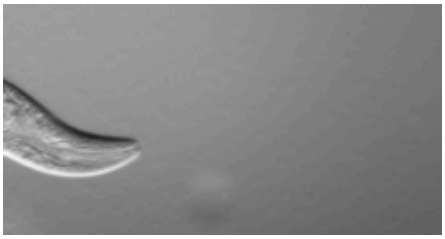
C. elegans (nematode worm)

Nematode worms are now used extensively in biological research, the most commonly used being the tiny roundworm *Caenorhabditis elegans*. It is easy to breed, has a well studied genome, and many generations are born in a time-frame of days. It is susceptible to environmental changes and mutations, and the effects of these can be seen on later generations within a short experimental timeframe. *C. elegans* has a small nervous system, with only 302 neurons making known synaptic connections. This tiny nervous system carries out many of the same functions as the nervous systems of higher organisms, and is often studied as a model to help understand the basic mechanisms behind complex behaviours.



Crawling *C. elegans* hermaphrodite worm

The discovery of novel antibiotics

C. elegans are used to identify chemicals with antibiotic properties through screening a vast array of possible candidates. The process involves infecting worms by laying them in a dish of pathogenic bacteria for 15 hours before a particle sorter is used to drop a precise number of the infected worms into small wells. Each of the 384 wells on a plate is loaded with a different, potentially-antibiotic chemical. The particle sorter distributes 15 worms into each well, and these are left for five days before they are examined to determine whether the worms survived their infection, which would indicate they were in contact with an antibiotic.

A new process involves exposing the worms to an orange dye at the end of five days. This dye is able to enter dead cells but not living ones, enabling researchers to identify the living worms more easily. The process of checking the wells can be easily automated as differences in contrast can be measured busing a camera and computer, in a process which allowed the team who developed it to identify 28 antibiotics (of 37,000 compounds tested). Importantly, some of these compounds work by different mechanisms to existing antibiotics, allowing them to bypass existing bacterial resistance.

How sensory input leads to behaviour

A team have investigated how *C. elegans* 'smells' food, triggering receptors, which in turn activate particular nerve pathways and lead to certain types of movement, enabling the worm to reach its food source. Despite the clear differences, this particular piece of 'circuitry' shares many features with the way that the retina senses light in mammals, and how this information is used by the brain to initiate other tasks.[1]

Lamarck's blacksmiths and the worm's genes

In the late 18th and 19th centuries it was observed that the son's of blacksmiths had stronger arms than the sons of weaver. Now, if you'd asked a biologistto explain that observation today you'd probably get a series of questions such as who observed this? Where did they grow up? What did the mother look like? What is the sample size? Or even, can we swap the children at birth?...

The muscles of the blacksmiths and their sons are responding to their environment, and although this represents quite a trivial example of such an interaction the phenomenon is widespread and there are many fascinating examples to be found in nature. For example, C Elegans has a quite

striking interaction with its environment, such that under conditions unfavourable for reproduction, instead of developing as an adult it can go into a form of stasis, developing into an arrested stage that is both environmentally resistant and long lived. Once environmental conditions improve the arrested worm resumes development, growing into a perfectly normal adult.

Extract from the full article in University of Bristol, Research News, May 2005.

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Tags

Research Fields: Anatomy & development, Biochemistry, Cell biology, Evolution & environment, Genetics, Infection and Immunity, Medical technologies, Disease characteristics, Brain & nervous system, Drugs & toxins

Animals Used: Nematode worm

Medical Applications: Basic research

Decapod Crustaceans

Decapods are the order of invertebrate crustaceans which includes crayfish, crabs, lobsters, prawns and shrimps. They are useful and appropriate models for many areas of biological research, and are also important to many economies as highly-valued edible shellfish.

© Andrew Simpson

Decapods and the economy

Global shellfish production is worth well over US $300 billion per year and shellfish farming is the fastest growing sector of the aquaculture industry worldwide. Decapod crustaceans account for about one third of this. Lobster production alone is valued at £30 million per year, with crab and cold water shrimp representing other important species.

One of the greatest problems facing aquaculture of shellfish is the control of infectious diseases. This problem is compounded by the fact that decapods, in common with other invertebrates, do not have the complex immune systems that permit the use of conventional 'antigen-antibody' vaccines used for humans and other mammals. Instead they rely on simpler inflammatory type responses that have no 'immune memory' component that would facilitate prolonged protection by specific vaccines.

At present there no good alternatives to live animal experiments to find solutions to this problem because there are no 'standard' cell lines available for 'replacement' *in vitro* work and there are no suitable alternative ethically acceptable un-regulated 'models'.

How do decapods differ from mammals?

Invertebrates are those animals without backbones and include many phyla with worm-like bodies as well as those with bodies covered by hard material. Most invertebrates are protostomes, whilst all vertebrates, including humans are deuterosomes. These two branches of the animal kingdom are defined by differences in their early embryonic development, indicating that they separated early in evolution. They have fundamentally different physiology, which makes it difficult to draw direct comparisons between their nervous systems and sensory organs.

Their small nervous systems can be compared with those of fish, which have the smallest brains among vertebrate species. While most vertebrates are thought to have some sort of consciousness, this is uncertain in both fish and invertebrates.

Arthropods are the most diverse phylum of the animal kingdom, and include insects, crustaceans and spiders. The basis for proposed legislation to protect the more complex decapod crustaceans, is that they may be capable of pain and suffering.

Decapod nervous systems

Crabs and lobsters have about 100,000 neurons, compared with 100bn in people and other

mammals. While this allows them to react to threatening stimuli, there is no current evidence that they feel pain. Their nerves are more primitive, lacking the myelin coating which allows fast conduction of signals (pain signals in humans travel exclusively through 'fast' fibres). The nerves of decapods therefore conduct at around 1ms-1, compared with 100ms-1 at which pain is conducted in mammals.

There is good evidence to suggest that decapods don't experience sensation the way we do. Their small number of neurons means they may not be able to be able to sense much at all. Additionally the organisation of their nervous system is very different from that of vertebrates: rather than having a spinal cord and brain where inputs from the sensory system are integrated, lobsters have a simple, system consisting of several discrete paired **clusters of nerve cell bodies** which are fused to a nerve cord which runs the length of the body. The largest of these ganglia control the mouthparts. In crabs, which have short bodies, the ganglia which would run through the thorax are fused into a single mass and there are fewer abdominal ganglia.

Do decapods feel pain?

This question is often debated by policy makers responsible for regulation of the food industry and science. It is important that animals are killed in a humane way and do not suffer, but the differences between decapod and human nervous systems complicate the issue.

Some recent research has focused on this debate. For example a study shows that crabs respond to and move away from an electric shock stimulus[1]. However, observations in humans with injured spinal cords and the experiments on animals tell us that you cannot infer that a vertebrate can "feel" anything simply by observing that it responds with movement to a stimulus.

Invertebrates use opioids as chemical transmitters, and this has also been cited as an indication of their capacity to feel pain. In mammals opioids form part of the pain pathway. They are released in response to pain signals in the spinal cord travelling through the spinal cord. Opioid transmitters administer the influence of higher brain functions on pain, ensuring that the severity of the pain is appropriate to the situation. The analgesic, morphine, acts on this system to relieve pain, and its effects are reversed by the opioid antagonist, naloxone.

Crabs respond with a threat display in response to electric shocks[2], or to striking the shell between the eyestalks[3]. Both of these experiments showed that the behaviour could be eliminated by administration of morphine and reinstated by injection of naloxone. It is difficult to know whether this implies that the stimulus is painful, but it is likely that the display is a simple response to a perceived threat in the environment rather than a sign of distress.

Neurochemicals often have very different roles in vertebrates and invertebrates, and their presence does not necessarily imply the perception of pain, since invertebrates lack the higher brain centres which suppress pain in mammals. In fact opioids are found all over the animal kingdom, in insects as well as crustaceans and mammals. Opioids have therefore governed responses to sensory input since early in evolutionary development, but all animals using opioid neurotransmitters are not necessarily able to experience pain.

We shouldn't lose sight of the fact that plants can sense and respond to innocuous and noxious stimuli. They can also communicate with members of their own species, as well as with plants of other species, sometimes miles away, yet we do not assume this to be evidence of sentience or their capacity to feel.

It is a huge stretch to extrapolate 'feelings' from humans to invertebrates when the two nervous systems are so different and pain is so subjective. The killing of crabs and lobsters by boiling them alive by the food industry presents an example of how our intuitive understanding of pain may not apply. The nerves of a crab from UK seawater (8-14˚C) fail irreversibly above 25˚C, a temperature reached very quickly in boiling water. This method of killing, is very rapid and humane, whereas freezing the animals slowly, as many people intuitively feel would be preferable, actually prolongs their death.

A report to the Norwegian Committee for Science and Food Safety [4], examined Sentience and Pain in Invertebrates. It concluded that there is little knowledge about the capacity for sentience in crustaceans and that their nervous and sensory systems appear to be less developed than those of insects. While lobsters and crabs have some capacity for learning, it is unlikely that they can feel pain.

Biomedical research

Advances in knowledge depend on scientific and biotechnological advances facilitated by basic research into decapod physiology, immunology, pathology, reproduction and endocrinology, and neurobiology amongst others. All entail to a greater or lesser extent on animal experimentation to understand basic biological processes, to develop veterinary products and find new biomedicines. Examples of important findings made from these animals are given below.

Neurology

The first clear demonstration of electrical, as opposed to chemical, synapses in the transmission of nerve impulses was made in crayfish. This work showed that **synapses** are not solely dependent on impulse transmission by **neurotransmitters** but can pass the junction simply by charge. This initial discovery has now effectively changed and extended our knowledge of brain function in humans. This landmark work still has enormous implications for tackling serious human illnesses, such as Alzheimer's and Hodgkin's Diseases as well as stroke and other paralysis conditions.

The existence of the important neurotransmitter **GABA** was also first demonstrated in crayfish. GABA is the most important inhibitory neurotransmitter in the central nervous system (CNS). In humans 60-75% of all synapses in the CNS respond to GABA. It is far less dominant in crayfish, because these invertebrates are neurologically far less complex, but during evolution its role in higher mammals has diversified and amongst other activities, GABA in humans is now known to regulate, mood memory and pain. It is the target of many pain-relieving drugs or anesthetic used in clinical medicine. Knowledge of the key role of this compound has had an enormous positive impact on human wellbeing.

Crayfish were also the experimental models in which behavioural-command neurons were discovered. These are important neurons which singly can stimulate quite complex patterns of behaviour. This has radically changed our understanding of human behaviour, in relation to addiction or certain types of drugs.

More recently, in the 1990s, neurobiological work on decapods has revealed that the level of **serotonin** (another very important neurotransmitter in humans) varies with dominance or social status. This discovery has led to the acceptance that not all individuals, even in higher animals, including humans, will respond identically to certain drugs or treatments but rather to according to the chemical status of their brain (mood, confidence, social standing and well being). This has revolutionised approaches to treatments of certain conditions, especially in elderly, depressed or young individuals.

Studies on limb and neural regeneration in decapods (crayfish and lobsters) as experimental models continue to inform us about basic biological processes that have relevance (and possibly biotechnological application) for recovery from trauma or stroke, or in coping with human disease states such Alzheimer's and other dementias.

Biochemisty

Landmark work on decapod immunity includes the discovery of a complex recognition and regulatory enzyme cascade in decapods, which bridges the complement system of more advanced organisms and the clotting systems of simpler ones. This work was the first to reveal the biochemical events that constitute the inflammatory pathways in arthropods. It opened up the work on insects that permitted genetics to be used to decipher complex pathways and signalling systems in fruit flies (relatives of decapods), and is the basis on which disease control strategies in aquaculture are being explored.

There have also been important discoveries of novel families of natural inducible antimicrobial proteins from decapods, many of which are salt-tolerant and active against some important human pathogens and even viruses. Most existing antibiotics are inactivated by salt (e.g. in sweat), so these natural proteins from marine animals have potential for exploitation as topical (skin surface) antiseptics.

Recently the implantation of certain genes into live crustaceans has been achieved. This technique now allows us to find ways to improve traits, such as disease resistance, colour, taste, growth rate and size in decapod shellfish reared in aquaculture. It could also be used to prevent the animals making the allergens to which so many people are sensitive and whose lives are put at risk from the presence of traces of decapod muscle in pre-prepared or restaurant foods.

Research has shown that many compounds marketed to shellfish producers as 'immune stimulating' have no real benefits in prolonging survival of infection. They appear to be a waste of money and when used routinely induce 'immune-fatigue' that impacts negatively on the wellbeing

of the stock.

Conservation and environment

Pathogen experiments on crayfish have demonstrated that some benign microbial commensals of exotic decapods, introduced for aquaculture purposes, jump host to native species that have no natural resistance to these bacteria. The effects on native crayfish populations are devastating. A good example is the near eradication of the UK crayfish by a fungal pathogen carried by imported Signal crayfish and transmitted into British waterways in the 1980s. The massive reduction in populations of the native crayfish has meant that waterways previously kept clear by their grazing activities have become choked with algae to the point of **anoxia**. This has had adverse knock-on effects to the small fish that populate the waterways, and Wetland birds that feed on them have suffered in turn.

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May 2009

Tags

Research Fields: Biochemistry, Infection and Immunity, Brain & nervous system

Animals Used: Crustaceans

Medical Applications: Medicine, Environment, Basic research

Drosophila melanogaster

Drosophila melanogaster, or fruit-fly is widely used in scientific and medical research. This 3mm-long insect usually accumulates around spoiled fruit. It has been used in genetics and developmental biology for almost a century, and today several thousand scientists are working on many different aspects of its biology.

Drosophila melanogaster
© istockphoto/janeff

The importance of Drosophila as an animal model was realised by Thomas Hunt Morgan, who was awarded the 1933 Nobel Prize for physiology or medicine after demonstating that genetic information is carried on chromosomes using drosophila. Since then this tiny insect, which breeds rapidly and is easily kept in a labaoratory, has performed a crucial role in genetics research.

Its importance for human health was recognised more recently by the award of the Nobel prize for medicine in 1995, for work on the genetic control of early embryonic development. Mutant flies with defects in any of several thousand genes are available, and the entire genome has recently been sequenced.

Drosophila helped in the development of drugs to combat pathogens responsible for a range of diseases from skin infections to pneumonia and meningitis. Recent research with fruitflies has focused on the pathology of Alzheimer's disease, for although the flies have a very simple brain they have highly developed muscles and nerves.

Tags

Research Fields: Anatomy & development, Evolution & environment, Genetics, Brain & nervous system, Drugs & toxins

Animals Used: Drosophila (fruit fly)

Medical Applications: Basic research

Zebrafish

The zebrafish (*Danio reiro*) has many features which make it an excellent model organism for studying development in vertebrates. The embryos develop externally to the mother and are transparent, so they can be easily viewed and manipulated.


Zebrafish in an aquarium

Compared to frogs the organization of the zebrafish embryo is simple, and they develop more quickly. Zebrafish grow to maturity and are able breed within 2 to 3 months. They also produce large numbers of offspring – a female zebrafish can lay up to 200 eggs a week.

Like the mouse, the zebrafish is suitable for genetic analysis, and is a valuable tool for creating genetic models of human diseases. The sequencing of the zebrafish genome began in 2001, and is currently ongoing.[1] Although the zebrafish genome is only half the length of the human genome, the genetic structure is remarkably similar. Genes responsible for human diseases often have equivalents in the zebrafish.

Creating transgenic models

It is easy to produce mutations in zebrafish, and screening programmes have been developed to find mutations which affect particular biological systems, such as the development of the nervous system. Transgenic zebrafish were cloned from cells cultured for three months in 2002.[2] Zebrafish mutants are currently used to model many human diseases, including Alzheimer's disease, congenital heart disease, polycystic kidney disease and some cancers.

ZF-models – zebrafish models for human disease is a project funded by the European commission, which began in 2004. Its aim is to use the zebrafish to produce new disease models, find new drug targets and learn more about the gene-regulation pathways involved in human development and disease. Among its research the project aims to produce 180 knockout strains of zebrafish, which will help understanding and modeling of human diseases.

Leukaemia

Using genetic techniques, a team of researchers stimulated the development of a type of leukaemia - T cell acute lymphoblastic leukaemia - in the fish.[3] They fused the gene *Myc*, which plays an important role in human leukaemia and lymphoma, to a zebrafish gene that works exclusively in lymphoid cells - which become diseased in leukaemia. They then tagged the fused gene with a third gene which caused leukaemia cells to glow green under fluorescent light, so they could observe the cancer as it progressed. The three-gene combination was then injected into embryonic zebrafish, so that the genes were incorporated in all developing fish cells. Cancer developed in virtually all fish that carried a functional *Myc* gene.

Creating zebrafish which develop leukaemia will enable researchers to screen thousands of zebrafish genes for mutations that contribute to the disease, and to test the effect of various anti-cancer agents.

The development of the heart

The transparent zebrafish embryo allowed researchers to study its beating heart, predicting that certain blood flow patterns in the organ are key to its normal, healthy development.[4] After

measuring the velocities and patterns of blood flow, the team calculated the expected forces imposed on the heart chamber walls by the flowing blood. They surgically blocked the incoming or outgoing blood flow and found that when flow was reduced, the smaller forces on the heart wall resulted in drastically altered development in the chambers, valves and orientation of the heart. Many of these changes were similar to those seen in cases of congenital heart disease and in zebrafish lacking key genes for heart development. The early development of the heart is similar in all vertebrates and these findings are likely to also be true of human embryos.

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Tags

Research Fields: Anatomy & development, Biochemistry, Cell biology, Evolution & environment, Genetics, Brain & nervous system

Animals Used: Zebrafish

Medical Applications: Medicine, Basic research

Frog

Frogs have been used as research models for many years. Their physiology is relatively simple when compared to mammals, and the study of frog muscles in the 1920's led to the discovery that the neurotransmitter acetylcholine was responsible for relaying nerve impluses signaling movement to the muscles.

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Frog's eggs are so large and resilient that they are often used to train cell biologists in genetic manipulation and other physiological techniques. It is possible to clone a frog easily, by using a syringe to carefully remove the nucleus, containing genetic material from one egg. The nucleus of another cell can then be injected it into the egg, where it will divide and eventually grow into a frog.

Studying embryonic development

The African clawed frog, *Xenopus laevis*, is often used in early studies of development. They produce very robust embryos, which can be easily observed at all stages. They have been particularly useful for studying very early events, such as the formation of the neural plate, which develops into the nervous system.

Although frog eggs, particularly *Xenopus laevis*, are relatively easy to manipulate, it is a tetrapoliod species - one whose cells have four copies of each chromosome. Most vertebrates, including humans, are diploid - having two copies of each chromosome. When genetics is an important part of the study the closely related species, *Xenopus tropicalis* is the prefered species.[1] These frogs have a shorter breeding cycle, and have diploid cells, so the study results can be compared with other species more easily.

A new type of antibiotic?

Another perculiarity of frogs which of interest to science is their ability to survive in bacteria-filled water without wounds becoming infected. Studying this property of *Xenopus laevis*, Michael Zasloff, discovered a new class of antibiotic compounds in 1987.[2] Their skin contains natural antibiotic peptides which he called 'magainins'. They are active against many disease-causing organisms, and may provide a future solution to the problem of antibiotic-resistant bacteria. They also present new questions about the immune system and whether higher animals have a similar front-line chemical defence against disease.

Transparent frogs

Recently, the first transparent animal with four legs was developed a Horoshima University in Japan. Japanese brown frogs *Rana japonica,* were bred, selecting for the recessive trait of light-coloured skin, until fully transparent frogs were born. The internal organs, eggs and other internal parts of the frog can be clearly viewed through the skin, making it a great model for studying many aspects of physiology in a whole, living body. By fusing genes for fluorescent proteins to the frog's genes, the research team responsible for this development hope to create frogs that glow. Glowing frogs help in the study of specific genes as the frogs will glow, providing a visual indication, when those genes become active.

"Transparent frogs will prove useful as laboratory animals because they make it easier and cheaper to observe the development and progress of cancer, the growth and aging of internal organs, and the effects of chemicals on organs." Masayuki Sumida, Hiroshima University

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Tags

Research Fields: Anatomy & development, Biochemistry, Cell biology, Evolution & environment, Genetics, Medical technologies, Drugs & toxins

Animals Used: Frog

Medical Applications: Medicine, Basic research

Quail

Over the last 50 years quails have proved research animals. The most commonly used is the Japanese quail, *Coturnix japonica*. It's relatively short lifespan and physiological similarities to humans make it useful in the study of aging and disease while its 16-day developmental period and easily accessible embryo make it a suitable model for developmental biology.

Background

The Japanese quail is considered a separate species from the common quail, *Coturnix corturnix* which is found throughout Europe, Asia, Africa and India, and is not a close relative of either the American bobwhite quail, *Colinus viginianus* or the Californian quail *Lorphortyx california*. The species is thought to have developed through the domestication of the common quail in China during the 11th Century, and to have been brought to Japan in the 12th Century, where they were bred as domestic songbirds. During the 1900s quails were bred in Japan for their eggs and meat, and while these strains were largely lost during the Second World War, new domesticated lines have been re-established, and are now used in laboratories all over the world.

C japonica was first described as a research model by Padgett and Ivey in 1959[1], who saw it as a practical animal for the study of development. In 1961 they published a detailed developmental atlas of the quail, charting the stages of quail development. There are now detailed studies of quail histology, anatomy and breeding that provide reliable reference texts for researchers, and the Japanese quail has become a common laboratory species. The American bobwhite quail is also frequently used in toxicity studies, particularly of chemicals which are intended for use, and which will therefore have environmental impact in North America.

Developmental studies

Unlike rodents, avian embryos can be easily studied and manipulated as they grow by removing a small section of the eggshell. This has made it possible to follow their development using time-lapsed video-microscopy, and watch the embryo as it grows[3].

The chicken and quail are both warm-blooded vertebrates which develop similarly to humans. The early development of quails is very like that of chickens, but the nucleus of quail cells contains condensed heterochromatin (a portion of the material making up the chromasomes) which is not found in chicken cells. This fundamental difference in makeup can be used to distinguish between tissue from the two species when tissue is transplanted between developing quails and chickens, to create a quail / chicken chimera. It has made the quail / chick chimera a successful model for determining the fates of particular cells as they develop[2].

Transgenic bird models

The quail has recently been used as a successful transgenic model for the production of a transgenic bird. A transgene coding for a green fluorescent protein maker was introduced into freshly laid eggs, which were then incubated until hatching. The fluorescent protein was visible in the offspring for several generations, showing that the new gene had been successfully introduced into the birds[4,5]. The quail is a suitable species for creating transgenic birds. It has a hardy embryo, which survives the introduction of the new transgene well.

Environmental Studies

Quails are often used in environmental toxicity testing. They eat many kinds of seeds and are used in palatability studies, which show the likelihood of, for example, a new pesticide, being eaten by birds. These birds also eat a variety of worms and insect larvae, and may be used to study the potential effects of a chemical substance on the food chain.

Their eggs can be grown in large batches in incubators, and allow the effects of particular substances on embryonic development to be studied. They are important models for reproductive studies that look at the effects of chemicals on the environment.

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Tags

Research Fields: Anatomy & development, Evolution & environment, Genetics, Medical technologies

Animals Used: Quail

Mouse

Mice, rats and other small rodents make up the majority of animals used in medical research, biological research and toxicity testing in the UK. Of these, most are mice representing 69% of the total animals used in 2006. They are small, inexpensive and easy to handle, making them ideal candidates for laboratory experiments. Their short life span and fast reproductive rate, makes it possible to investigate biological processes in many subjects, at all stages of the life cycle.

White mouse
© istockphoto/Brandon Laufenberg

The mouse makes an excellent model for human disease because the organization of their DNA and their gene expression is similar to humans, with ninety-eight percent of human genes having a comparable gene in the mouse. They have similar reproductive and nervous systems to humans, and suffer from many of the same diseases such as cancer, diabetes and even anxiety. Manipulating their genes can lead them to develop other diseases that do not naturally affect them, and as a result research on mice has helped the understanding of both human physiology and the causes of disease.

Mice are used in a vast range of experiments, many of which are classified as fundamental research, investigating the physiology of mammals. Inbred strains of mice were used as disease models, long before the mouse genome project and transgenics. There are a large number of laboratory strains available, and their long breeding history means that mice of a single laboratory strain are **isogenic**. This is useful in experiments, as it reduces natural variation between subjects. Some inbred strains are used for their predisposition to certain mutations or genetic diseases, while others are used for their general health and resistance to mutations.

It has been possible to clone mice since 1998.

Examples of inbred mouse strains

- NOD mice develop **type 1 diabetes**.
- Mice have been developed with unusual regenerative capacities.
- Waltzing mice have a mutation affecting their inner ear which causes them to walk in circles.

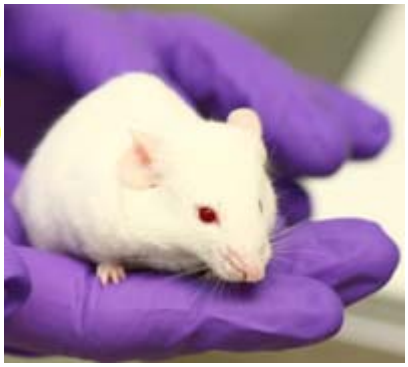
Tags

Research Fields: Anatomy & development, Biochemistry, Heart, lung & circulation, Cell biology, Endocrinology (hormones), Evolution & environment, Genetics, Infection and Immunity, Medical technologies, Disease characteristics, Brain & nervous system, Cancer research, Drugs & toxins, Psychology & behaviour, Bones & muscles

Animals Used: Mouse

Medical Applications: Vaccine, Surgery, Medicine, Basic research

Mouse (GM)



Following publication of the sequence and analysis of a mouse strain in December 2002[1] the mouse became the animal model of choice for most laboratory experiments. The potential of mice for genetic manipulation means that their use is now often favored over rats and other rodents both in safety testing and in fundamental research.

The mouse makes an excellent model for human disease because the organisation of their DNA and way their genes are expressed is very similar to humans. Their reproductive and nervous systems are like those of humans, and they suffer from many of the same diseases such as cancer, diabetes and even anxiety. Manipulating their genes can lead them to develop other diseases that do not naturally affect them, and as a result research on mice has helped understanding of both human physiology and the causes of disease.

Before genetic technology, mice were inbred to produce laboratory strains with particular characteristics. These inbred strains are very genetically similar, which makes them ideal for studying changes due to genetic modification.

Statistics

Our ability to manipulate their genes has made the mouse the most commonly used laboratory animal. In the UK their use has increased over the last 10 years, and a 5% increase was reported from 2005 to 2006. Most of these mice were used for breeding programmes and in fundamental biological research.[2] The recent increases in the number of mice used directly result from the development of new technologies that allow manipulation of their genes.

What is a transgenic mouse?

A transgenic mouse is one whose chromosomes have been altered so that its genes contain foreign DNA. These genes are found in the nucleus of every cell of the body, so all of the mouse's cells contain the new DNA. The foreign DNA can come any source, and may be human, from another animal or from another mouse.

The change in DNA usually means the cells gain a function, such as producing a new protein. For example, some transgenic mice produce contain proteins recognised by human immune cells, and these can be used to model particular aspects of disease. The foreign DNA can sometimes mean a loss, rather than a gain in function as the new DNA might interfere with a biochemical pathway or prevent production of a particular protein.

Transgenic mice are useful models for understanding how genes regulate processes in the body, because the effect of changing a particular gene can be seen on the whole organism. They are also used to study human diseases which are caused by 'errors' in the way that the body produces

certain proteins, for example in Haemophilia A, the crucial gene codes for a protein known as factor VIII which is needed for blood clotting.

Making transgenic mice

The two main techniques for introducing the foreign DNA into the mouse are either through pronuclear injection, or use of embryonic stem cells.

In pronuclear injection the foreign DNA is injected into the pronucleus of a mouse egg, which forms just after it has been fertilized. The foreign DNA integrates into the genome at a random position, usually after the first one or two cell divisions have occurred. This means that the mouse will not carry transgenic DNA in all of its cells, and so will only be partially transgenic. The transgenic eggs or sperm from these mice are then used to create the next generation of fully transgenic mice.

When DNA is introduced into embryonic stems cells it usually integrates randomly into the genome, but if it has a similar structure to an existing part of the genome it can be 'recognised' by the DNA – so that it undergoes homologous recombination, and a single copy becomes integrated into the genome at a specific location. These embryonic cells then need to grow, and are injected into a host embryo, becoming part of the mouse which grows from that embryo. The mouse grown from the host embryo is known as a chimera, and is formed from the embryonic cells of two different mice. Some of the sperm produced by the chimera will be transgenic – containing the foreign DNA, and when these sperm fertilise a normal egg, the mouse which grows from it will be fully transgenic with foreign DNA in every cell.

Examples of transgenic strains

- Large mice, with a rat hormone gene which makes them grow bigger than usual, and are used to study growth and development.
- Oncomice, have an inactivated oncogene, and are predisposed to developing cancer. These mice have been vital to the understanding of many cancers and the development of technologies to treat them.
- Doogie mice show improved memory and capacity for learning. These mice have enhanced function at NMDA receptors, which are needed for the brain to store new information.

Knockout mice

The more recent development of knock-out (or knock-in) strains of mice during the 1980's was a major advance for genetics. This technology allows particular genes on the DNA strand to be altered, usually removed, but they may also be inactivated or inserted. This allows researchers to determine the exact function of a particular gene, and these GM mice have provided excellent models of many human diseases, which could not be studied in animals before. The sequencing and analysis of the mouse genome has allowed many genes to be targeted and studied using this technology. The creators of the first knockout mice were awarded the 2007 Nobel Prize in medicine.

The background to gene-targeting

The technique which led to the creation of knockout mice was developed in bacteria by Joshua Lederburg, who received the Nobel Prize for his discovery in 1958. He discovered that bacterial strains could be crossed to give offspring with their own unique genetics, similar to sexual reproduction. This meant that when x-rays were used to produce mutations in their genetic structures, these changes could be passed on. Lederburg was first to describe the process of homologous recombination in bacteria, where chromosome pairs exchange genetic material, and found that during recombination, other pieces of genetic material in the bacterial body could be integrated into the genetic structure.

Two scientists in the USA, Mario Capecchi and Oliver Smithies were both working on ways to alter specific sequences on the mammalian genome. They both realised independently that Lederburg's technique could be used to introduce mutations into mammalian genes. Meanwhile, Martin Evans work on embryonic stem-cells provided a means to introduce the mutations into a living animal by altering the stem-cells, and then injecting them into a fertilized mouse egg.

Creating knock-out mice

Knockout and knock-in mice are produced by gene-targeting. This technique allows a specific gene on the mouse genome to be altered, by replacing it with a similar genetic sequence which has been modified to contain a mutation. The mutation often prevents the gene from functioning. When genes are knocked-in a mouse gene is often replaced by a similar gene from the human genome.

The mutant gene is created in a bacterial **plasmid**, which is injected into mouse embryonic stem cells, usually from a male mouse. These cells are from a very early mouse embryo, and will divide to form every type of cell in the body. The aim is for the mutant genetic material from the plasmid to form DNA in the sperm of the mouse when it is fully grown. Once the plasmid is inside a stem cell the two similar DNA sequences exchange genetic material by homologous recombination, which swaps the new, mutant-gene into the mouse genome. These stem cells are then implanted into a host embryo to grow. Mice with different coloured fur are usually selected as the hosts, so that it is clear which mice have the mutant genes.

When the mouse is born, it will be a chimera, as only some of its cells will be modified. This mouse will have fur of both colours. When sperm from the chimeric mouse fertilise a normal egg, some of its offspring will carry a single copy of the mutant gene. These mice will have the same coloured fur as the mouse whose DNA was altered originally.

Inbreeding between these mice gives offspring with two copies of the mutant gene. These are knockout mice.

Examples of knockout mice

There are many examples of knockout mice, as this technique has been used to study all aspects of physiology and to create models for many human diseases.

- Fat mice, prone to obesity due to a carboxypeptidase E-deficiency.
- Strong mice, with a disabled myostatin gene.
- Cold-tolerant mice, lacking a sodium channel which causes pain when exposed to cold.

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Tags

Research Fields: Genetics

Animals Used: Mouse (knockout/GM)

Medical Applications: Basic research

Mouse (immunodeficient)

Two major inbred strains of immuno-deficient mice are used to study the immune system and transplantation. These are nude mice, and SCID mice.

Nude mice

A nude mouse is an inbred strain whose thymus gland is missing or damaged, resulting in a deficient immune system. They have a very small number of T-lymphocytes, which are essential to the immune response. These mice are called “nude” because they also lack fur. With a very limited immune system, the nude mouse is able to receive many tissue and tumor grafts from many different species without suffering a rejection response. They are often used to grow grafted tissue, and are used in research to test new methods of imaging and treating tumors.

Nude mice were first discovered by Dr. N. R. Grist in 1962 at the virus laboratory at Ruchill hospital, Glasgow. They are pink-skinned and hairless, with oversized ears. Without a thymus gland, these mice are unable to kill virus infected or malignant cells, form most antibodies, reject tissue grafts or develop hypersensitivity responses – all of which require T-lymphocytes. The adaptive immune response of nude mice is so limited that they are even able to accept grafted tissue from other species. This makes them a vital model for cancer research, as they allow the study of human tumors, which retain their original characteristics.

Rosalyn Yalow realized that nude mice could be created using a radioimmunoassay techniques to remove the gene required for the thymus growth factor hormone. She was awarded the Nobel Prize in medicine for this discovery in 1977. An alternative method of creating nude mice is to remove the thymus from the mouse completely within 24 hours of birth. Nude mice are usually bred from the inbred strain, rather than created artificially by either of these methods.

In the early 1970s researchers saw the advantages that this model had to offer, but because their immune response was badly compromised, nude mice were prone to infections and often died before experiments were completed. Using sterile techniques to handle the mice, John Stehlin and Beppino Giovanella at the Stehiln Foundation for Caner Research, were the first researchers to keep nude mice alive for two years. In this time they were able to successfully grow and study human tumors.

Nude mice have allowed many insights into the immune system, leukemia, solid tumors, AIDS and other forms of immune deficiency.

[Link – good explaination of how T-lymphocytes function]

Tags

Research Fields: Biochemistry, Cell biology, Infection and Immunity, Medical technologies, Disease characteristics, Cancer research, Bones & muscles

Animals Used: Mouse (nude)

Medical Applications: Vaccine, Surgery, Medicine, Basic research

Rat

The laboratory rat has made invaluable contributions to the understanding and treatment of human diseases, in areas as diverse as cardiovascular medicine, neural regeneration, wound healing, diabetes, transplantation, behavioural studies and space motion sickness research. Rats have also been widely used to test drug efficacy and safety. Improved models in all these areas of research should result from our new knowledge of the rat genome.

The rat genome

The genome sequence of the Brown Norway rat was unveiled on 1 April 2004.[1] Almost 200 years after the brown rat, Rattus norvegicus, was first used by scientists to understand human physiology and medicine, with early studies concentrating on the effects of food and oxygen deprivation. Mazes to test rat intelligence were first built 100 years ago and the first albino Wistar rats were bred soon after. Since then the rat has become almost a byword for laboratory experimentation. The first knockout and cloned rats were produced recently, and new techniques for creating transgenic rats were announced last year, allowing powerful models of human diseases to be developed.

The rodent's DNA was deciphered and analysed by a collaborative network of researchers, known as the Rat Genome Sequencing Project Consortium, led by the US Baylor College of Medicine. To achieve its goal of producing a high-quality draft sequence, the Consortium developed a new, "combined" approach that used both whole genome shotgun (WGS) and bacterial artificial chromosome (BAC) clone sequencing techniques. To merge these into the final draft sequence, they developed a software package for genome assembly.

The sequence is a high-quality draft of 2.75 billion bases that covers 90% of the genome. It is the third mammalian genome to be decoded, allowing three-way comparisons to be made with the human and mouse genomes. The rat genome is smaller than its human equivalent, but larger than that of the mouse. All three encode a similar number of genes - between 25,000 and 30,000. The new information should enable researchers to determine which characteristics are specific to rodents and which are shared by all mammals.

Around 10% of the rat's genes are both shared with the mouse and absent in humans, including some that code for olfactory proteins. This may explain rodents' exceptional sense of smell. Rats have more genes for breaking down toxins than man. This means that rats may be better at removing toxins from their bodies than humans, so it may be possible to refine the use of rats in toxicology. There are significant distinctions, also, in the genes of the immune system.

Almost all disease-linked human genes have counterparts in the rat. Pinpointing these should help researchers to develop rat genetic models of human disease. Better rat models are likely to decrease drug failure in clinical trials - currently standing at about 90% - which will decrease development costs and time to market. The genome will also throw up new targets for drug intervention.

Rats and cardiac ischaemia

Recent research in rats may offer a new way of protecting people from cardiac ischaemia, a reduction of blood flow to the heart which can be life-threatening. Researchers at Stanford University in California found that rats with increased activity of the enzyme aldehyde dehyrogenase 2 (ALDH2) have reduced damage following cardiac ischaemia, and have isolated a compound, Alda-1, which activates the enzyme.

A mutation in the ALDH2 gene, which is particularly common in East Asian populations, leads to reduced enzyme activity, and therefore increases the risk of serious damage due to cardiac ischaemia. Giving Alda-1 can activate the mutant enzyme, restoring its activity to normal levels.

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Tags

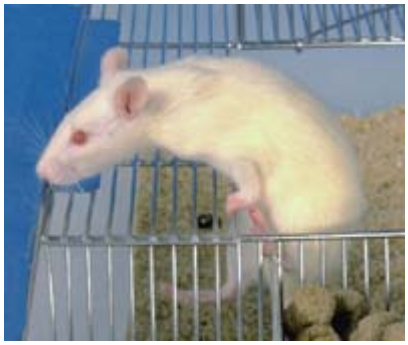
Research Fields: Anatomy & development, Biochemistry, Heart, lung & circulation, Cell biology, Endocrinology (hormones), Evolution & environment, Genetics, Infection and Immunity, Medical technologies, Disease characteristics, Brain & nervous system, Cancer research, Drugs & toxins, Psychology & behaviour, Bones & muscles, Digestion & nutrition

Animals Used: Rat

Medical Applications: Vaccine, Medicine, Basic research

Other rodents

Rodent models of depression



It is difficult to know whether animals suffer with psychiatric diseases like depression, so researchers aim to develop methods that examine specific aspects of the human disease. Diagnosing depression in humans uses a process of detailed questionnaires to determine the changes in the person's mood, motivation and the impact these have on their daily lives. This is clearly impossible in animals, and since depression is frequently studied in rodent species, signs and symptoms that might be common to a depressed state in any species have needed to be identified.

In animal studies, the researcher utilises the natural behaviour of the animal, such as how much they explore a novel environment. Drugs which improve the symptoms of depression in patients have also been shown to make the rats or mice more likely to explore a novel environment. This provides a way to test new drugs and compare findings with known antidepressants. Traditionally rats have been used in animal studies with behavioural components, and the majority of documented animal studies of depression concern rats. However, the ability to manipulate mice genetically means that their use is increasingly favoured in all aspects of biomedical research.

In depression, patients tend to feel negative about things in their lives and often find it difficult to see positive outcomes. This is something that can also be tested in rodents by measuring aspects of reward and motivation in tests such as the sucrose preference test, probability learning, cognitive bias test. The Porsolt forced swim test measures the length of time a rodent is prepared to swim for, with no apparent means of escape or reward. This test is also known as the despair test, and very low motivation to swim is a reliable model of many features of human depression.[1, 2]

Animal research into psychiatric disorders has also been very important to help us understand the way that different parts of the brain and different chemical messengers function. Studies in the normal brain provide information that enables researchers to understand what goes wrong in the diseased brain.

Rats and mice are used to directly examine the effects of drugs on the levels of neurotransmitters in particular brain regions through the use of *in vivo* microdialysis, which allows direct sampling of brain fluid between cells. Rodents are also used to look at the docking molecules or receptors that interact with the chemical signals to induce changes in the way the brain functions. These studies can use imaging techniques similar to those used in humans such as PET scanning. Alternatively, post mortem techniques can be used, for example, receptor autoradiography is used to show the precise location and activity of neurotransmitter receptors through the use of radioactive labels.

Much of the research carried out today focuses on understanding which parts of the brain go wrong and why this happens, providing the necessary knowledge base from which new drugs can be developed.

Non-human primates have been and are still used in this area but the numbers are very small when compared to the number of rodents used. Rats and mice are used the most but a small number of studies may use guinea pigs, gerbils, hamsters and tree shrews.[3, 4]

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Tags

Research Fields: Anatomy & development, Biochemistry, Heart, lung & circulation, Cell biology, Endocrinology (hormones), Evolution & environment, Genetics, Infection and Immunity, Medical technologies, Disease characteristics, Brain & nervous system, Cancer research, Drugs & toxins, Psychology & behaviour, Bones & muscles

Animals Used: Other or unspecified rodents

Medical Applications: Vaccine, Surgery, Medicine, Basic research

Rabbit

Improvements in the rabbits' health through better nutrition and sanitation has made the raising and maintenance of rabbit populations easier, making them increasingly useful as a research tool. These improvements to the way that we keep rabbits are largely due to the knowledge of rabbit physiology gained through medical research.

A New Zealand white - commonly used for antibody production and immunology research.
© istockphoto/Alistair Scott

The general physiology of rabbits is similar to that of humans, and like mice and rats, rabbits suffer from many diseases with human equivalents. Young rabbits often die from a disease called mucoid enteritis, which resembles cystic fibrosis and cholera. Rabbits are therefore used as models which can contribute to our understanding of these illnesses. Historically, Louis Pasteur used rabbits to develop his rabies vaccine and the rabbit has been important in the study of cardiovascular disease, particularly hypertension and atherosclerosis.

Studies in rabbits are key to many aspects of medical research, including cancer, glaucoma, ear infections, eye infections, skin conditions, diabetes and emphysema.

Antibody production

One of the most common uses of rabbits in the laboratory is for the production of antibodies, used to detect the presence or absence of disease and for research into infectious diseases and immunology. Antibodies are a key component of the adaptive immune system – the branch of the immune system which specifically recognises a foreign organism. They are complex molecules which can only be produced by the immune system of a living animal, and which recognise and bind to very specific protein sequences.

To produce antibodies the rabbit is injected with a protein sequence taken from the disease-causing organism to be studied. New Zealand white rabbits are generally used, as their large size ensures that plenty of antiserum is produced. Antibody is produced by the rabbit's immune system, and the progress of antibody production is monitored by taking small samples of blood at regular intervals. Once a sufficient level of antibody has been produced, blood is then taken from the rabbit under anaesthetic. The antiserum from a single rabbit keeps for a long time, and produces a large amount of antibody, which is often used for several years. There are currently no alternatives to using animals for antibody production, but there is ongoing research into developing a suitable method.

Laser surgery

The rabbit has provided an excellent model system to simulate the response of human tissue to the radiation produced by surgical lasers. Examples of laser advancements made possible by research on rabbits include eye surgery and the dissolving of plaque build-up on the walls of arteries.

High cholesterol

The genetic condition familial hypercholesterolemia causes blood-cholesterol levels three to seven times higher than normal in humans. High cholesterol causes atherosclerosis – a build up of fatty deposits in the arteries which greatly restrict blood flow, and those born with this condition usually die of heart attacks in childhood.

The Watanabe rabbit suffers from fatally high blood-cholesterol levels due to a genetic defect, which mirrors the fatal human condition and they suffer heart attacks by the age of two. These rabbits are used as a model to provide better treatments for children with this disease, and for

general research into high cholesterol. Research using these rabbits has included the development of an artificial liver to remove excess cholesterol from the blood of children suffering from hypercholesterolemia.

Tags

Research Fields: Anatomy & development, Biochemistry, Heart, lung & circulation, Cell biology, Endocrinology (hormones), Evolution & environment, Genetics, Infection and Immunity, Medical technologies, Disease characteristics, Brain & nervous system, Cancer research, Drugs & toxins, Psychology & behaviour, Bones & muscles

Animals Used: Rabbit

Medical Applications: Vaccine, Surgery, Medicine, Basic research

Sheep

Sheep are large mammals which have many similarities to humans in terms of physiology. They are easy to handle and suffer from many diseases which affect humans. They also have short gestation periods yet give birth to young of a similar weight to human babies, making them excellent for studying development and genetics. They are also used exensively in veterinary research, studies of digestion in ruminants, and research on the impact of farming on the environment. Sheep are frequently used as a model for cattle and other large mammals, as they are smaller, less expensive to keep and easier to breed.

Batten disease

Batten diseases (the neuroal lipofuscinoses or NCLs) are hereditary, fatal neurodegenerative diseases which affect around 1 in 12,500 children worldwide. These diseases cause progressive blindness, seizures, mental and motor retardation and changes in behaviour. Children with Batten diseases sleep poorly due to nightmares and hallucinations, suffer from frequent seizures and die between the age of 7 and adulthood, after a long period of 24 hour day-care. There is no effective treatment for these diseases.

They are caused by a malfunction of lysosomes, primarily in neurones. Lysosomes are organelles which digest and breakdown unwanted material within a cell, such as worn out organelles, unneeded chemicals or engulfed viruses and bacteria. Genetic defects in the digestive enzymes within the lysosomes mean that material is not degraded properly, and accumulates with them, damaging the cells. Six different NCLs are known, all caused by mutations to separate genes.

Batten disease also occurs in animals, and the New Zealand South Hampshire sheep is used as a model of the human disease. Sheep develop symptoms of the disease around 10 – 14 months and the disease follows a similar progression to the human form which is caused by a defect in the same gene. The sheep model has led to much greater understanding of the condition.

Tags

Research Fields: Cell biology, Genetics, Brain & nervous system

Animals Used: Sheep

Medical Applications: Medicine, Basic research

Cat

Cats share many cellular functions with humans. They have been domesticated for centuries, and have been used in scientific research from the early days of medicine. Since 1898, cats have contributed to the study of emotion, cardiac disease, spinal cord injury, cataract surgery, glaucoma, lupus, diabetes, spina bifida and more. These diseases are common to both humans and cats, and research in these areas has helped our understanding of human disease and the advancement of veterinary research.

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Cats are mainly used as models to study sensory systems and neuroscience. They have acute hearing, excellent eye-sight and highly developed balance and spatial awareness. These highly developed senses have always interested scientists, and more is known about the anatomy of the cat's sensory systems than those of any other animal. Cats also have well developed cognitive abilities and memories, and have often been used in laboratory tests of learning ability, with results which have been applied to human educational practices.

As reliable anaesthetic methods and delicate instruments to examine the nervous system have been developed, cats have been used to study a variety of neurological problems, such as epilepsy, deafness, and vision problems, making great contributions to our understanding of the nervous system.

The visual system

David Hubel studied the development and function of the visual system in cats, finding that all mammals, including humans, are born with a partially-developed visual system. He built on information from previous detailed studies of the nervous systems of kittens,[1] and found that proper development of the eyes, optic nerve and visual centres of the brain requires stimulation of the visual neurons by light. This work was awarded the Nobel Prize in medicine in 1981.[2]

Aging

The relatively long life span of cats, compared with mice and rats, makes it possible to observe the slower and more subtle effects of aging. Cats are known to reach the age of 16-20 years, and advances in treating many feline diseases have extended their life-expectancy.

Cancer research

Leukaemia, a malignant disease of the blood-forming organs, resulting in the uncontrolled production of abnormal white-blood-cells, is perhaps the most infamous of the diseases shared by cats and people. In cats, a condition similar to leukaemia in humans is caused by a retrovirus which was discovered in 1965. A vaccine for the feline leukaemia virus is available and has been refined over the years. Mammary cancer is also common in the cat, and many features of feline mammary cancer resemble human breast cancer which, among the human cancers, is the greatest killer of women.

AIDS research

For many years vets treated cats with symptoms of feline leukeamia, where they were unable to

isolate the virus. In 1986 researchers found that this disease was caused by a different retrovirus, which was similar to the HIV virus in humans. The retrovirus became known as feline AIDS (T-lymphotropic lenti virus),[3] and is thought to be transmitted through bite wounds, although the virus can remain dormant for years before causing disease, so there may be other routes of infection. FIV is not transmissible to humans, but the virus is genetically similar to HIV, and diseases caused by the virus are very similar. Cats which are naturally infected with the FIV virus are used as models to study effective anti-viral treatments for AIDS, which will benefit both cats and humans. The recent development of an FIV vaccine has given a potential new model for use in HIV vaccine development.

Detailed information about the FTLV assay and the disease can be found at Patents online.

Related link: Feline T-lymphotropic lentivirus assay patent information
http://www.freepatentsonline.com/5118602.html

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Tags

Research Fields: Anatomy & development, Brain & nervous system, Drugs & toxins, Psychology & behaviour

Animals Used: Cat

Medical Applications: Vaccine, Surgery, Medicine, Basic research

Guinea pig

Guinea pigs have biological similarities to humans, which make them useful in many fields of research. They have been used as experimental animals for centuries; hence the term 'guinea pig' for a human experimental subject. The Spanish conquistadors brought guinea pigs to Europe from South America, where they had been bred domestically, 400 years ago. Since vitamin C was discovered through research on guinea pigs, they have been important in nutritional research, and were also crucial to the development of: vaccines for diphtheria, TB, replacement heart valves, blood transfusion, kidney dialysis antibiotics, anticoagulants and asthma medicines.

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Statistics

Guinea pigs were used in just over 30,000 scientific experiments in the UK in 2006, representing less than 1% of total animal research. Over half of these were studies of the respiratory, nervous and immune system. The use of guinea pigs has fallen by over three-quarters since 1988, mostly due to a reduction in their use in safety testing. A recent contribution to this reduction has been the introduction of a milder test for the potential of chemicals to cause allergic skin reactions (skin sensitisation), which uses mice instead of guinea pigs. However, guinea pigs remain essential in many areas of research.

Guinea pig tissue

The guinea pig is also widely used to provide tissues and organs for research. Guinea pig blood components are widely used, and isolated organ preparations such as guinea pig lung and intestine are extensively used in research to develop new medicines. Such tissue and organ preparations were important in the discovery and early development of beta blockers to treat high blood pressure and drugs to treat stomach ulcers.

Numerous developments have used guinea pig intestine at some point in their development, for example the anti-nausea drugs used by cancer patients and the identification of naturally occurring pain killing substances known as enkephalins. Guinea pig intestine has also been extensively used to study the 'little brain' in the gut, which contains as many nerve cells as the spinal cord. This has given giving insights into not only the control of the gut itself but also the workings of nerve circuits. The information gathered from these studies is being used to develop computer models.

Allergies and respiratory diseases

The extreme allergic reaction, anaphylactic shock, has been studied extensively in guinea pigs, which display this reaction more readily and strongly than most other species. Guinea pigs' airways are sensitive to allergens, so it has been widely used in asthma studies. The inhaled medications that are the mainstays of asthma treatment were developed using guinea pigs as were orally-active drugs for asthma such as montelukast. Guinea pigs continue to be useful for the

development of improved treatments for asthma, and they are also used in the testing of vaccines against anthrax, currently an important area of biodefence research, and new medicines to treat drug-resistant tuberculosis.

Nutritional research

As well as requiring Vitamin C in their diet, guinea pigs also need high levels of folic acid, thiamine, arginine and potassium, which make them useful in nutrition studies. They also carry most of their plasma cholesterol in low density lipoprotein, so they are also useful in the study of cholesterol and lipoprotein metabolism.

Hearing

The structure of the guinea pig ear is similar to that of humans, meaning that their hearing range is also similar. Guinea pigs also display the Preyer reflex used in checking for deafness, in which the outer ear moves in response to a whistle. Guinea pigs are therefore a preferred animal model for studying auditory systems, and in 1961 Georg von Békésy was awarded a Nobel Prize for describing the mechanical mechanisms of the **cochlea** in guinea pigs[1].

Hair cells are sensory cells of the auditory system, which convert sound into an electrical signal. This signal can then be communicated through nerve cells. These cells do not re-grow when damaged, and loss of hair cells often leads to deafness as we age. The first successful attempt to regenerate hair cells in the inner ear of a mammal, announced in 2003, was achieved in guinea pigs[2].

Infectious diseases

The German scientist Robert Koch used guinea pigs to discover that TB was caused by the bacterium *Mycobacterium tuberculosis*, in 1882. The sensitivity of the guinea pig to this and other infections, and the similarities of its immune defence system to that of humans, has made it important in the study of infectious diseases.

In 1919 research showed that inoculation of guinea pigs with a small amount of blood from Yellow Fever patients produced a mild reaction in the animals. The same animals were later resistant to infection with *Leptospira icteroides*, while animals which had received no previous inoculation, or which had been inoculated with blood from malaria patients, died. The guinea pigs were said to have 'Aquired Immunity' to *L icteroides*[3].

Safety testing

The long gestation period of 59–72 days and mature central nervous system at birth means that guinea pigs are important in safety testing, particularly to prevent birth defects. However, it is the similarity of their immune system, and particularly their skin sensitivity, that led to their widespread use to test for allergic skin reactions.

This use of guinea pigs to test for skin allergies has now been largely replaced by the milder local lymph node assay (LLNA), which uses mice and fewer of them. In 1999 many of these tests were carried out on guinea pigs, mostly for non-medical products. Now guinea pigs are rarely used.

A brief summary of how guinea pigs are used in research is available from Understanding Animal Research

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Tags

Research Fields: Anatomy & development, Heart, lung & circulation, Infection and Immunity, Medical technologies, Disease characteristics, Brain & nervous system, Drugs & toxins, Digestion & nutrition

Animals Used: Guinea pig

Medical Applications: Medicine, Basic research

Primates

Monkeys and apes are our closest relatives in the animal kingdom. Since insights into human disease may be obtained from lower life forms including yeasts, nematode worms and fruit flies, it is no surprise that studies using primates are especially valuable. This is particularly the case in the quest to understand and treat infections and diseases associated with human physiological processes such as ageing, reproduction, endocrine function, metabolism, and neurology. Nevertheless, primates' high cognitive abilities and complex social behaviour mean that biomedical research using these animals requires additional justification and high welfare standards

Why use primates

The order Primates can be divided into 11 families. Man belongs to the family Pongidae, which is divided into four Genera: Pongo (orangutan), Pan (chimp and Bonobo), Gorilla and Homo. Excluding humans and the great and lesser apes (gorillas, chimpanzees, bonobos, orangutans, gibbons and siamangs), the members of the other 10 families can be roughly divided into prosimians (eg lemurs) and monkeys. Old World monkeys (baboons, macaques), also called true monkeys, are more closely related to humans than New World monkeys (marmosets, capuchins).

Due to the high degree of genetic, anatomical and physiological conservation, primates can be the best models for understanding human biological processes. Primates may be used to understand normal or abnormal structure and function or determine the efficacy of treatments where no other suitable animal models exist.[1] Their use has led to a number of valuable medicines and treatments. The chimpanzee, which shares over 98% of its genes with humans, is the most closely related primate, but the majority of biomedical research studies that require primates use the macaque monkeys.

Whilst genetic similarity to humans is high in non-human primates, it is also high in less developed species; for instance, we share 96% of our DNA with mice, 70% with fruit flies, and indeed 50% with crops such as bananas. In different species the same gene may be expressed in different ways or interact in different ways with other genes. Having genes in common may help with comparing and understanding some biological processes but is of limited relevance with respect to assessing welfare, social needs etc.

Despite their close relatedness, research with primates is not widespread and is only undertaken when other mammals are clearly inadequate. Primates are used in a small number of essential studies where only they share a particular biochemical or metabolic pathway with humans or where they model a human disease particularly well. For example atherosclerosis, osteoporosis and hypertension occur naturally in primates, which make them ideal animal models for those diseases. Without the use of primates, it would have been impossible to quickly identify the coronavirus responsible for the SARs outbreaks. Scientists then developed potential SARs vaccines that have provided protection against the disease in animals. Until we can develop other mammals with 'near-human' immune systems, primates are invaluable in safety testing potential

human vaccines.[15]

Toxicology

The majority of primates are used in the safety testing of medicines. Except in exceptional circumstances, new medicines must be tested in two species, a rodent and a non-rodent, before human clinical trials can take place.The purpose of these studies is not to prove a new medicine or vaccine is absolutely safe but rather to permit research to move on to human volunteers and patients. It is only after human clinical trials and licensing that a doctor can prescribe a new medicine.

The non-rodent species used in toxicology is usually the dog, but the type of the medicine being tested dictates the final choice. This is based on biological and pharmacological information eg the presence of a particular receptor. A test compound may elicit an immune response in one species but not in others, and differences in metabolic pathways may exist. For example, dogs are particularly sensitive to some test compounds (eg non steroidal anti-inflammatory drugs) and some drug vehicles (eg cremaphor, PVP). Other options for the second/non-rodent species include pigs and ferrets. The main reason why primates are considered is to assess safety of new vaccines or biologicals. In the development of specific vaccines, the interactions between parasites, viruses and their host are so specific that they must be studied in species closely related to humans to forecast the chance of unexpected or hyper-immune reactions. The science involved in selecting the second species is not always exact - a judgment is made on the balance of probabilities, and the question of what should count as sufficient/acceptable data to enable a properly informed choice about whether or not the drug should enter human clinical trials.[2]

Neuroscience

Due to the complexity of the brain, it is not possible to replicate its function in a test tube or rely on computer models. Thus, to develop new treatments for neurological disease it is necessary to use animals. Neuroscience research continues to produce important insights into the function of the human brain and associated disease states. Although there are some aspects of cognition that may be unique to humans, there is very strong evidence for structural, functional, behavioural and neurobiological commonalities that extend across species. The advantage of studying the monkey brain is that its connectivity, size, functional areas (reflected in its motor and behavioural capacities) and ageing processes are similar to ours. Nevertheless, there are gross anatomical differences between human and monkey brains - the gyri and sulci (ridges and valleys) of the human brain are much more pronounced; but the way the neurones themselves grow, develop, and send messages is common to all mammals. In fact, some of these more basic studies can be and are done in rodents, eg studying the effect of different concentrations of receptor agonists and antagonists on neuronal cell signalling in mouse brain slices by electrophysiology.

Some of the studies carried out on primates are behavioural studies, as monkeys are perceived to share similar emotions and are capable of carrying out similar actions. But to study how nerve cells work to produce behaviour it is necessary to examine their firing activity patterns using microelectrodes that are inserted painlessly into specific regions of the brain. This technique does not in any way incapacitate the animal and only causes minimal discomfort. The techniques are very similar to those used in certain human disorders, such as epilepsy and Parkinson's disease, where it is necessary to record brain activity. The vocal behaviour of primates and its underlying neural processes is another area of scientific investigation. Similarly, advances in the understanding of how the primate auditory cortex functions are leading to new hypotheses for the cause of deafness. Thus, researchers can learn about both normal and disordered brain functions underpinning higher cognitive and motor performance by using primates.

The use of primates in cognitive neuroscience research is covered further in Dick Passingham's article.

Alzheimer's disease

Alzheimer's disease affects more than 18 million people worldwide. It is a chronic debilitating disease that leads to irreversible memory loss, due to selective neuronal cell death. Accurate diagnosis, by autopsy, has revealed that the clinical features of Alzheimer's are the presence of beta-amyloid plaques and tau protein tangles in specific parts of the brain. Studies in macaque monkeys in the early 1990s led to the identification of the critical regions of the brain that are essential for cognition and memory and, like humans, ageing monkeys may show evidence of beta-amyloid plaques and lose neurones as they age.[3]

Partial models of Alzheimer's may also be created by priming monkeys with small amounts of human amyloid – they will develop plaques later whilst still reasonably young. As primates can be trained to perform memory-related tasks that permit the evaluation of changes in cognitive memory and emotional behaviour during ageing, they can be used to evaluate various treatment and prevention strategies. Recently, Bard and colleagues showed that they could prevent the build up of plaques and eliminate pre-existing plaques by treating mice with a beta-amyloid vaccine.[4] Beta-amyloid vaccines have been tested for tolerability in monkeys and humans and it is hoped that their use will lead to the alleviation of Alzheimer's symptoms.

Parkinson's disease

The therapeutic techniques that are currently used for Parkinson's disease and Essential Tremor would not have been possible without fundamental research on monkeys. The cause of Parkinson's disease was elucidated following the chance finding that Californian drug addicts who injected a home-made compound containing MPTP developed Parkinson's-like symptoms.[5] The suicide and subsequent post-mortem of one of the addicts revealed that the changes in the brain were identical to that of true PD patients. Shortly after, scientists showed that they could model the disease by giving MPTP to large primates. This enabled them to study how the symptoms manifested and to test new therapies.

Researchers in the UK found that in the primates with Parkinson's-like symptoms there is overactivity in a part of the brain that controls movement – namely the subthalamic nucleus – and that the overactivity is due to the selective loss of neurones in the substantia nigra that manufacture the chemical messenger dopamine.[6]

They were thus able to understand why the administration of L-dopa, a precursor of dopamine, was an effective treatment. To date, all the dopaminergic therapies that have been tested in the MPTP-treated primate have proven to be highly predictive of their clinical action in man.[7] However, L-dopa and related antiparkinsonian agents have side effects and their effectiveness wears off over long-term treatment.

Alim Benabid and colleagues in Grenoble, France were the first to find that by implanting an electrode into the subthalamic nucleus tremors could be controlled and normal movement restored.[8] This surgical technique, known as Deep Brain Stimulation (DBS), has been approved in Canada, Europe and Australia since 1998 for the treatment of PD and some tremor-like disorders. The procedure involves implanting electrodes into the patients' skull whilst they are awake. A battery-operated pacemaker that sends continuous electrical pulses is also placed beneath the skin. The patient can turn off the generator, eg at night, with the use of a special magnet. The high frequency stimulation 'paralyses' the overactive nerve cells. Indeed, two thirds of patients have a significant reduction in their tremor. So far, worldwide, around 40,000 patients have been treated with this technique which often reduces or eliminates the need for antitremor medication.[9]

Another intervention derived directly from primate research is constraint-induced movement therapy (CI therapy), which effectively strengthens weak limbs. This form of rehabilitation for stroke patients arose from the finding that if the sensory nerves supplying one arm in an adult monkey were severed, the brain would undergo long-term massive reorganisation of neuronal circuits.[10, 11 (review)] Many years later non-invasive techniques showed that something similar happens in the stroke-damaged brains of humans.

This fundamental observation in the primate led to the development of CI therapy, which involves restricting movement of the less affected arm while intensively training the more affected arm. Over the two years of their randomised controlled CI therapy studies, Taub and Uswatte showed large improvements in upper limb motor function after stroke.[11]

HIV & AIDS

Initial hopes that primates could be used in the development of a Human Immunodeficiency Virus (HIV) vaccine were dashed when it was found that the virus did not cause disease in chimpanzees. However, primates do have their own species-specific immunodeficiency virus, Simian Immunodeficiency Virus (SIV), and they develop an AIDS-like condition when infected with SIV ie they have similar nervous system changes, develop dementia and exhibit behavioural changes similar to those seen in HIV-infected patients.[12] This is not surprising given that HIV and SIV have similar genes and properties, and both attack T helper (CD4) immune system cells.

It takes just months for SIV infection to progress to simian AIDS as opposed to the many years usually seen in HIV-infected humans. Human studies have shown that the majority of HIV

infections occur when the virus crosses mucosal membranes, typically during sex or birth. Further studies, in female primates, led to the identification of the mucosal cells that are initially infected during heterosexual transmission of the virus. The SIV model also confirmed that the virus could be transmitted to newborns that swallow amniotic fluids or breast milk from infected mothers.[13] These discoveries open new opportunities for blocking HIV transmission with drugs, vaccines, or other precautions. For example, a humanised monoclonal antibody-based therapeutic approach, that in vitro inhibits HIV and SIV replication, has been shown to be safe when administered to rhesus monkeys.[14] For ethical and legal reasons, therapeutics such as these can not be directly tested in humans in case they provoke a severe reaction, hence they are first administered to primates that have a similar immune system.

Polio

Primates were and continue to be essential for the development and testing of the oral polio virus vaccine (OPV), also known as the Sabin vaccine, and the Salk vaccine. The OPV consists of several strains of live attenuated virus and the Salk vaccine of 'killed virus'. Until recently, each lot of vaccines had to be tested on monkeys to ensure that they were safe. However, in the last couple of years the WHO has approved and recommended that a transgenic mouse test for OPV be implemented as an alternative to the monkey neurovirulence test.[15] Interestingly, humans are not the only mammals to have benefited from the development of a polio vaccine - it has also been used to protect a wild colony of East African colony of chimpanzees from a potential epidemic.[16]

Malaria

Primates are extremely valuable models for understanding malaria pathogenesis, screening anti-malarial drugs and vaccine development. Malaria is caused by a protozoan parasite that is carried by mosquitoes. Interestingly, primates do not die from malaria although they may harbour the parasite. The reason why primates resist disease when infected, whereas humans do not, is an important question for researchers to answer. Additionally, the fact that primates can harbour an infection without becoming seriously ill makes them ideal for research into vaccine and drug development.[17]

The reproductive system

Some female primates menstruate and undergo menopause in the same way as women.[18] Specifically, the manner in which pregnancy is maintained following fertilisation and implantation of the embryo into the uterus is shared by all primates. During the first trimester, the corpus luteum (which develops after ovulation from the residual follicules) is responsible for synthesising progesterone, a hormone without which a pregnancy would not continue. Therefore, the corpus luteum has to be switched on at the right time and just as importantly switched off. This is key to understanding how the body maintains a pregnancy. Another example relates to the hormone prolactin, which, if locally active in primate uteri, has an immunoprotective effect. If the key to pregnancy loss is to be found, one must therefore use the primate model alongside normal cultured human uterine tissue. This human tissue is quite difficult to obtain as samples from healthy young women who are not on the contraceptive pill are needed. Tissue from women with a pre-existing pathology who have had a hysterectomy is not appropriate for these studies. These are just two examples of studies in the area of in vitro fertilisation (IVF) area which could not advance without using primates.

In vitro studies

Not all primate experiments involve the whole animal. Some studies make use of primate tissue and cells in culture, eg primate stem cells, but while these in vitro studies do reduce the number of animals needed in a study, they by no means replace them. This is partly because in the laboratory the properties of cells and tissues change over time. The conditions in a glass dish do not replicate the conditions in the 3D normal body environment where cells are exposed to a mass of circulating hormones and other substances. Indeed, many of the proteins in our body have not yet been identified. Thus the cell and tissue culture methods of research should be viewed as complementary approaches not exclusive ones. These in vitro methods will not, in the foreseeable future, replace the need for whole animal experiments whether on rodents or primates.

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Tags

Research Fields: Cell biology, Genetics, Infection and Immunity, Disease characteristics, Brain & nervous system, Drugs & toxins, Psychology & behaviour

Animals Used: Primates

Medical Applications: Medicine, Basic research