.

CERVICAL CANCER

Cervical cancer kills more women worldwide than any other type of cancer apart from breast cancer. It accounts for nearly 230,000 deaths and about 500,000 new cases every year, according to the International Agency for Research on Cancer. It is usually triggered by the human papillomavirus (HPV), one of the world's most common virus groups. There are more than 100 different types of HPV, including 30 that are sexually transmitted.

Many animal species, from birds to whales, are also susceptible to papilloma infection. The cottontail rabbit papillomavirus (CRPV) was the first animal model of cancer caused by a mammalian virus. Fortunately – for research into human disease – animal and human papillomaviruses have strong similarities.

CRPV played a critical part in the development of vaccines against cervical cancer because HPV cannot be replicated in cell culture, nor can it be transmitted to other animals. The fact that fully infectious HPV induces cancer ruled out direct human experimentation. Using CRPV, canine oral papillomavirus and BPV (bovine papillomavirus) researchers found that, whichever animal was used, it was possible to protect against infection by papillomaviruses. Thus they could stop the development of papillomas or cancer through various modes of immunisation.

The first of two vaccines to be developed became available in 2006, but Dr Richard Shope of the University of Rochester actually discovered CRPV in 1933. Alerted by a friend to cottontail rabbits with 'horns' (which were actually large warts), Shope ground up the horns, filtered them through porcelain that let only tiny virus-sized particles through, and injected the filtrate into other rabbits. In turn these rabbits grew horns³⁹.

Two years later, Shope's Rochester colleague Dr F Peyton Rous described the progression of papilloma warts into cancer⁴⁰. Rous is famous for an earlier study in 1910 with chickens which established the first link between tumours and viruses.⁴¹

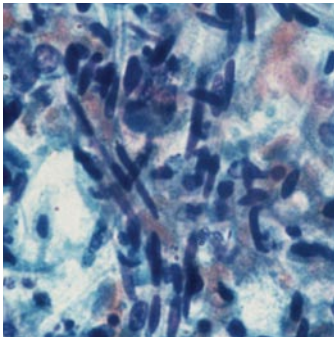
This groundbreaking work initially attracted little interest. The prevailing attitude was that animal cancers bore little resemblance to human cancers and that viruses could not transmit them. But Rous was eventually vindicated and won a Nobel Prize in 1966 for his early discovery. Nonetheless, Dr Saverio Campo, of Glasgow University, noted in 2002 that it still took "a paradigm shift in the late 1970s for some viruses to be recognised as 'tumour viruses' in humans"⁴².

In 1977 a German researcher, Dr Harald zur Hausen, published the first research linking the papilloma virus to cervical cancer⁴³, but his views – like those of Rous before him – were discounted. His controversial hypothesis finally turned into experimentally proven certainty in the early 1980s when he and his team isolated two previously unknown virus types, HPV-16 and HPV-18, from tumour tissue⁴⁴.

Why did it take so long to confirm HPV as a cause of cervical cancer? One important discovery was that the virus did not always cause cancer. Here again there was an important clue in animal research. For example, BPV had been known for many years to cause cancer in cattle, but only in those that also ate bracken. In women, HPV also needs some kind of 'push' or 'help' to trigger cervical cancer. About half of sexually active women are estimated to be infected with HPV yet only a very small proportion of these develop cervical cancer.

This is partly because only certain HPV types are dangerous. There are more than 100 different types, but types 16 and 18 are responsible for 70% of cervical cancer. However, not all women infected by types 16 and 18 develop the disease. Some women shake off HPV infection altogether, perhaps because of a genetic predisposition or perhaps because they have fewer risk factors than other women. Risk factors include smoking, a poor diet and a weakened immune system.

It is easy to see therefore why the primary cause of cervical cancer eluded science for so long. In 2002, Dr Campo also noted that the World Health Organization did not officially declare that HPV-16 and HPV-18 were carcinogenic (cancer causing) until 1995. Animal papillomavirus studies, he added, were "a determining factor" in that decision.

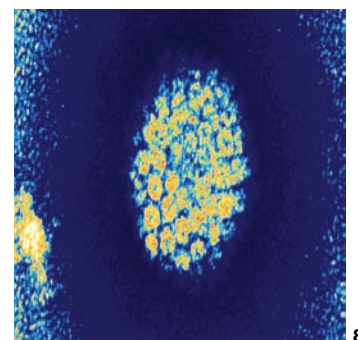


7 The polio vaccine alone has saved millions of lives. Forty years of research using monkeys and mice led to the introduction of the vaccine in the 1950s. The World Health Organization launched a vaccination programme to eradicate polio worldwide in 1988, which resulted in a 99% reduction in cases by 2000.

8 The primary cause of cervical cancer in women is the human papillomavirus (HPV), but only certain types are dangerous. Research on papillomaviruses (ABOVE) from rabbits, dogs and cattle showed that it was possible to stop the development of papillomas or cancer (RIGHT) through immunisation.

COMMON SEXUAL INFECTION

- Human papilloma virus (HPV) is the most common sexually transmitted infection diagnosed in genito-urinary medicine clinics in the UK. HPV is linked to cervical cancer.
- About 40 women die from cervical cancer each day in Europe⁴⁵.
- An estimated 70% of sexually active people are exposed to HPV at some time⁴⁶.
- HPV is usually cleared by the body within about one year with no symptoms, but it can cause cervical lesions which can progress to precancerous lesions. In turn these may progress to cancer when infection persists.
- Cervical cancer screening resulted in a 42% fall in deaths in England and Wales between 1988 and 1997⁴⁷. Vaccines have the potential to prevent the cancer altogether.



FIGHTING DISEASE IN THE DEVELOPING WORLD. MILLIONS OF PEOPLE – MOST OF THEM CHILDREN – DIE EACH YEAR IN DEVELOPING COUNTRIES FROM DISEASES THAT ARE PREVENTABLE AND TREATABLE. THE THREE BIGGEST KILLERS IN THE DEVELOPING WORLD ARE TB, AIDS (SEE PAGE 10) AND PARASITIC DISEASES LIKE MALARIA, WHICH CAUSES ABOUT THREE MILLION DEATHS A YEAR.

14

Other parasitic diseases that cause disability or death include schistosomiasis (bilharzia), which affects about 200 million people in tropical countries, and leishmaniasis (kala-azar), which affects about 12 million people.

RIVER BLINDNESS

The anti-parasitic medicine ivermectin, originally one of the world's leading animal treatments, has saved millions of people in Africa and South America from the scourge of river blindness, which is estimated to affect about 17 million people. Ivermectin became available to people in 1987 thanks to a hunch by a researcher⁴⁸. But it has to be taken once or twice a year on an ongoing basis and is not easy to distribute in remote rural settings.



River blindness is spread by a small black fly (buffalo gnat) that breeds in rapidly flowing rivers and streams. Its bite introduces parasites into the skin that grow and reproduce, which in turn liberate millions of microscopic offspring that can cause incessant itching and disfiguring skin lesions as well as blindness. People in endemic areas may be bitten by infected flies hundreds or thousands of times each year.

Also known as onchocerciasis, the disease has been blamed on the parasitic worm, *Onchocerca volvulus*. Antibiotic therapy for this and elephantiasis, caused by another parasitic worm, is now being tried in Africa. Use of antibiotics for treating both these severely disfiguring diseases could be the biggest breakthrough in the field since ivermectin.

The antibiotic trials follow work on rodents in Germany and on cattle at the University of Liverpool⁴⁹. Scientists were surprised to find that common antibiotics could kill worms.

The explanation may be that the real culprits in river blindness are *Wolbachia* bacteria that live inside the worm. The worms depend upon the bacteria to live and reproduce. This was shown by work with two groups of mice. The first was infected with normal worms. The second group, infected and treated with tetracycline antibiotics, were subsequently found to have fewer visual problems and less inflammation⁵⁰. It seems that a receptor in the eye is particularly susceptible to the bacteria.

Another possible solution is a vaccine. Vaccination against a parasite similar to that which causes river blindness in people has been successful in cattle. A team of Liverpool scientists developed the vaccine after discovering that some cattle have natural immunity to the parasite. Known as *Onchocerca ochengi*, it causes lumps to appear on animal skin, but does not cause blindness or illness. After two years of natural exposure to infected black flies, the number of worms in vaccinated animals was far lower than that in unvaccinated animals.

Professor Sandy Trees, of the University of Liverpool Faculty of Veterinary Science, said in 2006⁵¹: "Although the immunisation method that we tested in cattle would not be suitable for human use, this research provides the first proof that immunisation against onchocerciasis is possible and hence it may be feasible to protect humans from the parasite using some form of vaccination."

The Liverpool team is trying to find out how some cattle develop natural immunity while others do not.

NEW TARGETS FOR ANTI-MALARIAL MEDICINES

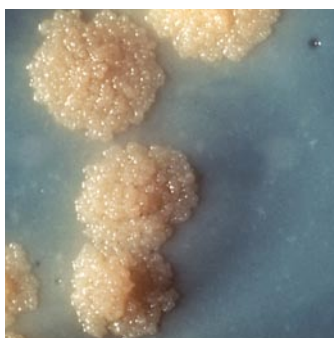
New medicines are desperately needed because the malaria parasite has become resistant to existing therapies except the plant product artemisine and its derivatives⁵².

Researchers are targeting a number of unique features in the parasite such as its food vacuoles (storage bubbles) and a mini bacterium called the apicoplast, a kind of cell-within-the-cell. A promising new medicine, fosmidomycin, is known to affect an enzyme pathway in the apicoplast⁵³.

A new antibiotic, azithromycin, has been shown to be effective in mice and monkeys, and has undergone a successful trial to prevent malaria in humans⁵⁴. An antibiotic called triclosan – used in mouthwashes, anti-acne preparations and deodorants – could also be effective. It clears the parasite from infected mice⁵⁵ by blocking a parasitic enzyme called Fab I.



10



TUBERCULOSIS

About a third of the world's population is infected by tuberculosis (TB), which causes about two million deaths* and eight million new infections a year. Infected people have a 10% chance of developing the disease and a 1% chance of dying.

These figures belie the true scale of TB which goes hand in hand with poverty, malnutrition, poor sanitation and severe immune deficiency. People with HIV are at 50 times greater risk than the general population.

TB in humans is caused by the bacterium *Mycobacterium tuberculosis* and is spread by sneezing or coughing, or by infected cows' milk. In up to 95% of cases, the immune system suppresses the infection without killing the bacteria, which can lie low in human lung tissue for decades before striking.

Vaccination has been the mainstay of protection. More than three billion doses of BCG vaccine have been administered since 1921. BCG was named after its French inventors, Albert Calmette, a pupil of Louis Pasteur, and Camille Guérin, who found that a close relative of the TB bacterium carried by badgers and cattle was weakened when it was grown on a beef bile medium.

They spent 13 years re-culturing the *Mycobacterium bovis* every three weeks until, 231 strains later, they had a strain that was mild enough to work as a vaccine but not strong enough to provoke disease. BCG provides substantial protection in the developed world and against disease which has spread beyond the lungs in children, but its effect wanes after 10 years, leaving adults at risk. It does not confer consistent protection against lung disease in the developing world.

A new vaccine has recently undergone successful preliminary human trials in the UK after studies on mice, guinea pigs and monkeys⁵⁶. Research leader Dr Helen McShane, of the University of Oxford, said: "These results are very exciting. This is one of the major advances in the field for over 80 years". Further human trials are underway.

Medicines are effective, but TB bacteria undergo random mutations that eventually cause resistance to all drugs. In 1943 the Russian-born scientist Professor Selman Waksman isolated the antibiotic streptomycin⁵⁷, a discovery which was to save millions of lives. It has been estimated that between 1700 and 1900, TB accounted for one in seven of all deaths in the world, killing about a billion people⁵⁸.

As early as 1952, in accepting his Nobel Prize, Waksman warned of resistance of bacteria to streptomycin. The challenge is to develop new drugs to keep ahead of *Mycobacterium tuberculosis*.

Exposing further weak links in the bug is the way forward. For example, a mouse study showed that spread of TB from the lungs depends upon interaction between one of the bug's surface proteins, called HBHA, and the epithelium, a layer of cells lining vessels and tissues⁵⁹. Another mouse study uncovered a biochemical pathway which may hold the secret to the bacteria's long term survival strategy⁶⁰. Further mouse research showed how TB bacteria inhibit the normal immune response⁶¹.

Researchers are also turning a speculative eye towards gene therapy in light of animal research showing that absence of a gene (*CCR2*) increases susceptibility to TB⁶². This may explain why only a small proportion of infected people develop what was known, before the antibiotic era, as 'the great white plague'.

BEATING THE LORD OF THE FLIES

Leishmaniasis is spread by sandfly bites and causes ugly boil-like scars, usually facial, and may also trigger fever, anaemia and damage to the liver and spleen. It has been found in nearly 90 countries – from the rainforests in Central and South America to the deserts of Asia.

A study has identified a weak link which might prove useful in designing an anti-leishmaniasis drug⁶³. The sandfly saliva contains an immunosuppressant, which stops the victim's body from mounting a defence and allows the parasites to multiply.

Millions of leishmaniasis parasites can be injected into mice with no ill effect if the immunosuppressant is absent. A hundred or so parasites injected alongside the immunosuppressant are enough to cause infection. The study found that parasites engineered to lack the molecule known as lipophosphoglycan (LPG) were 10 times more vulnerable to attack by white blood cells.

9 The tiny parasitic worms thought to cause river blindness are spread by small back flies known as buffalo gnats. The medicine ivermectin, originally developed to treat parasitic infections in animals, has probably saved the sight of millions of people. New research in mice and cattle has shown that the real culprit may be a bacterium that lives in the worm, opening up the possibility of antibiotic or vaccine treatment.

10 A hundred years ago, the *Mycobacterium tuberculosis* bacterium was a major cause of death and disease, and isolation hospitals to contain the infection were common. Nobel Prize-winning research on guinea pigs in the 1940s led to the first antibiotic effective against TB, streptomycin. The challenge for research today is to develop a more effective vaccine and new treatments to keep ahead of drug resistance.

FOOTNOTE

* Some estimates put the annual death rate at about three million

BEYOND MEDICINES AND SURGERY. ANIMAL RESEARCH HAS BECOME SYNONYMOUS WITH ADVANCES IN MEDICINES AND SURGERY, BUT ANIMAL STUDIES HAVE ALSO HELPED TO DEVELOP LIFE SUPPORT SYSTEMS FOR PREMATURE BABIES, TECHNOLOGY FOR INTENSIVE CARE UNITS, POSTOPERATIVE CARE, DIAGNOSTIC EQUIPMENT AND NUTRITIONAL SUPPLEMENTS.

16

BLOOD TRANSFUSION

The UK national blood transfusion services, which distribute 8,000 donations a day, recently celebrated their 60th anniversary, a landmark in a long chain of discovery underpinned by animal research.

It started in the 17th century with animal experiments witnessed by Britain's most celebrated diarist Samuel Pepys and it continues today, with new challenges replacing old ones. Nucleic acid amplification testing of donated blood, for example, is now being used routinely to screen blood donations for viruses such as HIV.

But it took more than 200 years to make blood transfusion part of routine clinical practice. This goal would probably never have been reached without animal research and earlier 17th century groundbreaking work by William Harvey, the first scientist to demonstrate that blood circulates around the body⁶⁴. He used 50 different animal species in his work, laying the foundations of modern medicine.



Early in the 19th century, James Blundell of London was one of several scientists to show that transfusion from one species to another did not work. He went on to establish human-to-human blood transfusions as a sound clinical practice, performing 11 transfusions in women with *post partum* haemorrhage (post birth bleeding). Rallying after receiving eight ounces of blood, one patient commented that she had "felt as if life were infused into her body"⁶⁵.

Blundell's resounding success must have been attributable to the fortuitous use of compatible donors: it occurred before Karl Landsteiner, the central figure in transfusion medicine, discovered in 1901 that blood comes in four groups – A, B, AB and O⁶⁶. This Nobel Prize-winning discovery showed where at least some transfusions had been going wrong for more than 200 years.

Mixing incompatible blood from two individuals will lead to blood clumping or agglutination when mixed outside the body. If incompatible blood is transfused, the red cells will be destroyed, leading to serious and sometimes fatal reactions.

The discovery in 1939 of the Rh blood group system⁶⁷ showed the important role of rhesus factors in causing 'haemolytic disease of the newborn' when fetal red blood cells are destroyed by the mother's antibodies crossing the placenta. It was shown that incompatibility of these groups between mother and child was the cause of this disease.

11 Studies in Landsteiner's laboratory showed that injection of rhesus monkey red blood cells into rabbits produces an antibody that agglutinates red blood cells of many humans⁶⁸. This was the basis of research that was to show that 84% of white people have a 'Rh factor' on the surface of their red blood cells. They are 'Rh-positive'. The remaining 16% are 'Rh-negative'. If they are transfused with Rh-positive red cells, they can make anti-Rh, which will destroy Rh-positive red cells. So Rh compatibility is also considered in blood transfusion, especially for women. It was later discovered that, though similar, the rabbit anti-Rh and the human anti-Rh factor were not the same – but it was too late to change the name.

Initially there was no way to store donor blood or stop it clotting, but research reported in 1915 showed that citrate could prevent blood from clotting and that citrated blood could be safely transfused into dogs⁶⁹. Even more exciting was the discovery that citrated blood could be stored for two days and was still effective when transfused into guinea pigs and dogs that had lost blood⁷⁰. Further work with rabbits extended the shelf life of blood for transfusion to 14 days⁷¹.

DISCOVERY VIA SERENDIPITY

Animal research in 1914 showed that it was possible to remove toxic metabolites from blood using special membranes containing hirudin – an anticoagulant from crushed leech heads – and return the blood safely to the body.

John Abel gave the first demonstration of so called 'continuous dialysis of the blood' using anaesthetised dogs and rabbits and dialysis membranes made from treated parchment⁷². The animals recovered consciousness and were fine within three hours.

Abel's original idea was to develop a technique to measure the blood concentrations of substances like hormones - but he recognised the potential to develop 'an artificial kidney'. This paved the way towards the first successful treatment of a patient with acute kidney failure in 1945.

Dialysis has since saved hundreds of thousands of lives. In the UK alone, nearly 20,000 patients had kidney dialysis in 2005. **SOURCE:** UK Renal Registry.



PREVENTING BLOOD CLOTS

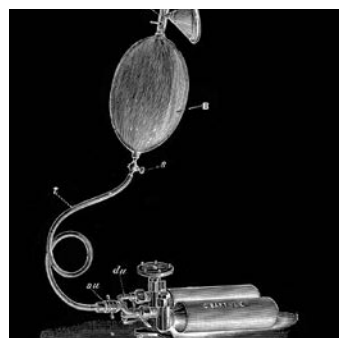
Blood clotting (coagulation) disturbs blood flow. Anticoagulants are used to prevent clotting which can lead to deep vein thrombosis, pulmonary embolism, heart attacks and strokes.

Heparin, an anticoagulant occurring naturally in mammals, is one of the main blood-thinning medicines. It was first isolated from dog liver by Jay Maclean, an American medical student at Johns Hopkins University, in 1916⁷³. Early extracts were impure and unsuitable for human use, but in 1937 purified extracts were found to cause no ill effects in dogs, rabbits, guinea pigs, mice and, subsequently, human patients⁷⁴.

As well as being administered directly to patients, heparin is also used in medical devices such as test tubes, renal dialysis machines and blood transfusion bags. But it is expensive and must be injected or infused.

While doctors were learning to make the best use of heparin, researchers at the University of Wisconsin discovered another potent anticoagulant, dicoumarin, in rotted sweet clover, which had been killing cattle. The Wisconsin labs synthesised more than 100 related substances, and one proved to be a deadly rat poison which works by making the animal bleed to death. They called it warfarin (from the initials of the Wisconsin Alumni Research Foundation, plus the -arin ending of the coumarin family).

In small doses, however, warfarin is an effective anticoagulant and is now the most commonly used. It can be used in smaller doses than dicoumarin and unlike heparin it can be given by mouth.



ANAESTHETICS

The first use of anaesthetics in human patients over 150 years ago - nitrous oxide (1844), ether (1846) and chloroform (1847) - was a turning point that was to change clinical practice for ever.

Fifty years before an anaesthetic was first used in patients, Humphry Davy, a surgeon's apprentice, demonstrated that nitrous oxide or 'laughing gas' could produce a state of reversible unconsciousness in animals⁷⁵. It also relieved his own toothache and he wrote in his book *Researches* in 1800: "Nitrous oxide . . . may probably be used with advantage during surgical operations in which no great effusion of blood takes place."

11 Early studies involving both animals and people led to better understanding of blood circulation and transfusion. But blood transfusion only became routine when modern apparatus was introduced and stored citrated blood was shown to be safe for transfusion in dogs. Later, the rhesus factor was discovered in rhesus monkeys.

12 Early anaesthetics such as nitrous oxide, or laughing gas, were tried out on animals in the early 19th century. Nitrous oxide became popular for human operations in 1868 when it was made available as a compressed gas in cylinders. Modern anaesthesia owes much to the early pioneering work and further animal studies.



In 1821, the surgeon Henry Hill Hickman claimed to have performed painless experiments on animals under carbon dioxide, but the medical establishment ignored his work⁷⁶. In 1844, the dentist Horace Wells had a wisdom tooth removed under nitrous oxide. He went on to demonstrate the gas at Harvard Medical School, but the patient had been too lightly anaesthetised and Wells was dismissed as a fraud⁷⁶. Negative reports about nitrous oxide generated interest in ether, but nitrous oxide returned to favour in 1868 when it became available in compressed form in cylinders⁷⁵.

The search by James Simpson for a non-flammable alternative to ether to relieve the pain of childbirth provided a dramatic example of the benefits of animal testing. Simpson changed his mind about testing ethylene dibromide on himself when two rabbits died in safety tests⁷⁷.

The latter half of the 19th and the early 20th centuries saw refinements in delivery of inhaled anaesthetics and the emergence of safer mixtures of gases and methods to measure their flow. Development from the 1950s of non-flammable, safer, inhaled anaesthetics depended as much on animal research as did the early anaesthetics pioneered by Humphry Davy. Experiments on rodents, rabbits, dogs, cats and monkeys showed that the volatile liquid halothane was easy to use, quick acting, had a muscle-relaxing effect but minimal side effects.

Carl Koller pioneered local anaesthesia in a partnership with Sigmund Freud, who was then a neurologist. Finding that applying cocaine to his tongue deadened sensation, Koller investigated its pain-killing (analgesic) effects on animals, on himself again, on his friends and finally on his patients in the early 1880s⁷⁶.

Spinal analgesia began when Leonard Corning accidentally pierced the dura (the membrane surrounding the spinal cord) of a dog in a cocaine experiment. He deliberately repeated the injection in a patient - calling it spinal anaesthesia⁷⁸. Corning's and Koller's studies were the prelude to modern epidural anaesthesia and analgesia and the introduction of highly effective local anaesthetics like lignocaine. Tests on rabbits' corneas had shown lignocaine to be more effective and quicker acting than other agents⁷⁹.

Benefits of animal studies extend far beyond the operating theatre to off-shoots of modern anaesthesia, such as postoperative and intensive care, and to emergency care in the community. For example, rabbit and dog studies have led to advances in the understanding and treatment of electric shock-induced ventricular fibrillation (a dangerous cardiac arrhythmia)⁸⁰.

GENE THERAPY – A REVOLUTION IN THE MAKING. THE MAIN AIM OF GENE THERAPY IS TO PREVENT OR TREAT DISEASE: BY REPLACING DEFECTIVE OR MISSING GENES OR BOOSTING BENEFICIAL GENES.

18

Not only are there natural animal strains for many inherited human conditions, but new technology makes it possible to insert, very precisely, faulty human genes into mice and other animals to mimic human diseases. Such animals are used to study these diseases and to test gene therapy. Current research is looking into how to get the therapy to the right tissues in the body and how to eliminate unwanted effects – such as the body ‘turning on’ cancer genes.

MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) is the most common inherited form of muscular dystrophy. It usually affects boys – two are born with it every week in the UK – and leads to difficulty in walking in infancy, progressive muscle wasting and death in early adulthood.

DMD is caused by errors in the largest known gene in the human body – that for dystrophin. Without this, muscle cells weaken with continuous contraction and eventually die. There is no cure yet, but researchers are optimistic that animal work will lead to gene therapy and stem cell therapy which could have a major impact on patients’ quality of life.

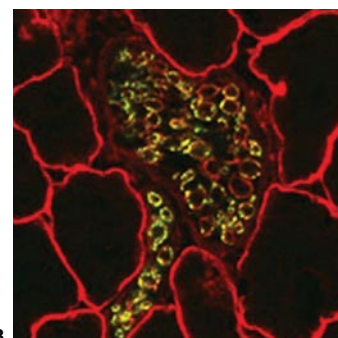
The ‘*mdx* mouse’, a naturally occurring strain with a mutation similar to that in people, has made a major contribution to research enabling scientists to test a variety of potential therapies.

Because the dystrophin gene is so large, it is difficult to get it into the appropriate muscle fibres. One solution is a new approach which quite literally patches up the problem. A ‘molecular patch’ of synthetic genetic material (antisense oligonucleotide) injected into affected muscle bypasses the genetic error causing DMD. This allows key parts of the dystrophin protein to be made so that it works almost normally, relieving many symptoms of the disease⁸¹.

In mice, these patches have led to improved muscle function: a single injection resulted in production of the corrected dystrophin for more than three months⁸². Obviously, it is vitally important not only to test for dystrophin protein production, but also to find out if it has a long-term effect.

At the Hammersmith Hospital in London, patches are now being tested in human muscle cells, reducing reliance on animal research. Muscle cells isolated from biopsies taken when the condition is diagnosed are grown in culture to assess the effectiveness of newly developed patches.

However, as Professor Dominic Wells of Imperial College, London, points out: “Although cell culture experiments can help us to assess the potential of novel approaches to treatment, only by understanding and testing such therapies in the complex environment – the whole organism – can we develop clinically useful protocols. As clinical trials develop into effective treatments, the DMD community will have a lot to thank the *mdx* mouse for.”



13

Doctors began recruiting boys for a UK clinical trial early in 2007. During the clinical trial the molecular patches will be injected into a small muscle on the top of the foot. Human studies have shown that this small foot muscle is relatively well preserved compared with other muscles in boys up to the age of around 15.

ANOTHER APPROACH TO MUSCULAR DYSTROPHY

An Italian team has found that a type of stem cell called a mesoangioblast, extracted from the lining of blood vessels, could be the basis of another treatment for muscular dystrophy⁸³ (see page 22 for detailed explanation of stem cells). These were tested in golden retriever dogs with natural mutation causing muscular dystrophy. The cells allowed the dogs with the crippling genetic disorder to walk and even jump again.

Talking about one retriever, Dr Giulio Cossu of the San Raffaele Scientific Institute, said: “One of the most emotional moments I had was when I saw the severely impaired dog running again. I couldn’t have anticipated it going so well. I hope that this result can be transferred to humans.”

SICKLE CELL DISEASE

About 250,000 babies are born across the world each year with sickle cell disease. Most are of African or Caribbean descent, although it also affects babies from Asia, the Middle East and the eastern Mediterranean. Sickle cell disease is a painful, debilitating and potentially fatal condition caused by a mutant gene affecting haemoglobin, the oxygen-carrying component of red blood cells.

The abnormal haemoglobin causes normally round and flexible red blood cells to become deformed and sickle-shaped. They stick together and cannot easily pass through small blood vessels, causing a



14

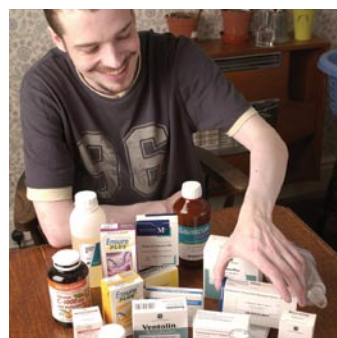
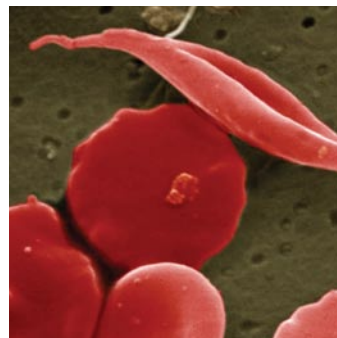
blockage of blood supply which in turn stops oxygen getting through, leading to damage in critical organs. The disease was first described in 1910⁸⁴ by an American doctor, James Herrick.

Treatments include antibiotics, pain-relieving medications, red blood cell transfusions and oxygen therapy, but the only cure is a bone marrow transplant. This treatment is recommended only for severely ill patients because of the risks of the procedure. Of the 22 children in the first multi-centre study of bone marrow transplantation, two died and four (18%) rejected their grafts⁸⁵.

13 In muscular dystrophy, the naturally occurring mutant *mdx* mouse has made a major contribution to research. A gene patching technique is now being tried in patients with this debilitating and fatal muscle-wasting condition.

Scientists believe that gene and stem cell therapy will lead to significant advances, but progress has depended on developing a good mouse model of sickle cell disease. Early mouse models with human sickle cell genes developed only mild symptoms of the disease because normal mouse genes counteracted the defective human genes.

In 1997 scientists finally succeeded in developing genetically engineered mice that could mimic all symptoms of the human disease⁸⁶. But gene therapy proved far more elusive than initially imagined, according to Philippe Leboulch of the Massachusetts Institute of Technology and Harvard University.



15

"Everybody thought it would be the first genetic disorder cured by gene therapy, that it would be simple, but it turned out to be completely different. It was a real challenge".

14 Sickle cell disease and cystic fibrosis are two relatively common serious inherited diseases. In sickle cell, mis-shaped red blood cells get stuck in blood vessels resulting in pain and damage to critical organs. The only cure to date is bone marrow transplantation, but this carries serious risks.

15 Cystic fibrosis affects the lungs and digestive system; patients have to take many different medicines to keep the symptoms at bay. For both diseases, GM mice have been crucial in understanding and developing gene therapies for patients.

Four years later, for the first time, scientists corrected sickle cell disease in mice with gene therapy⁸⁷. They removed bone marrow from mice with sickle cell and then transferred a new antisickling gene using a virus to carry it into the bone marrow. This gene incorporated itself into the stem cells that are responsible for producing red blood cells (see page 22 for more on stem cells). Ten months later, nearly all mice transplanted with this bone marrow had the anti-sickling gene in their red blood cells.

Progress in refining these gene therapy techniques over the last few years means that a small trial with patients is now taking place in France.

Another promising therapy involves turning off the defective gene while reactivating another gene responsible for production of fetal or zeta globin, which inhibits the formation of sickle cells. In one study reported in 2004, researchers genetically modified (GM) mice with sickle cell disease to produce zeta globin⁸⁸. These GM mice had normal blood counts and were no longer anaemic. The lifespan of their red blood cells was extended almost five-fold to normal levels.

In 2006 another team produced embryos from GM mice with the sickle cell mutation⁸⁹. They extracted stem cells from the embryos and replaced the defective genes in these with healthy ones, from which they then grew healthy blood cells.

The hope is that this work will lead to better solutions than bone marrow transplantation, especially for severely afflicted patients who do not have compatible donors. Some scientists are concerned about the lack of progress, but it is only 10 years since scientists developed mice to mimic all the symptoms of the human disease.

CYSTIC FIBROSIS

Cystic fibrosis (CF) is an incurable genetic disorder that mainly affects the lungs and digestive system. It affects about one in 2,000 children in the UK. Major improvements in treatment have increased average life expectancy to about 30 years from about four years in the 1950s. Nevertheless, quality of life is still severely affected.

The condition is due to a defect in a single gene, the *CFTR* gene, which was identified in 1989. In 1992 four research teams in the USA and UK reported on mice bred with defects in their cystic fibrosis gene⁹⁰. These mice showed that it is possible to correct the biochemical defect by introducing a 'good' copy of the *CFTR* gene to the respiratory tract⁹¹. This paved the way for clinical trials of non-viral gene therapy in the UK⁹² which reported evidence of gene correction, albeit short-lived.

Now working together as a consortium backed by the Cystic Fibrosis Trust, three UK groups, in London, Oxford and Edinburgh, have made substantial improvements to the gene therapy protocol. This has been tested in mice and sheep in preparation for further clinical trials. Fifteen CF volunteers are to join a single dose gene therapy trial in 2007, while a further 200 people are to be monitored. In 2008 up to 100 of these people will be recruited into a multi-dose gene therapy trial.

FROM OPEN HEART SURGERY TO A PHARMED FUTURE FOR PROTEINS AND ORGANS. USE OF ANIMALS IN MEDICINE IS NOT RESTRICTED TO THE STUDY OF DISEASE AND DEVELOPMENT OF NEW MEDICINES. ANIMAL TISSUE ALSO HAS IMMENSE THERAPEUTIC VALUE. ANIMAL HEART VALVES HAVE SAVED HUNDREDS OF THOUSANDS OF LIVES, THANKS TO OPEN HEART SURGERY.

20

Researchers are now investigating new therapeutic uses of animals such as genetically altering livestock to produce new medicines. 'Transgenic pharming' could produce, for example, insulin-producing pig cells to treat diabetes and a blood clotting factor to treat haemophilia.

OPEN HEART SURGERY – OPENING MORE DOORS

The advent of human open heart surgery in 1953 was made possible by the development of heart-lung bypass technology. This enabled surgeons to stop and re-start the heart to repair congenital abnormalities and replace diseased or deformed valves with either animal or mechanical substitutes.

The story began when a young patient's death stirred the surgeon John Heysham Gibbon to develop a heart-lung bypass machine, which he tried out on a cat in 1935. After further studies with dogs after World War Two he finally performed the world's first open heart operation on a human patient in 1953⁹³.

Until then little could be done for serious heart valve defects. Average life expectancy for patients with untreated heart valve disease is two years⁹⁴. Today about 6,500 patients a year in the UK alone receive replacement heart valves⁹⁵. Worldwide, animal heart valves have saved hundreds of thousands of lives.

The many attempts to mimic the anatomy of heart valves with artificial or mechanical materials in early animal studies convinced scientists that trying to copy natural designs would not work. It was hard to get the anchoring mechanism right and lack of strength was a major problem⁹⁶. Natural valves look delicate but in fact are very strong and durable.

So, instead of trying to mimic nature, scientists opted to use animal valves directly. Valves from pigs, sheep, calves and goats were transplanted into dogs in the early 1970s⁹⁷. Researchers overcame potential rejection problems by washing, 'denaturing' and tanning processes to make the animal tissue biologically inert. The first transplant of a pig heart valve into a human patient took place in 1975 and of a cow heart valve in 1981.

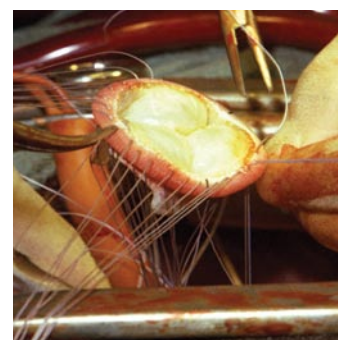
Both mechanical (artificial) valves and valves taken from animals are used today. Each has its drawbacks. Mechanical valves can last indefinitely but are prone to cause blood clots, leaving patients dependent on anti-clotting or blood-thinning medication for the rest of their lives. In contrast, biological valves rarely cause clotting but may gradually deteriorate and need to be replaced every 10 to 15 years.

Techniques described as 'tissue engineering' may produce a new generation of valves with all the benefits and none of the drawbacks of existing options. Japanese researchers have used rabbit cells to grow heart valve-shaped tissue inside the animal's body⁹⁸. The process may make it possible to grow rejection-proof replacement human valves with the patients' own cells, a technique called autologous tissue engineering.

Dr Kyoko Hayashida of the National Cardiovascular Center Research Institute in Osaka, Japan, told the American Heart Association in 2006: "It's the first fabrication of an autologous heart valve inside a living body."

She added: "If every body organ could be recreated by using autologous cells, it would solve the current shortage of donated organs available for transplantation and the use of costly and harmful anti-rejection drugs."

Xenotransplantation – the transplant of living organs from animals to humans – has been heralded as a potential solution to the worldwide shortage of human organs for transplantation. For example, in the UK in 2002, 401 people were reported to have died while on the transplant waiting list⁹⁹. Optimism was sparked in the 1990s by the genetic modification of pigs which succeeded in overcoming the serious rejection problems of trans-species transplants.



16

However, in December 2006 the UK Department of Health highlighted concerns about a possible risk that infectious diseases/agents might be transmitted from animals to people and potentially to the wider population. It recommended that any xenotransplants should be carried out within a research protocol approved by a research ethics committee. At that time there were no xenotransplant trials in the UK; nor had there been previous transplants of solid organs from animals to humans in the UK¹⁰⁰.

AN END TO INJECTION THERAPY?

Insulin from pigs closely resembles human insulin and has been used by patients for many years for routine injections. Within three to five years, subject to safety tests, researchers hope to treat patients with insulin-producing islet cells from pigs, thus ending their need for regular insulin injections. This follows the successful transplantation of pig cells into monkeys¹⁰¹.

Islets are also available from donated human pancreas. A transplant patient will typically receive islets from up to three donated pancreases. New donor sources are thus needed to help the millions of people with type 1 diabetes and the pig route could be the answer.

16 Heart valve defects can be overcome by implanting animal valves or artificial valves. One day it may be possible to use tissue engineering to grow living human replacement valves, or even whole hearts.

17 One of the first medical applications of transgenic technology was human insulin from bacteria. Other therapeutic proteins are now being produced in transgenic hens' eggs and in the milk of transgenic farm animals. For example, a clot buster from goats helps patients with an inherited deficiency of an anti-clotting factor. A protein produced in sheep's milk is being tried for an inherited lung and liver disease, A1AD, and for cystic fibrosis.

A NEW CLOT BUSTER

Most medicines are chemically synthesised, but biotechnology offers science the dazzling opportunity to use the body's own proteins as medicines. The first 'therapeutic protein', human insulin from bacteria, was launched in 1982¹⁰² – thanks to recombinant DNA technology which combines genetic material from two different sources *in vitro* (in a test tube).

Most therapeutic proteins are produced by microbial or mammalian cell culture. However, some proteins are difficult to express in cell culture, but relatively easy to express in animal milk.

Atryn is a medicine extracted from the milk of goats whose DNA has been modified by the introduction of a human gene. It is designed to treat an inherited deficiency of the antithrombin protein which can cause life-threatening blood clots, especially during surgery and childbirth.

In 2006 Atryn became the first medicine derived from transgenic animals (animals with externally introduced genes) to be licensed by the European Medicines Agency¹⁰³. It is expected to become available in the UK in mid-2007.

GTC Biotherapeutics created the first transgenic goats 15 years ago by injecting embryos with the human gene for antithrombin. The gene was incorporated into the DNA of the embryonic goats which were then implanted into surrogate mothers¹⁰⁴.



17

Cows, pigs and sheep are also used to produce pharmaceutical human proteins, but GTC says: "As a dairy breed goats show efficiency of milk production that is unrivalled." It has about 2000 goats on a Massachusetts farm established in 1995: each can produce approximately 2.5litres of milk a day containing 2g of protein per litre, and hence about 1kg of human antithrombin per animal per year.

The transgene is expressed in milk because it is joined with a DNA signal called a 'promoter' which is only active in the mammary gland. Thus the transgene, while present in every cell of the animal, is only active where the milk is made. The fact that the mammary gland is not part of the animal's main life support system minimises any potential risk to the animal. A successful transgenic animal will produce the desired protein without any risk to its own health and, moreover, pass this ability to its offspring.

Eggs provide another non-invasive harvesting medium. In 2007, UK researchers reported that significant quantities of two human proteins, interferon beta-1a and a humanised monoclonal antibody (miR24) were expressed in the whites of eggs laid by transgenic hens¹⁰⁵. Interferon-beta is a key component of the human immune system and an active ingredient in several multiple sclerosis therapies. miR24 is being developed for malignant melanoma, the most severe form of skin cancer.

TRANSGENIC ANIMALS

These are animals whose genetic composition has been altered to include selected genes, by methods other than those used in traditional breeding. Transgenic animals are used to:

- Study how a disease develops and reacts to medicines
- Test gene therapies
- Produce human therapeutic proteins
- Evaluate the purity of human proteins.

Most medicines are synthetically produced. This will almost certainly continue to be the case for the foreseeable future. However, the pharmaceutical industry is set for major change with the development of so called 'biotech medicines' which include therapeutic proteins such as enzymes and antibodies.

BROAD SPECTRUM APPLICATION

Researchers are using transgenic animals to develop therapies for a wide range of diseases including:

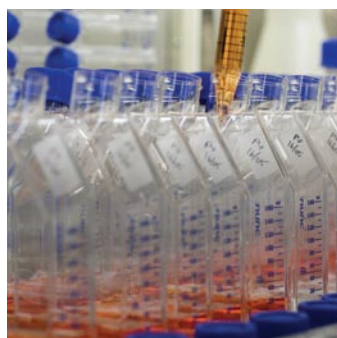
- Alzheimer's Disease
- Anaemia
- Cystic fibrosis
- Cancer
- Emphysema
- Haemophilia
- HIV/AIDS
- Malaria
- Rheumatoid arthritis

STEM CELLS – THE MASTER BUILDERS. OUR BODIES ARE REMARKABLE FOR THEIR THRIFTY DESIGN. FOR EXAMPLE, WE USE OUR MOUTHS TO BREATHE, EAT AND SPEAK. BUT ANIMAL RESEARCH HAS HELPED TO IDENTIFY WHAT MAY BE THE ULTIMATE IN BIOLOGICAL THRIFT – STEM CELLS.

22

There are two types of stem cells: embryonic and adult. Human embryonic stem cells can be obtained from aborted fetuses or very early-stage embryos left over from *in vitro* fertilisation. An embryonic stem cell is a non-specialised cell which can make copies of itself indefinitely and become any one of the 216 different types of cell found in the body. Adult stem cells taken from and put back into the same patient are not rejected by the immune system. While they are less versatile than those from embryos, they do not elicit such heated debate.

Stem cells have set the stage for a revolution in medicine and biology. More than a decade of research on the biology of stem cells in mice has helped to pave the way for developing human cell lines to treat disease. They have immense potential to treat a wide range of disorders, from paralysis to blindness.



SPINAL CORD REPAIR

Imagine if you were paralysed with a spinal cord injury, and were having cells taken from your nose so that you could walk again. This is not science fiction. Using small animals like mice and rats, scientists can easily mimic the kind of human spinal damage that is caused by road traffic or climbing accidents.

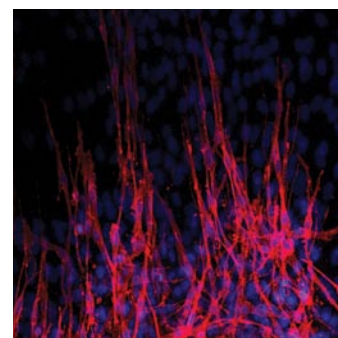
After discovering in 1985 that nasal stem cells constantly regenerate themselves¹⁰⁶, Professor Geoffrey Raisman of University College London and colleagues showed that transplanting nasal stem cells into paralysed rats can help them walk and climb again.

How does this happen? Spinal cord injury can sever the links between the cord and the brain with devastating effect. This may include loss of sensation due to destruction of fibres conveying sensory input from the body to the brain, and loss of control of movement, breathing, bladder, bowels, sexual function, blood pressure and body temperature.

Research has shown that if transplanted cells are implanted at the site of the damage in animals, they have the potential to build a bridge across the injured spinal cord, allowing the severed nerve fibres to knit back together again¹⁰⁷. Stem cells migrate up the spinal cord and develop into different types of cell. These include oligodendrocytes, which help to maintain the nervous system's electrical conduction.

The overall effect of this extensive stem cell activity is to put a patch over the original injury and 're-open' the communication chain between brain and spinal cord. This repair can be achieved in rats by transplanting stem cells two months after the original accident, by which time scar tissue covers the original wound¹⁰⁸.

Late in 2005 scientists announced a plan to carry out a preliminary safety study of the technique on about 10 patients at the National Hospital for Neurology and Neurosurgery in London. They plan to start it in 2007. It will be restricted to patients with injuries affecting movement and sensation in the upper limbs, but the ultimate aim is to free people from life in a wheelchair. The research also has the potential to treat nerve injuries associated with stroke and deafness.



18 Stem cells are set to revolutionise medicine, thanks to more than a decade of research in mice. Stem cells from the nose, grown *in vitro*, can mend spinal cord damage in rats and are now being tried in patients. Similarly, small trials of stem cell therapies are being conducted for blindness and heart attacks.

18

NEW FRONTIER FOR HEART PATIENTS

In 2006 UK doctors announced a pioneering trial to treat heart attacks. Patients are to have stem cells from their bone marrow injected into their heart muscle within five hours of their heart attacks. Research has shown that it is possible after a heart attack to use the healthy parts of the heart to regenerate some of the damaged parts.

This study is the first to be sponsored by the UK Stem Cell Foundation. It is combining stem cell therapy with primary angioplasty, a technique which involves opening up blocked arteries with a balloon-tipped catheter. Balloon-angioplasty also has its origins in animal research. (See footnote)

Being treated with angioplasty immediately after a heart attack instead of with clot-busting medication has led to marked increases in survival, but a significant proportion of patients are still left with heart damage.



The hope is that the stem cells will lead to further improvements in quality of life and delay or prevent heart failure, a frequent complication of heart attacks. Dr Anthony Mathur, senior lecturer and consultant cardiologist at Bart's Hospital, London, pointed out¹⁰⁹ that there was less likelihood of rejection complications with cells taken from the patients themselves.

The trial follows extensive animal research. In one study reported in 2006, US cardiologists took samples of heart tissue no bigger than rice grains from the healthy parts of hearts of pigs that had had heart attacks¹¹⁰. In the lab they grew large numbers of cardiac stem cells from the samples and implanted them into the pigs' hearts one month later.

Dr Eduardo Marbán of the Johns Hopkins University School of Medicine said: "Starting with just a very small amount of tissue, we demonstrated that it was possible, very soon after a heart attack, to use the healthy parts of the heart to regenerate some of the damaged parts."

"This is a relatively simple method of stem cell extraction that can be used in any community-based clinic, and if further studies show the same kind of organ repair we see in pigs, it could be performed on an outpatient basis."

BLINDNESS

Retinal cell transplants carried out by UK scientists have restored vision in blind mice which have a genetic defect that causes the loss of photoreceptors (light-sensing cells in the retina) and affects the eye's response to light¹¹².

This development may help many thousands of blind people to regain their sight. Photoreceptor loss also occurs in many human eye diseases, including diabetes and age-related macular degeneration, the most common causes of severe vision loss in the western world.

Photoreceptor loss-induced blindness used to be considered irreversible because the mature retina was thought to be unable to repair itself or support the development of new photoreceptors.

The retina might be one of the best places to try stem cell therapy because photoreceptor loss initially leaves the wiring to the brain intact. Earlier studies involving transplants of stem cells failed, however, because the transplanted cells did not integrate into their new environment and develop into photoreceptor cells.

There was speculation that this may have been because transplants were carried out too early in the cellular life cycle. London eye surgeon Robert MacLaren said: "We worked on the theory that cells at a later stage of development might have a higher probability of success." He was part of the team which restored vision to blind mice. It also included scientists at London's University College, and the Institutes of Ophthalmology and of Child Health.

It may be possible to use embryonic stem cells to achieve similar results in people, but the solution could be far closer to hand – within the eyes of the patients themselves.

Research team leader Professor Robin Ali said: "Recent research has shown that a population of cells can be found on the margin of the adult retina which has stem cell-like properties. In other words they are capable of self-renewal. These could be harvested through minor surgery and grown in the lab to become photoreceptor precursors before being re-implanted on the retina."

EXTENDING THERAPEUTIC POTENTIAL

Stem cells could be used in many different ways. For example:

- Nerve cells for stroke, Parkinson's, Alzheimer's, multiple sclerosis, spinal cord damage, and brain damage
- Skeletal muscle cells for muscular dystrophy
- Insulin-producing cells for diabetes
- Cartilage cells for osteoarthritis
- Blood cells for cancer, immune deficiencies, inherited blood diseases, and leukaemia
- Liver cells for treating hepatitis and cirrhosis
- Skin cells for burns and other skin wounds, and repairing scars
- Bone matrix cells for osteoporosis
- Retinal cells for blindness.

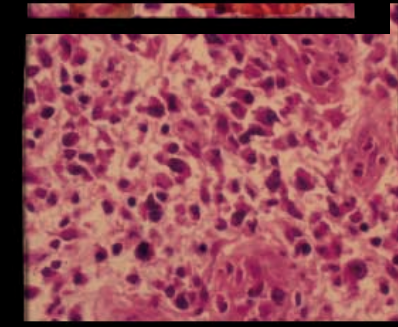
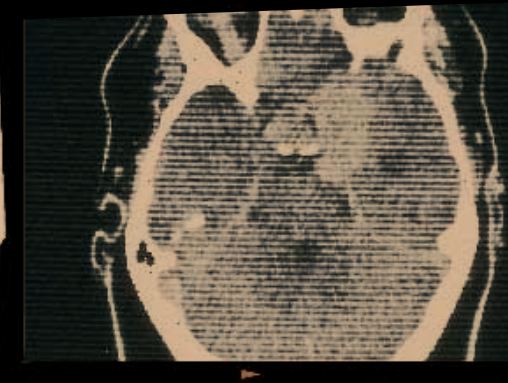
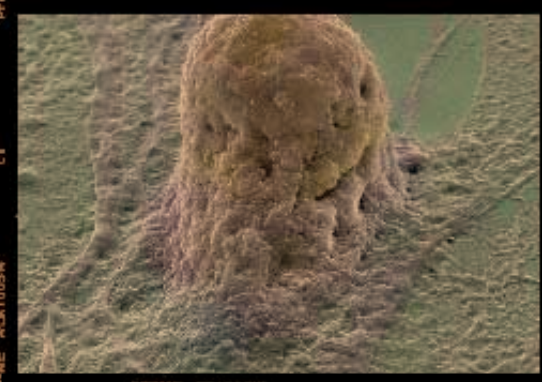
FOOTNOTE

Andreas Gruentzig, a young German physician, pioneered balloon angioplasty, presenting the results of the first animal studies to the American Heart Association in 1976. He performed the first balloon angioplasty on a 37-year-old man on 1977¹¹¹.

REFERENCES

- ¹ Statement of the Royal Society's position on the use of animals in research (2006) September, Ref 16/06.
- ² Illman J (2000) *The Expert Patient*, Association of the British Pharmaceutical Industry.
- ³ Kotas R, Avery ME (1971) Accelerated appearance of pulmonary surfactant in the fetal rabbit *Journal of Applied Physiology* **30** 358.
- ⁴ Reynolds, LA, Tansey, EM (eds) (2005) *Prenatal Corticosteroids for Reducing Morbidity and Mortality after Preterm Birth*. Wellcome Witnesses to Twentieth Century Medicine **25** 97, Wellcome Trust Centre for the History of Medicine at UCL.
- ⁵ *Ibid.*
- ⁶ Notter RH (2000) *Lung Surfactants: Basic Science and Clinical Applications*, Marcel Dekker, New York.
- ⁷ *Ibid.*
- ⁸ Crowther C *et al* (2006) Neonatal respiratory distress syndrome after repeat exposure to antenatal corticosteroids: a randomised controlled trial *The Lancet* **367** 1913.
- ⁹ Ferreira SH *et al* (1970) Isolation of bradykinin-potentiating peptides from *Bothrops jararaca* venom *Biochemistry* **9** 2583.
- ¹⁰ Vane JR (1999) The history of inhibitors of angiotensin-converting enzyme *Journal of Physiology and Pharmacology* **50** 489.
- ¹¹ Prichard BNC *et al* (2001) Beta-blockers in the third millennium – when are they really indicated *Journal of Clinical and Basic Cardiology* **4** 3.
- ¹² Prichard BNC (1982) Propranolol and beta-adrenergic receptor blocking drugs in the treatment of hypertension *British Journal of Clinical Pharmacology* **13** 51.
- ¹³ Jackson R *et al* (1993) Management of raised blood pressure in New Zealand: a discussion document *British Medical Journal* **307** 107.
- ¹⁴ Tunstall-Pedoe H *et al* (1999) Contributions of trends in survival and coronary event rate to change in coronary heart disease mortality: 10 year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease *The Lancet* **353** 1547.
- ¹⁵ Brodie A *et al* (2003) Predictions from a preclinical model: studies of aromatase inhibitors and anti-estrogens *Clinical Cancer Research* **9** (supplement) 455s.
- ¹⁶ Schuetz K (2004) See Translating Basic Research into Patient Care: An Interview with Dr. Angela Brodie www.umm.edu/cancer/brodie.html (accessed 16 February 2007).
- ¹⁷ Coombes RC *et al* (2007) Survival and safety of exemestane versus tamoxifen after 2–3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial *The Lancet* **369** 559.
- ¹⁸ Piccart-Gebhart M *et al* (2005) Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer *New England Journal of Medicine* **353** 1659.
- ¹⁹ Bazell R (1998) *Her-2: The Making of a Revolutionary Treatment for Breast Cancer*, Random House, New York.
- ²⁰ FDA Centre for Drug Evaluation and Research (1998) FDA Oncology Tools Product Labels for Trastuzumab.
- ²¹ Friedberg JW (2004) Developing new monoclonal antibodies for aggressive lymphoma *Clinical Cancer Research* **10** 5297.
- ²² Hale G & Waldmann H (2000) From laboratory to clinic: the story of Campath-1, *Methods in Molecular Medicine* **40** 243.
- ²³ Bliss M (1983) *The Discovery of Insulin*, Paul Harris, Edinburgh.
- ²⁴ Gallo RC (2002) The Early Years of HIV/AIDS *Science* **298** 1728.
- ²⁵ *Ibid.*
- ²⁶ Rausch DM *et al* (1999) The SIV-infected rhesus monkey model for HIV-associated dementia and implications for neurological disease *Journal of Leukocyte Biology* **65** 466.
- ²⁷ Veazey RS *et al* (1998) Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection *Science* **280** 427.
- ²⁸ Goffinet C *et al* (2007) HIV-susceptible transgenic rats allow rapid preclinical testing of antiviral compounds targeting virus entry or reverse transcription *Proceedings of the National Academy of Sciences* **104** 1015.
- ²⁹ Ehringer H, Hornykiewicz O (1960) Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behaviour in diseases of the extrapyramidal system *Klinische Wochenschrift* **38** 1236.
- ³⁰ Cotzias GC *et al* (1967) Aromatic amino acids and modification of parkinsonism *New England Journal of Medicine* **276** 374.
- ³¹ Tropini G (2007) The shaking palsy: a review of Parkinson's Disease *The Science Creative Quarterly*, 16 January (<http://www.sq.ubc.ca/?p=632>, accessed 8 February 2007).
- ³² Langston JW, Palfreman J (1995) *The Case of the Frozen Addict* Pantheon Books, New York.
- ³³ Aziz TZ, Peggs D *et al* (1991) Lesion of the subthalamic nucleus for the alleviation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the primate *Movement Disorders* **6** 288.
- ³⁴ Clarke RH, Horsley V (1906) On a method of investigating the deep ganglia and tracts of the central nervous system (cerebellum) *British Medical Journal* **2** 1799.
- ³⁵ Speigel EA, Wycis HT, *et al* (1947) Stereotaxic apparatus for operations on the human brain. *Science* **106** 349.
- ³⁶ Salisbury DM, Begg NT (eds) (1996) *Immunisation against Infectious Disease*, HMSO.
- ³⁷ Sood S, Daum R (1991) In *Vaccines and Immunotherapy* edited by Cryz S, Pergamon, New York.
- ³⁸ Florey H (1953) The advance of chemotherapy by animal experiment, *Conquest*, **XLI** 4.
- ³⁹ Shope RE (1933) Infectious papillomatosis in rabbits *Journal of Experimental Medicine* **58** 607.
- ⁴⁰ Rous P, Beard JW (1935) The progression to carcinoma of virus induced rabbit papillomas *Journal of Experimental Medicine* **62** 523.
- ⁴¹ Rous P (1911) A sarcoma of the fowl transmissible by an agent separable from the tumour cells *Journal of Experimental Medicine* **13** 397.
- ⁴² Campo MS (2002) Animal models of papillomavirus pathogenesis *Virus Research* **89** 249.
- ⁴³ zur Hausen H (1977) Human papillomaviruses and their possible role in squamous cell carcinomas *Current Topics in Microbiology and Immunology* **78** 1.
- ⁴⁴ Boshart M (1984) A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer *The EMBO Journal* **3** 1151.
- ⁴⁵ Ferlay J *et al* (2004) *Globocan 2002: Cancer incidence, mortality and prevalence worldwide* International Agency for Research in Cancer (IARC) Press, Lyon, France.
- ⁴⁶ Koutsky LA *et al* (1988) Epidemiology of genital papillomavirus infection *Epidemiology Review* **10** 122.
- ⁴⁷ Swerdlow A *et al* (2001) *Cancer Incidence and Mortality in England and Wales. Trends and Risk Factors*, Oxford University Press.
- ⁴⁸ Vagelos PR (2001) Social benefits of a successful biomedical research company: Merck *Proceedings of the American Philosophical Society* **145** 575.
- ⁴⁹ Langworthy NG, Renz A *et al* (2000) Macrophilicidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship *Proceedings of the Royal Society B: Biological Sciences* **267** 1063.
- ⁵⁰ Saint André A *et al* (2002) The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness *Science* **295** 1892.
- ⁵¹ Scientists step closer to new treatments for river blindness (2006) University of Liverpool http://www.liv.ac.uk/newsroom/press_releases/2006/04/river_blindness.htm accessed 13 March 2007.
- ⁵² Bathurst I, Hentschel, C (2006) Medicines for Malaria Venture: sustaining antimalarial drug development *Trends in Parasitology* **22** 301.
- ⁵³ Jomaa H *et al* (1999) Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science* **285** 1573.
- ⁵⁴ Taylor WR *et al* (2003) Tolerability of azithromycin as malaria prophylaxis in adults in northeast Papua, Indonesia. *Antimicrobial Agents and Chemotherapy* **47** 2199.
- ⁵⁵ McCleod R *et al* (2001) Triclosan inhibits the growth of *Plasmodium falciparum* and *Toxoplasma gondii* by inhibition of apicomplexan FabI. *International Journal of Parasitology* **31** 109.
- ⁵⁶ Goonetilleke NP *et al*, (2003) Enhanced immunogenicity and protective efficacy against *Mycobacterium tuberculosis* of Bacille Calmette-Guérin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara *The Journal of Immunology*, 2003, **171** 1602; Williams A *et al* (2005) Boosting with poxviruses enhances *Mycobacterium bovis* BCG efficacy against tuberculosis in guinea pigs *Infection and Immunity*, **73** 3814; Verreck F *et al*, unpublished data.
- ⁵⁷ Waksman S (1954) *My life with microbes*. Simon and Schuster.
- ⁵⁸ http://nobelprize.org/nobel_prizes/medicine/laureates/1952/waksman-bio.html accessed 6 March 2007.
- ⁵⁹ Pethe K *et al* (2001) The heparin-binding haemagglutinin of *M tuberculosis* is required for extrapulmonary dissemination *Nature* **412** 190.
- ⁶⁰ McKinney JD *et al* (2000) Persistence of *M tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase *Nature* **406** 735.
- ⁶¹ Giacomini E *et al* (2001) Infection of human macrophages and dendritic cells with *M tuberculosis* induces a differential cytokine gene expression that modulates T cell response *Journal of Immunology* **166** 7033.
- ⁶² Peters W *et al* (2001) Chemokine receptor 2 serves an early and essential role in resistance to *M tuberculosis*. *Proceedings of the National Academy of Sciences* **98** 7958.
- ⁶³ Späth GF *et al* (2003) The role(s) of lipophosphoglycan (LPG) in the establishment of *Leishmania major* infections in mammalian hosts. *Proceedings of the National Academy of Sciences* **100** 9536.
- ⁶⁴ Porter R (1997) *Medicine: A History of Healing* Michael O'Mara Books.
- ⁶⁵ Blundell J (1828) Successful case of transfusion. *The Lancet* **1** 431.
- ⁶⁶ Landsteiner K (1930) On individual differences in human blood. Nobel Lecture. http://nobelprize.org/nobel_prizes/medicine/laureates/1930/landsteiner-lecture.html accessed 8 March 2007.
- ⁶⁷ Levine P, Stetson R (1939) An unusual case of intragroup agglutination *Journal of the American Medical Association* **113** 126.
- ⁶⁸ Landsteiner K, Wiener AS (1940) An agglutinable factor in human blood recognized by immune sera for rhesus blood *Proceedings of the Society of Experimental Biological Medicine* **43** 223.
- ⁶⁹ Lewisohn R (1915) Blood transfusion by the citrate method. *Surgery Gynecology & Obstetrics* **21** 37.
- ⁷⁰ Weil R (1915) Sodium citrate in the transfusion of blood *Journal of the American Medical Association* **64** 425.
- ⁷¹ Rous P & Turner J (1916) The preservation of living cells *in vitro* - Methods of preservation *Journal of Experimental Medicine* **23** 219.

- ⁷² Abel JJ *et al* (1914) On the removal of diffuse substances from the circulating blood of living animals *Journal of Pharmacology and Experimental Therapeutics* **5** 275.
- ⁷³ Beck E (1984) The treatment of thrombosis in *Discoveries in Pharmacology* Vol 2, ed Parnham MJ, Bruinvels J, Elsevier.
- ⁷⁴ Murray D *et al* (1937) Heparin and the thrombosis of veins following injury *Surgery* **2** 163.
- ⁷⁵ Livingston A (1983) from *Discoveries in Pharmacology* Vol 1 ed Parnham MJ, Bruinvels J, Elsevier.
- ⁷⁶ Rushman GB, Davies NJH, Atkinson RS (1966) *A Short History of Anaesthesia. The First 150 Years*, Butterworth Heinemann.
- ⁷⁷ Youngson A J (1979) *The Scientific Revolution in Victorian Medicine* Croom Helm.
- ⁷⁸ Corning JL (1885) Spinal anesthesia and local medication of the cord *New York Medical Journal* **42**, 483.
- ⁷⁹ Wiedling S (1952) The specificity and non-specificity of substances having local anaesthetic action *Acta Pharmacology et Toxicology* **8** 117.
- ⁸⁰ Lee WR *et al* (1971) An experimental comparison in dogs of expired air and oxygen ventilation during external cardiac massage *British Journal of Anaesthesia* **43** 38.
- ⁸¹ Lu QL *et al* (2003) Functional amounts of dystrophin produced by skipping the mutated exon in the mdx dystrophic mouse *Nature Medicine* **9** 1009.
- ⁸² *Ibid.*
- ⁸³ Sampaolesi M *et al* (2006) Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs *Nature* **444** 574.
- ⁸⁴ Herrick JB (1910) Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anaemia *Archives of Internal Medicine* **6** 517.
- ⁸⁵ Walters MC *et al* (1996) Bone marrow transplantation for sickle cell disease *New England Journal of Medicine* **335** 369.
- ⁸⁶ Pászty C *et al* (1997) Transgenic knockout mice with exclusively human sickle haemoglobin and sickle cell disease *Science* **278** 876.
- ⁸⁷ Pawliuk R *et al* (2001) Correction of sickle cell disease in transgenic mouse models by gene therapy *Science* **294** 2368.
- ⁸⁸ Zhenning HE, Russell JE (2004) Antisickling effects of an endogenous human-like globin *Nature Medicine* **10** 365.
- ⁸⁹ Chang JC *et al* (2006) Correction of the sickle cell mutation in embryonic stem cells *Proceedings of the National Academy of Sciences* **103** 1036.
- ⁹⁰ Snouwaert JN *et al* (1992) An animal model for cystic fibrosis made by gene targeting *Science* **257** 1083; Clarke LL *et al* (1992) Defective epithelial chloride transport in a gene targeted mouse model of cystic fibrosis *Science* **257** 1125; Dorin JR *et al* (1992) Cystic fibrosis in the mouse by targeted insertional mutagenesis *Nature* **359** 211; Colledge WH *et al* (1992) Cystic fibrosis mouse with intestinal obstruction *The Lancet* **340** 680.
- ⁹¹ Alton EW *et al* (1993) Non-invasive-liposome mediated gene delivery can correct the ion transport defect in cystic fibrosis mutant mice *Nature Genetics* **5**, 135; Hyde SC *et al* (1993) Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy *Nature* **362** 250.
- ⁹² Caplen NJ *et al* (1995) Liposome-mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis *Nature Medicine* **1** 39; Gill DR *et al* (1997) A placebo-controlled study of liposome-mediated gene transfer to the nasal epithelium of patients with cystic fibrosis *Gene Therapy* **4** 199; Porteous DJ *et al* (1997) Evidence for safety and efficacy of DOTAP cationic liposome mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis *Gene Therapy* **4** 210; Alton EW *et al* (1999) Cationic lipid-mediated CFTR gene transfer to the lungs and nose of patients with cystic fibrosis: a double-blind placebo-controlled trial *The Lancet* **253** 954; Hyde SC *et al* (2000) Repeat administration of DNA/liposomes to the nasal epithelium of patients with cystic fibrosis *Gene Therapy* **7** 1156.
- ⁹³ Naef AP (2004) The mid-century revolution in cardiovascular and thoracic Surgery: Part 6 *Interactive Cardiovascular and Thoracic Surgery* **3** 535.
- ⁹⁴ Freeman RV, Otto CM (2005) Spectrum of calcific aortic valve disease: pathogenesis, disease progression and treatment strategies *Circulation* **111** 3316.
- ⁹⁵ UK Heart Valve Registry (2007).
- ⁹⁶ Frater RWM, Ellis FH Jnr (1961) Problems in the development of a mitral valve prosthesis. In *Prosthetic valves for cardiac surgery*, 244. Ed Merendino KA, Thomas Springfield.
- ⁹⁷ Paton B (1972) in *Biological Tissues in Heart Valve Replacement* Edited by Ionescu M, Ross D, Wooller G, Butterworths, London.
- ⁹⁸ Hayashida K (2006) Presentation, American Heart Association Scientific Session.
- ⁹⁹ British Medical Association (2004) *Human tissue and organs – shortages*. Briefing paper.
- ¹⁰⁰ Department of Health (2006) Xenotransplantation guidance, Gateway reference number 7345.
- ¹⁰¹ Cardona K *et al* (2006) Long term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways *Nature Medicine* **12** 304.
- ¹⁰² Draznin B, Leroith D (1994) *Molecular biology of diabetes: autoimmunity and genetics; insulin synthesis and secretion*. Totowa, New Jersey: Humana Press.
- ¹⁰³ European Medicines Agency (2006) *European Public Assessment Report: Atryn* EMEA/H/C/679.
- ¹⁰⁴ Echelard Y *et al* (2006) Production of recombinant therapeutic proteins in the milk of transgenic animals. *BioPharm International* **19** 36.
- ¹⁰⁵ Lillico SG *et al* (2007) Oviduct-specific expression of two therapeutic proteins in transgenic hens *Proceedings of the National Academy of Sciences* **104** 1771.
- ¹⁰⁶ Raisman G (1985) Specialised neuroglial arrangements may explain the capacity of vomeronasal axons to reinnervate central neurons *Neuroscience* **14** 237.
- ¹⁰⁷ Li Y *et al* (1998) Regeneration of adult rat corticospinal axons induced by transplanted olfactory ensheathing cells *Journal of Neuroscience* **18** 10514.
- ¹⁰⁸ Keyvan-Fouladi N *et al* (2003) Functional repair of the corticospinal tract by delayed transplantation of olfactory ensheathing cells in adult rats *Journal of Neuroscience* **23** 9428.
- ¹⁰⁹ UK Stem Cell Foundation funds heart repair project (2006) http://domain883347.sites.fasthosts.com/news/news_items/item23.html accessed 13 March 2007.
- ¹¹⁰ Johnston P (2006) Findings presented to the American Heart Association, Chicago.
- ¹¹¹ Hurst JW (1986) The first coronary angioplasty as described by Andreas Gruentzig *American Journal of Cardiology* **57** 185.
- ¹¹² MacLaren RE *et al* (2006) Retinal repair by transplantation of photoreceptor precursors *Nature* **444** 203.



In 2008, RDS and CMP merged to form Understanding Animal Research
www.UnderstandingAnimalResearch.org.uk info@uar.org.uk

