Chapter 5

The use of animals in basic biological research

The use of animals in basic biological research

Introduction

5.1 In this chapter, we are primarily concerned with the use of animals for basic, 'blue-sky' or curiosity-driven research (see Paragraphs 3.53–3.54). This kind of research aims to help us understand how animals develop and function at the behavioural, physiological, cellular and molecular levels. Knowledge produced in basic research has also contributed to medical advances. Several different types of animals are used, including invertebrates such as fruit flies and nematode worms, non-mammalian vertebrates (frogs, fish and chickens) and mammalian vertebrates such as mice, rats, rabbits, cats, dogs and primates. Almost all of the animals used are specially bred for this purpose, and approximately 80 percent of animal experiments carried out on vertebrates in the UK in 2003 involved mice or rats.[1]

5.2 A wide range of different experiments are performed in basic research, and we can only give selected examples here. For the sake of simplicity, we divide our discussion into the use of animals for the following purposes, which cover most types of research in this area:

- behavioural studies;
- physiological studies;
- studies on development;
- genetic studies; and
- the development of research tools and techniques, for example, antibody production, biopharmaceuticals and cloning.

Comments on the use of animals in basic research from respondents to the Consultation

'…major developments in medicine and surgery have often been based on fundamental understanding of biological premises. These have required 'blue-skies' research, which, by definition, has no immediate or obvious application.'
Biosciences Federation

'…the genetic mechanisms of many species (nematode worms, fruit flies, fish or mice) work in precisely the same manner as in humans, and in the mouse there are counterparts for most human genes.'
Sarah Johnson, member of the Ethical Review Panel at the MRC NIMR

'The number of GM animals we use is rising fast. This process is best described as commodification. The moral problem is that animals are not computers or areas of land or other "resources".'
Shaun Carey

'The production of GM animals is not a perfect science and there are often many animals produced to develop the specific modifications that are required to meet research objectives. This results in a large number of mice required to be bred and many to be culled that do not have the specific genetic manipulation.'
Canadians for Health Research

Behavioural studies

5.3 One of the great challenges to life scientists is to understand the biological basis of animal behaviour. Why do some birds sing when others do not? Why are some animals monogamous, and others promiscuous? What cues do birds use to navigate when they migrate over long distances?[2] How do animals learn and remember? There are many different types of behaviour to understand and many ways to study them. In the category of behavioural studies, we arbitrarily focus on observational research that does not usually involve injections, drawing blood, surgery, dietary manipulation or chemical treatment. They comprise studies in which animals are observed in their natural habitat or

1 Home Office (2004) *Statistics of Scientific Procedures on Living Animals Great Britain 2003* (London: HMSO).

2 Invasive research on animals that has no expected or direct application to the human species raises different ethical issues than research which has possible application (see paragraphs 3.52–3.55). See Schrag B, Freeberg T and Anestidou L (2004) The Gladiator Sparrow: Ethical Issues in Behavioral Research on Captive Populations of Wild Animals: A Case Study with Commentaries Exploring Ethical Issues and Research on Wild Animal Populations *Science and Engineering Ethics* **10**: 717–34.

in an environment that has been changed for the experiment. In some cases, the welfare of the animals is unaffected. In other cases, some distress may be caused, for example, when animals are tagged in some way. This may involve catching and restraining them for the duration of the identification process. Tagging itself can be invasive and potentially harmful (such as the amputation of a toe, as sometimes done to amphibians, or the use of 'patagial tags', which are attached to the muscle of fish or blubber of cetaceans) or non-invasive (such as use of a leg ring for birds).[3] An animal's welfare may also be affected if it is released into a foreign environment where it may have to re-establish its territory (see paragraph 4.48).

Observational research

5.4 As an example of observational research, a songbird might be reared in the absence of other birds in order to determine whether the bird would normally learn to sing by hearing the song of other birds, or whether it has an innate ability. In other examples, rats or mice are observed as they run in mazes, swim to rafts or associate a sound or coloured light with the delivery of a 'reward', such as food, an aversive stimulus in the form of, for example, a bitter-tasting substance or a 'punishment', such as a minor electric shock, to investigate aspects of learning and memory. The exploratory behaviour of animals on exposure to a novel environment might be studied in order to distinguish the bold from the timid. When behavioural studies are undertaken in a laboratory, an animal's welfare may be affected if the experimental environment is incompatible with its species-specific needs; for example, if space or environmental enrichments are insufficient or lacking. To explore the cellular and molecular basis of behaviour in more detail, scientists whose work involves animals not only observe the influence of environmental manipulation, but also seek to directly manipulate the animal as we discuss in the following section (see Box 5.1).

Physiological studies

5.5 We include here experiments involving surgical, dietary or drug treatment of animals that are directed at understanding biological processes at the physiological, cellular or molecular

Box 5.1: Example of research – Manipulation of circadian rhythms and comparison of gene expression in the liver and heart of mice

Storch KF, Lipan O, Leykin I *et al*. (2002) Extensive and divergent circadian gene expression in liver and heart *Nature* **417**: 78–83.*

In many mammalian tissues, the expression of genes that are responsible for the daily timing of physiological processes is controlled by biological timing mechanisms called circadian clocks. In this study, researchers used mice to compare gene expression in the liver and heart. They found that many of the genes expressed were under circadian control, although there were substantial differences between the two organs with regard to the kinds of genes affected. The authors hypothesised from their results that circadian clocks have a specialised role in each tissue, and that the extent of circadian gene regulation meant that it influences many different processes. They concluded that their work addressed important aspects of circadian gene regulation that applied to all mammals and made comparisons between the genes in mice and those in plants and fruit flies.

The following methods were used: mice were synchronised to a 12-hour light/dark cycle for at least two weeks and then placed in constant dim light for at least 42 hours. The mice were subsequently killed at various intervals of a light/dark cycle and their tissues collected and analysed. The mice would have experienced mental and physical disruption in their daily rhythms for the period that they were kept in constant dim light.

* This is an example of animal research that has been carried out in the UK and published in a peer-reviewed journal. Details relate to this specific example and should not be taken to represent a 'typical' animal experiment. It is important to note that individually published experiments usually form one part of a continuing area of research, and the significance of the results may therefore be difficult to interpret.

3 These identification methods have recently been criticised on scientific and animal welfare grounds, since there is some evidence that they can lead to increased mortality after release. See May RM (2004) Ethics and amphibians *Nature* **431**: 403.

levels. Better understanding of these processes has historically contributed to the body of scientific knowledge on animal and human biology. It has played an important role in the discovery of treatments for diseases, usually as a result of systematic methodological enquiry, and in some cases serendipitously (see Box 5.2).

Box 5.2: Examples of how basic research has lead to unexpected clinical benefit

Narcolepsy

Narcolepsy is a disabling sleep disorder estimated to affect between three and five people per 10,000 in European populations.* Affected individuals have overwhelming feelings of sleepiness and fatigue. They may also experience dream-like hallucinations and the sudden onset of paralysis lasting for a few seconds, usually brought on by strong emotion. The cause and nature of narcolepsy were unknown until recently. In 1998 two groups, neither of which was working on narcolepsy, independently identified a neurotransmitter made by the hypothalamus in the brain; one group called it hypocretin and the other called it orexin. When the gene encoding the neurotransmitter was experimentally inactivated in mice, the mice developed narcolepsy.† The following year, a group studying an inherited form of narcolepsy in dogs isolated a defective gene, and found that it encoded a membrane receptor for one of the two forms of orexin/hypocretin.‡ Based on the evidence that defects in the orexin/hypocretin signalling system caused narcolepsy in mice and dogs, two research groups examined the brains of deceased humans who had suffered from narcolepsy. They found that orexin/hypocretin-producing cells in the hypothalamus were greatly decreased or absent.∫ It is now thought that narcolepsy in humans is usually caused by the autoimmune destruction of these cells in the brain, much as type I diabetes is usually caused by the autoimmune destruction of the cells that produce insulin in the pancreas. Identification of the biological basis of narcolepsy is thus a significant step in developing more effective ways of treating the disorder.

Myasthenia gravis

Myasthenia gravis is a life-threatening disease in which muscles become progressively weaker with exercise. The annual incidence of new people diagnosed with the disease is between 0.25 and two per 100,000.** A crucial discovery relevant to the pathology of this disease was made in 1973 by researchers who were studying the structure and function of receptors of the chemical transmitter acetylcholine. They isolated and purified the receptors from the electric organ of electric fish (eels, skates and rays) and injected them into rabbits to raise antibodies against them for use in their research (see paragraphs 5.24–5.25). Unexpectedly, the rabbits developed what was identified to be myasthenia gravis.†† It was found that patients with myasthenia gravis make antibodies against their own acetylcholine receptors and that these 'auto-antibodies' are usually causally linked to weakening of their muscles. The receptors are normally on the surface of muscle cells and are activated when motor nerves release acetylcholine to stimulate the muscle to contract. In patients with myasthenia gravis, the anti-receptor antibodies inactivate the receptors so that acetylcholine is relatively ineffective. The presence of anti-acetylcholine receptor auto-antibodies is now widely used in the diagnosis of myasthenia gravis, and treatment is directed at removing or inhibiting the production of the antibodies. As a result of these pioneering studies, a number of other muscle and neurological diseases, such as Lambert–Eaton myasthenic syndrome and acquired neuromyotonia, were also found to be caused by the inactivation of receptors and channels by auto-antibodies.

* Zeman A, Britton T, Douglas N *et al*. (2004) Narcolepsy and excessive daytime sleepiness *BMJ* **329**: 724–8.

† Sakurai T, Amemiya A, Ishii M *et al.* (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour *Cell* **92**: 573–85; De Lecea L, Kilduff TS, Peyron C *et al*. (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity *Proc Natl Acad Sci USA* **95**: 322–7.

‡ Lin L, Faraco J, Li R *et al*. (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene *Cell* **98**: 365–76.

∫ Peyron C, Faraco J, Rogers W *et al*. (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains *Nat Med* **6**: 991–7; Thannickal TC, Moore RY, Nienhuis R *et al*. (2000) Reduced number of hypocretin neurons in human narcolepsy *Neuron* **27**: 469–74.

** Vincent A, Palace J and Hilton-Jones D (2001) Myasthenia gravis *Lancet* **357**: 2122–8.

†† Patrick J and Lindstrom J (1973) Autoimmune response to acetylcholine receptor *Science* **180**: 871–2; See also pages of the Myasthenia Gravis Association website, including http://www.mgauk.org/mganews/0203-01.htm. Accessed on: 23 Apr 2005.

Study of the endocrine system

5.6 Most of what we know about the endocrine system (which produces and releases hormones), has resulted from studies involving animals. Typically, hormone-producing endocrine glands, such as the thyroid, were surgically removed or chemically inactivated in adult animals. The effects of this treatment on the behaviour and physiology of the animals were analysed, and attempts were made to reverse them by administering extracts of the gland. If successful, the next step was to purify the active hormone(s) from the extracts. Most of the known hormones in humans were discovered in this way. Even today, newly discovered molecules that are thought to be responsible for signalling between cells are often tested by injecting them into

a living animal (usually a rodent). This is because those who undertake such research believe that this procedure is the most scientifically valid, and often the only way of determining hormone function in physiology and development. The welfare implications for the animals involved will vary depending on the kind of hormone and the dose administered. In humans, hormonal imbalances can cause unpleasant symptoms, including lethargy and headaches.

Box 5.3: Example of research – How do monkeys view faces?

Guo K, Robertson RG, Mahmoodi S *et al*. (2003) How do monkeys view faces? – a study of eye movements *Exp Brain Res* **150**: 363–74.*

Perception of faces plays a crucial role in social communication. The aim of this research was to study accurately how faces are viewed by primates. The researchers investigated the organisation of eye movements in two adult male rhesus macaque monkeys in response to facial images. Previous studies had suggested similarities between humans and monkeys in the neural mechanisms responsible for the perception of faces. Thus, it was concluded that the results of this study could be compared to findings obtained from humans by less invasive means.

The monkeys underwent an operation under anaesthesia to implant a head-restraint device (see paragraph 4.47). Coils were then surgically implanted into the white, outer layer of the eyeball (the sclera) so that eye movements could be recorded. During experiments, the monkeys were seated in 'primate chairs' (see also Box 5.5), which enable the head of the monkey to be fixed. The monkeys' eye positions were recorded while images of monkey and human faces were presented on a computer screen.

It was already known that when monkeys are shown faces of other monkeys, their eyes fix on the eyes in the image. This particular experiment investigated the visual process that occurs when the faces were unfamiliar to the monkeys, and when the images were inverted or scrambled. Differences in perceptual processing when either a monkey or a human face was shown were also assessed. It was found that the monkeys exhibited similar eye scan patterns while viewing both familiar and unfamiliar monkey faces, or while viewing monkey and human faces. There was a greater incidence of fixation of the eye region of all the face images, and particularly re-fixation of the eyes of unfamiliar faces during the first few seconds, confirming that the eyes are important for initial identification. However, it was found that the eyes in the scrambled face images were much less of a focus than those in the upright or inverted faces. The researchers concluded that, while viewing faces, the eye movements in non-human primates are controlled by more than one level of perceptual processing; i.e. that the targeting of the eye region may occur at a relatively low level of visual processing (before identification of the object) and that the probability that the eyes will become the eventual target in the image is affected by higher levels.

With regard to welfare implications, the implants could have caused discomfort; the monkeys would also have needed to be carefully trained to avoid psychological distress caused by the restraint during the experiment. No reinforcements in the form of 'rewards' or 'punishments' were given during this procedure.

* This is an example of animal research that has been carried out in the UK and published in a peer-reviewed journal. Details relate to this specific example and should not be taken to represent a 'typical' animal experiment. It is important to note that individually published experiments usually form one part of a continuing area of research, and the significance of the results may therefore be difficult to interpret.

Study of the immune system

5.7 Many studies on living animals, involving mainly mice and rats, have been conducted to examine the vertebrate immune system, and most current knowledge is based on this research. The immune systems of animals and humans protect them from infection. If the adaptive immune system is challenged by a particular infectious agent that it has previously overcome, it is able to do so on subsequent occasions much more quickly and effectively. Research on the adaptive immune system usually involves an initial immunisation of animals with foreign (from another animal) biological molecules or cells or microorganisms such as bacteria. Immune responses are characterised by the production of immune cells and antibodies, which specifically recognise and help eliminate the foreign molecules, cells or microorganisms (all referred to as antigens). Experiments of this kind provided the first evidence that the cells responsible for adaptive immune responses were a class of white blood cells called lymphocytes. In these experiments, rats or mice were irradiated with X-rays to kill most of their white blood cells, including lymphocytes, rendering them unable to make adaptive immune responses. When different cell types were transferred into these animals, only lymphocytes were found to reverse this deficiency. The welfare of the animals

was usually affected because of increased susceptibility to infections, particularly in the gut, due to the destruction of the lining of mucosal cells caused by the irradiation. These infections were usually treated with antibiotics. In the first series of experiments of this kind, significant numbers of animals died, most likely due to diarrhoea. In general, it can be assumed that the experiments entailed at least some malaise for the animals involved.

5.8 These irradiation experiments depended on the availability of inbred strains of rats and mice, which are produced by repeated rounds of inbreeding until the animals within each strain are nearly genetically homogeneous. The use of these strains allows cells to be transferred between animals of the same strain without the problems of immunological rejection. If cell transfers are attempted between animals of different strains or species, the transferred cells are recognised as foreign by the immune system and the body mounts a reaction and tries to destroy them. Experiments in which skin grafts were transplanted between mice of different strains established that graft rejection is an immunological response. Studies of these immune responses, and the development of medicines that are able to overcome them, eventually facilitated organ transplantation in humans. Transplantation experiments cause some distress to the animals involved, partly because of the anaesthesia used and partly because bandaging the grafts may cause irritation.

5.9 The approach of transferring lymphocytes into the same inbred strain of irradiated mice or rats has also been used to show that different classes of lymphocyte mediate different types of immune responses. New subclasses of lymphocyte and response are still being discovered in this way. Since immune responses in mice and rats are remarkably similar to those in humans, many researchers have applied the knowledge gained from research in rodents to humans. It is also possible to transfer human lymphocytes to immunodeficient mice to enable the study of 'human' immune responses using mice. Such 'humanised' mice have been important in understanding the function of a range of viruses, including how HIV/AIDS destroys the human immune system and eventually causes the death of the patient. Since mice without a functioning immune system are highly susceptible to infections, they are usually kept in sterile environments, and enrichments are not commonly provided.

Study of cell differentiation

5.10 Similar experiments involving cell transfer in mice are currently being carried out to study the potential of unspecialised stem cells to develop into various specialised cell types. Stem cells isolated from adult organs are called adult stem cells, whereas those isolated from early embryos are called embryonic stem (ES) cells. Experiments involving the transfer of mouse stem cells into irradiated, or otherwise injured, mice have contributed to knowledge about the potential of using human stem cells to treat conditions in which cells die, such as strokes, heart attacks, diabetes and Parkinson's disease (see paragraph 5.26).[4] Blood-forming stem cells from bone marrow have long been used to treat patients whose own blood cells had been destroyed by disease, irradiation or anti-cancer medicines. Welfare problems for the animals used in the experiments referred to above could result from the underlying disease, as well as from the cell transplantation procedure itself, which involves an injection of cells through the lining of the abdominal cavity or into the bloodstream or an organ.

Study of the nervous system

5.11 Much of our knowledge about the functioning of the central nervous system (CNS) has come from invasive animal experiments in which parts of the nervous system are electrically monitored, stimulated or destroyed. Many studies have been undertaken in primates, as the

4 Nuffield Council on Bioethics (2000) *Stem cell therapy: the ethical issues* (London: NCOB).

cerebral cortex, which is responsible for most higher brain functions such as thought and speech, is very poorly developed in animals other than primates. For example, individual nerve cells or groups of cells in the cortex of a conscious monkey that are involved in anticipating a movement before it occurs can be distinguished from those cells that send the signal for the movement itself. In a similar way, it is possible to distinguish areas of the cortex involved in recognising the colour of an object from those involved in recognising motion of that object. Although non-invasive imaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) now allow the physiological activity of large groups of nerve cells in the human brain to be studied, the resolution of these methods is still too poor to study individual nerve cells, or even small groups of nerve cells. Currently, therefore, the only way in which individual or small groups of cells can be studied is by inserting needle electrodes into the brain (see Box 5.4). Nonetheless, imaging techniques are rapidly improving and are likely to provide increasingly powerful alternatives to invasive animal research of this type (see Box 11.1).

Box 5.4: Example of research – Studying control and function of the hand using primates

This is an example of animal research witnessed by some members of the Working Party during a visit to a research establishment.

The objective of this research involving macaque monkeys was to increase understanding of how a stroke can impair use of the hand in humans. It sought to investigate how activity in groups of brain cells in a part of the brain called the motor cortex controlled specific hand and finger movements. Primates were used because only these animals have a sufficiently similar brain structure, function and cognitive ability to ensure that the results were relevant to humans. Research of this type has recently made significant contributions to the diagnosis and therapy of movement disorders and has been crucial to the development of deep brain stimulation (DBS), a new treatment for Parkinson's disease.*

The monkeys were procured from a breeding colony in the UK where normal practice was to rear them in groups of 16–18 animals and accustom them to contact with humans. In the laboratory, the animals were housed in pairs from the age of 18 months. The cages measured approximately 2.40x1.80x1.20m (width/height/depth) and contained objects for enrichment such as toys, mirrors, puzzle boxes and swings. Foraging material was provided in one part of the cage. The room was lit with natural daylight through windows on two walls. In winter, the light was regulated on a 12-hour scheme with fading transitions. Researchers reported that they maintained frequent social contact with the monkeys.

This project and the procedures were classified as 'moderate' by the Home Office. The first procedure was usually an MRI scan. Under anaesthesia, three-dimensional scans of the monkey's skull and brain were taken. These pictures aid the accurate targeting of areas of the brain from which recordings are made.

The animals then underwent a period of training and learned that they would be rewarded with treats such as fruit, nuts and biscuits when they performed certain tasks correctly. Over a period of time, which varied between six and 12 months, they were also trained to remain still while the research was being carried out, which required the use of some degree of restraint to which they became accustomed.

The primary surgical intervention was the implanting of devices necessary to record specific nerve-cell and muscle activity. Under general anaesthesia, a head-restraint device and recording chamber were fitted. The implants weighed 150g and consisted of a metal ring of approximately 10cm in diameter and 1mm in thickness, which was attached to the monkey's head by means of four bone screws of about 3mm diameter. The screws were inserted through holes made in the skull and were fixed on the inside. These screws were subsequently used to attach the head of the monkey to a specially designed primate chair during an experimental procedure. During surgery, electrodes were also implanted to record the activity of the various nerve cells and muscles that are involved in moving the hand and arm.

After surgery, monkeys received post-operative care including pain relieving medicines and antibiotics and were monitored according to a regime approved by the named veterinary surgeon (NVS). The average recovery time to normal behaviour was two to three days. The recording procedure itself, which involved introducing very fine microelectrodes into the brain, is not painful, because the brain itself has no pain receptors. With regard to the psychological effects on the animals, there was usually a period of two to three days during recovery from surgery when the monkeys touched the implant. They then became accustomed to it and stopped doing so.

In order to allow for the recording of neural and muscular activity, the monkey was placed in a primate chair. This is a steel device, measuring approximately 70x30x30cm. Once the monkey was seated in the chair, a metal disk was put over the ring attached to its skull, thereby immobilising the head by connecting it to the chair. This is required to allow for the stable recording of the activity of single neurones. The monkey remained able to move its jaw and chew, and the rest of

Continued

the body was free to move. The monkey appeared not to resist this procedure (see paragraph 3.34). The multiple electrodes inserted through the implanted recording chamber into the monkey's brain were connected with wires to a computer, and to devices recording the activity of muscles in the arm and hand.

With regard to the experimental procedure itself, the standard task required the monkey to perform a highly skilled hand movement, using its thumb and index finger to squeeze two levers into precise target zones. Each time it squeezed the levers successfully, it was given a food reward by an animal technician sitting next to the monkey. Once researchers had obtained sufficient data on the connection between certain neural areas of the motor cortex and hand movements, the electrodes were inserted into a new area of the brain. There were typically three to five sessions per week, with regular breaks of three to four weeks. Each session lasted approximately three hours, during which a monkey received around 600 food rewards. On average, each monkey provided 100–200 fully analysed neurones over 18 months. Animals were killed at the end of this period by administering deep general anaesthesia from which they did not recover. This allowed electrophysiological and neuroanatomical investigations of brain pathways involved in hand control which enabled the scientists to verify the anatomical position of the electrodes that had been inserted during the research. At this particular laboratory, approximately one monkey per year was used for this type of research.

* DBS involves the implantation of small stimulating electrodes of approximately 1x3mm in the brain circuits of patients suffering from Parkinson's disease. The electrodes are connected with wires to a unit implanted close to the collar bone. This unit generates electrical impulses in a method similar to pacemakers. To date, approximately 22,000 patients have been treated with DBS. The technique helps to reduce dramatically the manifestation of tremors, episodes of spasticity and other forms of abnormal movement typically experienced by sufferers of Parkinson's disease. See Rodriguez-Oroz MC, Zamarbide I, Guridi J, Palmero MR and Obeso JA (2004) Efficacy of deep brain stimulation of the subthalamic nucleus in Parkinson's disease four years after surgery: double blind and open label evaluation *J Neurol Neurosurg Psychiatry* **75**: 1382–5; Kumar R, Lozano AM, Kim YJ *et al*. (1998) Double-blind evaluation of subthalamic nucleus deep brain stimulation in advanced Parkinson's disease *Neurology* **51**: 850–5.

Studies of animal development

5.12 Developmental biologists often carry out experiments on embryos to determine the cellular and molecular basis of animal development. Parts of an embryo (often chick embryos) are removed to learn about how different tissues develop (see Box 5.5). In some cases, a fragment of tissue is transferred to a new location in the embryo to observe its development. The outcome indicates whether or not the tissue was already irreversibly programmed for development into a particular tissue or organ at the time of transfer. A dye might also be injected into one or more cells, to enable observation of their stages of development. Zebrafish embryos are often used because they are transparent, which is a useful property with regard to monitoring the development of injected cells in the living embryo.

Box 5.5: Example of research – Developmental studies involving amphibians

This is an example of animal research witnessed by some members of the Working Party during a visit to a research laboratory. The main focus of the research was to improve understanding of the processes that determine cell differentiation during the early stages of embryonic development. Researchers used two different species in order to provide comparable information. Amphibian embryos were preferred to mammalian models such as the mouse because amphibians produce a large number of eggs that develop externally to the mother, are of a size which allows experimental reagents to be injected easily, and develop fairly rapidly. The research was undertaken on embryos of the frogs Xenopus laevis and Xenopus tropicalis. In general, the results gained from developmental studies on these frogs are considered to be readily transferable to mammals, including humans, as most of the basic developmental mechanisms have been highly conserved in evolution.

The stimulation of egg-laying was the only procedure undertaken in this study that fell under the A(SP)A. Adult female frogs were injected with a hormone that caused them to lay large numbers of eggs within 3-12 hours. This involved a subcutaneous injection just over the dorsal lymph sac. The eggs were fertilised artificially to ensure synchronous development. In order to do so, a male frog was killed by methods referred to in Schedule 1 of the A(SP)A, and its testes was removed and used to fertilise the eggs. Female frogs generate more eggs over a four month rest period and are reused in the procedure described above for the production of new eggs.

The frogs were kept in a windowless room in three rows of five basins, each measuring approximately 60x40x30cm. There were between five and 25 frogs per tank, each frog having a minimum of one litre of water. The water was changed daily. No enrichments were provided in the tanks. The room light operated on a 12-hour cycle, with gradual transitions between light and darkness.

Study of gene function in embryos

5.13 Developmental biologists often seek to determine the roles of single genes in animal development. A useful way of doing this is to create GM animals in which the expression of a specific gene is increased or decreased (see paragraphs 5.19–5.20). For example, in some experiments, molecules of ribonucleic acid (RNA, an intermediary involved in the transfer of genetic information between DNA and proteins) are injected into early frog or fish embryos. This will transiently increase or decrease the expression of a specific protein, thereby helping to determine how that protein (and thereby the gene that codes for it) normally functions in early development. The welfare implications of such experiments are difficult to predict and, depending on the gene, could range from no adverse affects to severe developmental abnormalities and disability (see paragraph 4.57). It is for this reason that in this, and similar types of genetic research, endpoints are defined in licence applications and research should be stopped humanely if they are exceeded (see paragraphs 5.22 and 12.21).[5]

5.14 Embryologists who study early development in mice sometimes mix cells from embryos of two different mouse strains to form a mouse that is made up of cells from the two strains. If a specific gene in one of the sets of cells is altered before mixing them, the influence of that gene on the development of the altered cells (and the cells that derive from them) can be determined in an embryo in which many of the cells are unaltered. The mixed embryos need to be implanted into the uterus of a surrogate mother in order to develop. The mothers may then be killed in order to obtain the embryo at different stages of development. The welfare implications for the animals relate to the anaesthesia and implantation procedure for the surrogate mother and to any developmental abnormalities in the chimeric offspring.[6]

Study of development after birth in mammals

5.15 Since development continues after birth in mammals, many studies in this area involve research on animals after they are born. Neurophysiologists, for example, first demonstrated the importance of a critical period in visual development by patching one eye of newborn cats and monkeys.[7] If this is done for one week during the first six months after birth, the covered eye becomes permanently blind as a result of alterations in the way in which nerve cells are interconnected in the brain. Patching after this time does not produce the same effect. The same phenomenon was later found in children with one lazy eye. These children are now treated with alternating left and right eye patching to maintain vision in the affected eye until after the critical period, as first demonstrated in kittens.[8]

Genetic studies

Selective breeding

5.16 Selective breeding has been used for many decades to create more productive, or higher yielding farm animals, and for the breeding of animals with particular features and characteristics, including some companion animals and 'show' animals. It has also been used

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5 See Wolfensohn S and Lloyd M (2003) *Handbook of Laboratory Animal Management and Welfare*, 3rd Edition (Oxford: Blackwell Publishing Limited), Chapter 4.

6 See Morton DB and Hau J (2002) Welfare assessment and humane endpoints, in *Handbook of Laboratory Animal Science: Essential principles and practices*, Volume I, 2nd Edition, Hau J and Van Hoosier GL (Editors) (Seattle, WA: CRC Press), Chapter 18, pp457–86.

7 Early work includes, for example, Wiesel TN and Hubel DH (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens *J Neurophysiol* **28**: 1029–40; Wiesel TN and Hubel DH (1965) Extent of recovery from the effects of visual deprivation in kittens *J Neurophysiol* **28**: 1060–72.

8 Many of those opposed to animal research are concerned about certain types of basic research, and argue that the alleged benefits cannot justify the suffering involved (see paragraphs 3.52–3.55).

in medical research to investigate basic biological processes. Many mouse mutants have arisen spontaneously in colonies maintained specifically for experimental purposes. Some of these have been used as models of human disease, including diabetes, obesity and neurodegenerative diseases.[9]

'Forward genetics'

5.17 Other techniques seek to deliberately change the genetic complement of animals, in order to observe the consequences of these alterations. Classical genetic experiments (also called 'forward genetics') are performed by inducing random mutations. The animals are treated with mutagens such as X-rays, chemicals that alter genetic information or viruses that insert DNA into the host genome. Offspring are screened for abnormal features in development, physiology or behaviour. The advantage of this approach is that when a mutated gene is found, it is likely to be important for the feature that is abnormal in the mutant. The mutant gene can then be identified, by comparing gene sequences from the mutated animal to those from normal animals. This procedure has become much more straightforward since the genomes of a number of animals have been mapped and sequenced.

5.18 Until recently, these studies were mainly carried out in fruit flies and nematode worms, organisms which are small, low cost and have rapid generation times. These are crucial features for large-scale genetic studies that involve many thousands of animals. Genetic screens in flies and worms have contributed to many important advances in our understanding of animal development. Many of the genes identified were later shown to be common to all animals, including humans, and they often function in very similar ways. The conserved functions of particular genes have been demonstrated by transferring them, for example, from humans to worms or flies, and showing that they function in the same way. This research has revealed a remarkable degree of conservation of genetic information during evolution. More recently, large-scale genetic screens have been carried out using zebrafish and mice, primarily to discover the genes responsible for a particular developmental or physiological process. The welfare implications of such experiments are difficult to predict and, depending on the genes involved, could range from no adverse affects to severe developmental abnormalities and disability (see paragraph 5.13).

'Reverse genetics'

5.19 Another genetic approach, called 'reverse genetics', is mainly applied to mice. Researchers can alter a specific gene of unknown function either by over-expression (in *transgenic* mice), elimination (in *knock-out* mice) or replacement with an altered form of the gene (in *knock-in* mice). The genetic change is then passed on from generation to generation in the new, genetically engineered mouse strain, in which the function of the gene under study can be analysed.

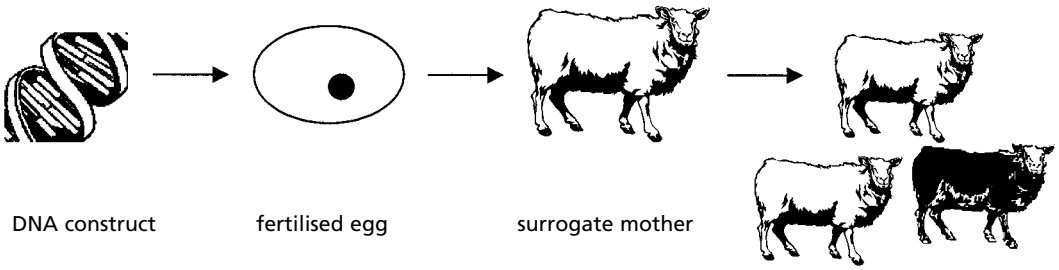
5.20 In order to over-express a gene, DNA is injected into the nucleus of a fertilised egg, which is then implanted into the uterus of a surrogate mother. A gene might also be eliminated (knocked out) or altered (knocked in) in ES cells, which are then injected into an early mouse embryo so that the cells derived from the modified ES cells develop into the tissues of the developing mouse. If cellular descendents of the ES cells form germ cells (sperm or eggs), these chimeric mice will produce offspring that have the eliminated or altered gene. Further breeding will produce some mice in which the gene has been completely eliminated or in which only the altered form of the gene is present (see Box 5.6).

9 See Schuler AM and Wood PA (2002) Mouse models for disorders of mitochondrial fatty acid ß-oxidation *Inst Lab Anim Res* **43**: 57–65.

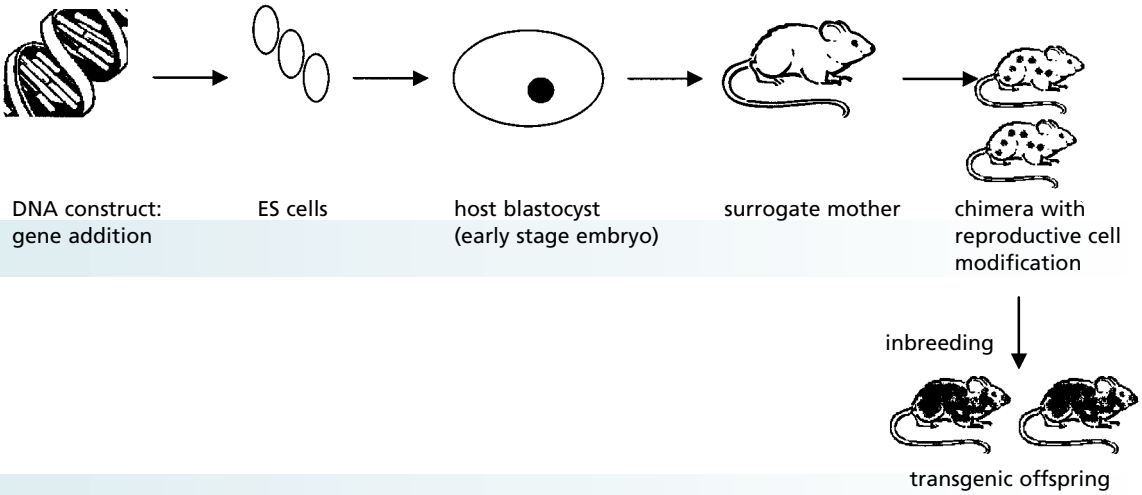
5.21 A specific gene can also be altered, over-expressed or deleted in particular cell types or at specific times, providing even more precise ways of studying gene function in animals. There

Box 5.6: Common techniques for creating transgenic animals

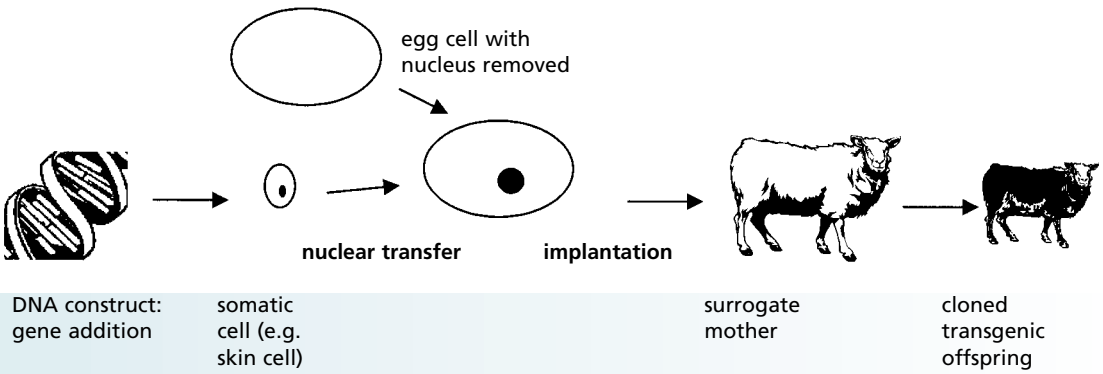
Pro-nuclear injection



Embryonic stem cells



GM followed by nuclear transfer



Pro-nuclear injection

In the 1980s the first transgenic animals were created by pro-nuclear injection, which allowed only random introduction of new DNA sequences into the genome.* DNA is injected into a fertilised egg that is then transferred to a recipient female. Only a small proportion of the injected eggs will produce a first-generation ('founder') transgenic animal containing the gene of interest. Therefore the resulting offspring need to be selectively bred in order to obtain a line of animals all with the desired traits. This method has been used in

Continued

mice, rats, pigs, sheep, cattle and goats. The efficiency is low as approximately three to five percent of the animals born as a result carry the transgene.*

Embryonic stem cells

ES cells can be used to modify the animals' own genes in a targeted way, although as yet this has only been successfully carried out in mice. DNA is manipulated in the ES cells before they are transferred to developing embryos. The technique allows for specific gene targeting, enabling the precise deletion or modification of specific genes. Correctly modified ES cells are identified and injected into a host blastocyst (an embryo at an early stage of development). This will develop into a chimeric animal consisting of both the host's original cells and the modified ES cells. Chimeric mice whose reproductive cells (sperm and egg cells) have arisen from the modified ES cells are then used as founder animals in selective breeding.*

Nuclear transfer

Nuclear transfer techniques (or 'reproductive cloning', see Figure 5.1) have been adapted to allow more precise modifications of the genome, allowing researchers to target specific genes. GM is carried out in a cultured cell before nuclear transfer. The nucleus from the modified cell is transferred to an oocyte (immature egg cell) which has had its nucleus removed. The oocyte and modified nucleus are combined through a process called 'cell fusion' and the resulting cell transferred to a recipient female. Viability and survival rates of embryos generated by nuclear transfer are low and it is estimated that less than three percent of the nuclear transfer embryos result in live offspring† (see paragraphs 5.28-5.29).

A relatively new technique involving the use of viruses to transfer DNA into the genome has the potential for much higher efficiency. It has been reported that 80–100% of the mice born following this technique are transgenic.*

* See Clark J and Whitelaw B (2003) A future for transgenic livestock *Nat Rev Genet 4:* 825–33.

† Roslin Institute (2002) Somatic Cell Nuclear Transfer (Cloning) Efficiency, available at: http://www.roslin.ac.uk/public/webtablesGR.pdf. Accessed on: 25 Apr 2005.

are between 22,000 and 25,000 genes in the mouse genome, and several hundred have already been specifically eliminated in mice. In principle, all of the remaining genes could be deleted in further studies, alone or in combination with other genes. Not all of these procedures would result in viable offspring, as the elimination of some genes would lead to the death of the developing embryo. However, more sophisticated techniques have been developed, such as producing 'conditional knock-out' animals, in which the gene deletion is only triggered for experimental purposes or in specific tissues.[10]

5.22 The welfare implications for animals used in these kinds of experiments cannot be predicted because it is not known beforehand what type of defect may be produced by the genetic modification (see paragraph 4.57). As we have said, licences require that research is stopped and animals are killed humanely if defined thresholds of pain or suffering are exceeded (paragraphs 5.13 and 12.21). Although many of the mice created have no obvious abnormality, others have severe developmental defects. For example, mice in which a growth factor receptor gene was knocked out had severe abnormalities including skeletal defects and profound deafness.[11] The methods by which GM animals are produced also have the potential to be painful and distressing (paragraph 4.58). Large numbers of animals are used to produce a single GM strain due to the relatively low efficiency of the methods used to achieve genetic modification. Usually, the majority of the animals that are produced do not have the desired genetic traits and are usually euthanised (see Box 5.6). More efficient methods would be desirable. Many strains of GM animals are expected to be established in the future. For example, it has been predicted that 300,000 new genetic lines of mice could be created over the next two decades.[12]

Study of protein and cellular function

5.23 Genetic modification can also be used to produce mice that express a fluorescent form of a particular protein under study. This intervention allows researchers to observe the location

10 For a review, see Cohen-Tannoudji M and Babinet C (1998) Beyond 'knock-out' mice: new perspectives for the programmed modification of the mammalian genome *Mol Hum Reprod* **4**: 929–38.

11 Colvin JS, Bohne BA, Harding GW, McEwen DG and Ornitz DM (1996) Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3 *Nat Genet* **12**: 390–7.

12 Abbot A (2004) Geneticists prepare for deluge of mutant mice *Nature* **432**: 541.

of specific proteins in living cells and to analyse their activity. The cells expressing these fluorescent proteins can be readily visualised in tissue using a fluorescence microscope and purified using a fluorescence-activated cell sorter. Fluorescent proteins themselves are not known to cause adverse welfare effects. Mice can also be engineered to express a toxic protein in a specific cell type so that cells of this type can be eliminated by the body. This technique is used as an effective way of determining the normal function of a particular type of cell.[13] Adverse effects on the animal would depend on the cell type that is eliminated.

Research tools and techniques

Production of antibodies

5.24 Antibodies are proteins that are widely used in many areas of biomedical research, as well as in clinical medicine. They are highly useful tools, as each antibody type recognises the specific 'foreign' molecule (antigen) against which it was produced. Antibodies of a particular type can therefore be used to identify, localise, quantify or purify an antigen. For example, antibodies might be labelled with fluorescent dyes and then used to locate specific molecules by fluorescence microscopy of tissues *in vitro* (i.e. in a tissue sample in the laboratory). They can also be labelled with enzymes and used to quantify specific molecules in blood or other fluids or tissues, as for example in the common pregnancy test. Antibodies are also used to purify cells or molecules by attaching them to magnetic beads. The antibodies bound to the cells or molecules of interest can then be 'attracted' out of solutions.

5.25 Antibodies are made by B lymphocytes, which develop in the bone marrow. In order to produce antibodies against an antigen of interest, an animal (usually a mouse, rabbit, sheep or goat) is administered with the antigen one or several times (immunised), together with a stimulant (an adjuvant), and the antibodies that are activated in response are then collected from the blood. Adverse effects depend on the dose, frequency of injections and the use of adjuvants, which can lead to irritation and the formation of an abscess. Immunisation can also occasionally lead to a severe allergic reaction (anaphylaxis), which can be fatal. If animals are used for the production of purified *monoclonal* antibodies (the ascites method), then serious adverse effects can occur. This procedure is rarely used in the UK, although antibodies made by this method may be imported from abroad.[14]

Animal cloning

5.26 The term cloning refers to the process of creating an identical copy of a gene, cell or a whole animal. Two types of cloning need to be distinguished: *reproductive* and *therapeutic*. The former is used to produce an animal that is virtually genetically identical[15] to the predecessor from which it was cloned (see Figure 5.1 and Box 5.6).

5.27 The main purpose of developing *reproductive cloning* techniques is to facilitate the targeted genetic modification of animals.[16] Research also seeks to explore their potential for novel medical applications such as providing organs for xenotransplantation (see paragraph 1.18). In addition, cloned animals could be used to rapidly increase the number of animals of a genetically identical strain and therefore might replace repeated inbreeding (paragraph 5.8). Cloned animals are being used to study age-related changes in cells, including cancers,

13 The specific use of GM animals as disease models is discussed separately (see Chapter 7).

14 No procedures were performed during 2003 in the UK using the ascites model. See Home Office (2004) *Statistics of Scientific Procedures on Living Animals Great Britain 2003* (London: HMSO).

15 A very small fraction of DNA (16,500 base pairs out of a total of 3,000 million base pairs in the human genome) is external to the nucleus, and therefore comes from the donor egg rather than the donor nucleus.

16 See Clark J and Whitelaw B (2003) A future for transgenic livestock *Nat Rev Genet* **4**: 825–33.

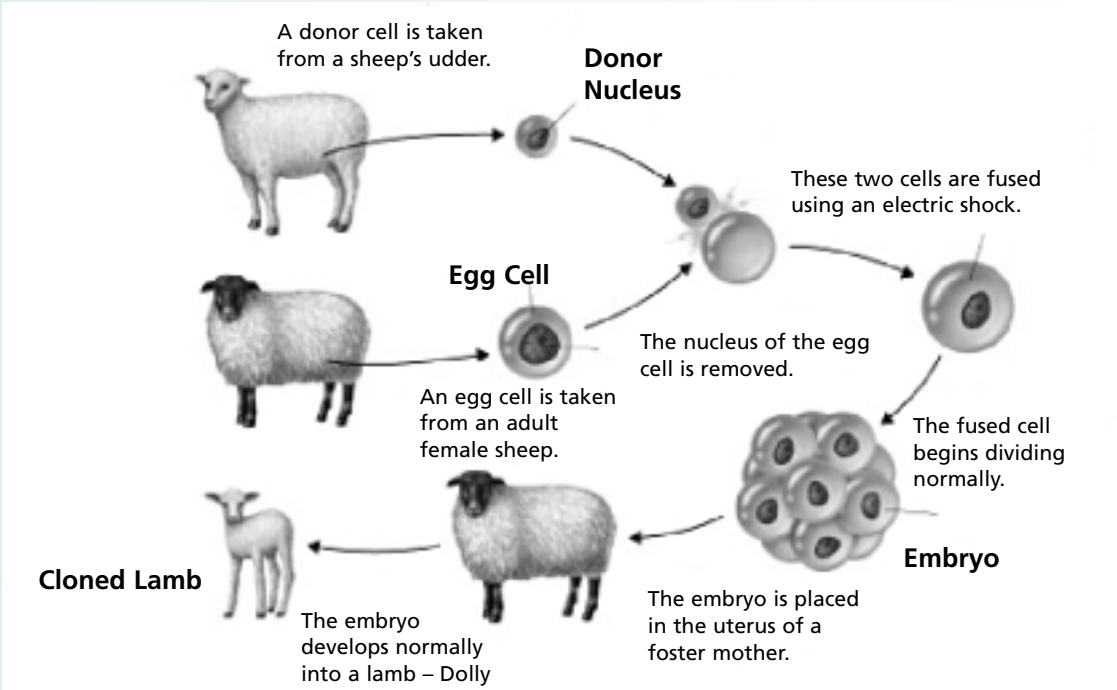


Figure 5.1 Reproductive cloning of a sheep using nuclear transfer*

Dolly the sheep was produced using nuclear transfer. If the embryo is used to make ES cells for research rather than a new individual, the procedure is called 'therapeutic' cloning (see paragraph 5.30).

* Miller KR and Levine J (2003) Biology (New Jersey: Pearson Prentice Hall).

and some people are hopeful that the approach could help to conserve endangered species. A range of other purposes are possible in principle, such as the breeding of champion racehorses, the replacement of deceased pets or 'pharming' (see paragraph 5.31).

5.28 The first animals to be cloned from the nuclei of adult somatic cells were amphibians. This significant work using tadpoles in the 1970s showed that somatic cells (and not only reproductive cells) contained all the information required to develop into the organism.[17] In 1996, Dolly the sheep was the first mammal to be cloned from a cell from an adult animal, and the event attracted worldwide media attention (see Figure 5.1). Other animals that have now been cloned include the mouse, rat, cow, goat, pig, cat, rabbit, mule and horse.[18] Certain cloned animals have also been 're-cloned' to produce a second generation of clones.[19] Scientists are using these animals to study the longer-term effects of cloning, in order to assess any possible developmental abnormalities and welfare implications.

5.29 Reproductive cloning of animals raises a number of concerns. The method is currently highly inefficient, requiring repeated attempts to remove eggs and implant embryos to obtain even a single viable clone. The cloning of Dolly the sheep, for example, required the production of 277 fused embryos. Of this number, 29 cloned embryos were transferred into surrogate ewes, from which one pregnancy resulted.[20] More recently, 358 eggs fused with skin cells from a cloned animal yielded two re-cloned bulls, one of which died shortly after

17 Gurdon JB, Laskey RA and Reeves OR (1975) The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs *J Embryol Exp Morphol* **34**: 93–112.

18 See UN Educational, Scientific and Cultural Organization (2004) *Human Cloning* (France: UNESCO).

19 Kubota C, Tian XC and Yang X (2004) Serial bull cloning by somatic cell nuclear transfer *Nat Biotechnol* **22**: 693–4.

birth. Attempts to create a third generation of clones failed after 248 embryos were fused, six of which resulted in pregnancies, but all failed to develop into viable calves.[21] Cloning also has implications for animal health. Large offspring syndrome, in which the animals are too large for normal birth, occurs frequently, and cloned animals may also show signs of early aging. Dolly the sheep was euthanised in March 2003, six years after her birth, after suffering progressive lung disease and arthritis. These conditions are not uncommon in sheep of this age, and it is uncertain whether cloning was a factor.

5.30 The term *therapeutic cloning* is used to refer to the technique of producing ES cells that are genetically identical to the donor of the nucleus. ES cells, isolated from developing embryos, have the unique potential of being able to develop into different types of cells and to reproduce indefinitely. Therapeutic cloning could improve the prospects for the development of cell replacement therapy in humans. Genetically foreign cells (from another person) would be rejected unless the immune system was suppressed with powerful pharmaceuticals that may need to be taken for many years. However, if ES cells were produced from a cloned embryo made with the nucleus from one of the patient's own cells, they will be almost genetically identical. Cells and tissues made from these ES cells would not be rejected if transplanted into this patient (see paragraph 5.8). Advocates of this technique, currently being used in research with animals, hope that it could be used to treat patients suffering from conditions such as Alzheimer's disease and Parkinson's disease (see paragraph 5.10). Some preliminary work with cloned human embryos in the first few days of development has recently been licensed in the UK.[22]

'Pharming'

5.31 The term 'pharming' refers to the production of pharmaceuticals in plants or animals. Although, strictly speaking, pharming does not fall within the category of basic research, given its potential applications we consider it here as research in the area is still in its infancy. In plants, pharming generally involves the genetic modification of a crop plant in order to produce substances which can be extracted and processed into refined compounds. In animals, a potential pharming technique involves the transfer of human genes that encode specific therapeutic proteins. If the method is successful, the proteins which would be produced in milk, eggs or blood could be isolated for further processing. Sheep, goats and cows are used the most frequently in research on pharming as they produce relatively large quantities of milk. The production of these therapeutic proteins by other means can be technically difficult, expensive and time-consuming.

5.32 Clinical trials to test pharmed medicines have been initiated. The company PPL Therapeutics produced alpha-1 anti-trypsin (AAT), a treatment for emphysema and cystic fibrosis which was used in trials at hospitals in Europe, Canada, Australia and New Zealand. It was initially hoped that genetically engineered AAT would be on the market by 2007 but the project ceased in 2003. The European Medicines Agency (EMEA) is currently reviewing a Market Authorization Application for the pharmed pharmaceutical ATryn (human anti-thrombin).[23] It was developed to treat patients with hereditary anti-thrombin deficiency, a condition resulting in vulnerability to deep-vein thrombosis. The human gene for the required protein

20 Wilmut I, Schnieke AE, McWhir J, Kind AJ and Campbell KHS (1997) Viable offspring derived from fetal and adult mammalian cells *Nature* **385**: 810–3.

21 Kubota C, Tian XC and Yang X (2004) Serial bull cloning by somatic cell nuclear transfer *Nat Biotechnol* **22**: 693–4.

22 Human Fertilisation and Embryology Authority (HFEA) (2004) Press release *HFEA grants the first therapeutic cloning licence for research*, available at: http://www.hfea.gov.uk/PressOffice/Archive/1092233888. Accessed on: 24 Apr 2005; HFEA (2005) Press release: *HFEA grants embryonic stem cell research licence to study motor neuron disease*, available at: http://www.hfea.gov.uk/PressOffice/Archive/1107861560. Accessed on: 24 Apr 2005.

was inserted into an egg cell from a goat and activated only in udder cells so that it was possible to extract it from the goat's milk (see Box 5.6).[24] A number of other companies are also developing transgenic animal proteins.[25]

5.33 With regard to implications for animal welfare, there is some uncertainty as to whether the GM process may cause unexpected side effects. Genes may not always be expressed in the intended tissues or at appropriate levels, since insertion of microinjected DNA into the genome can be random (see Box 5.6). Advances in the process are aiming to overcome this problem, for example by designing the inserted DNA to ensure that it is only expressed in the intended tissue (a technique used in the production of ATryn). There are also concerns that the pharmed proteins might cause a toxic reaction.

Summary

5.34 In this chapter we have discussed five areas of basic research: behavioural studies, physiological studies, studies on development, genetic studies and the use of animals in the development of research tools and techniques such as the production of antibodies and biopharmaceuticals. Research in all of these areas has provided much of what we know about biological systems and their functioning. While most of this activity has sought to contribute to the body of scientific knowledge, it has also led to the discovery of treatments for human diseases (see Boxes 5.2 and 5.4).

5.35 Basic research has enabled scientists to relate knowledge about animal behaviour to knowledge of animal physiology and, more recently, genetics. The results have been compared to human data to further knowledge of human biology and medicine. Genetic studies using animals have enabled the discovery of the location and function of individual genes, many of which play similar roles in a range of different species. We have discussed how research tools such as antibodies have proved invaluable for the development of molecular biology and how new techniques in genetics, including cloning and pharming, may allow advances in treatments for human diseases.

5.36 The impact of basic research on the welfare of the animals that are used is as varied as the types of research. It ranges from little impact to serious consequences for the animals' welfare. The former comprises research such as the observation of animals in their natural habitats, whereas the latter comprises research that changes the normal functioning of an animal through, for example, surgery or infection with a disease. New technologies, including genetic modification, cloning and pharming, also have the potential to adversely affect welfare. For example, the technical difficulties involved in cloning mean that a great number of animals are necessary to produce a single cloned animal. The number of animals that will be used in genetic research is expected to increase very substantially in the next few years. We noted, for example, that 300,000 new transgenic mouse lines could be created over the next two decades. A particular cause of concern regarding GM is that any implications for welfare are difficult to predict and that current techniques are relatively inefficient, requiring large numbers of animals for the production of a single GM strain.

23 GTC Biotherapeutics (2004) *ATryn® – Recombinant Human Anti-thrombin*, available at: http://www.transgenics.com/products/atryn.html. Accessed on: 24 Apr 2005.

24 See (2004) Down on the pharm *The Economist: Technology Quarterly Supplement* 16 Sept, pp34–5.

25 These include Nexia and Viragen. See Viragen *Avian Transgenic Technology*, available at: http://www.viragen.com/avian_intro.htm. Accessed on: 24 Apr 2005; Nexia Biotechnologies *Protexia™ – A Bioscavenger*, available at: http://www.nexiabiotech.com/en/01_tech/09.php. Accessed on: 24 Apr 2005.