Chapter 6

The use of animals in the study of human disease

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Introduction

6.1 In this chapter, we consider some of the principles and rationales of using animals as disease models. We examine in more detail two areas of recent medical advance: new therapeutic strategies for rheumatoid arthritis (RA), which also illustrates the contribution and use of non-animal models of disease, and the development of the scientific understanding of transmissible spongiform encephalopathies (TSEs), including bovine spongiform encephalopathy (BSE) and variant Creutzfeld–Jakob disease (vCJD). We also describe the role of animal research in the implementation of public health policies for protecting humans from exposure to TSE agents. These examples are followed by brief discussions of historically important animal disease models for hepatitis C and polio. We then consider two cases of diseases that have proved difficult to treat and cure, despite the availability of animal models: Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) and cancers.

The pathogenesis of disease

6.2 The study of the causes of disease is known as *etiology*. The mechanisms by which a disease develops, causes tissue damage and spreads within the body are known as *pathogenesis*. Understanding the etiology and pathogenesis of a disease is usually necessary in order to develop strategies to either prevent or limit disease. For example, a disease may be prevented by vaccination or the use of antibiotics. The effects of a disease may be limited by means of therapies and therapeutics that reduce inflammation or stop further tissue degeneration.

6.3 Most diseases are complex and involve dynamic interactions between molecular and cellular systems, which influence the development of the disease process.[1] Biologists who study a particular disease often use a variety of methods, both animal and non-animal, to investigate its mode of action. For example, pathogenesis studies with animal models are generally complemented by clinical, epidemiological and imaging studies using humans. While all of these areas are very important, researchers whose work involves living animals consider that their research plays a special role in the study of the pathogenesis of diseases of animals and humans, because it is often the most effective method of studying the complex interactions between molecules, cells and organs that occur in disease processes. For example, transferring a disease from one animal to another is commonly held to be the most reliable way to establish that a disease is caused by an infectious agent. This principle was first demonstrated in the 19th century when mice were injected with blood from cows infected with anthrax. The research showed clearly that the mice subsequently developed the disease.[2]

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1 Examples include diseases in which high levels of antibodies and microbial or tissue antigens form immune complexes (a complex of antigen and antibodies in the blood circulation). These complexes can activate powerful inflammatory systems (the complement or coagulation cascades) that recruit different molecular and cellular systems into the process of pathogenesis. Effects include widespread damage to blood vessels (vasculitis), the kidney (nephritis), skin (dermatitis) or brain (meningitis).

2 The Nobel Foundation (1967) *Nobel Lectures, Physiology or Medicine 1901–1921* (Amsterdam: Elsevier Publishing Company), see *Robert Koch – Biography*, available at: http://nobelprize.org/medicine/laureates/1905/koch-bio.html. Accessed on: 12 Apr 2005.

Comments on the use of animals for the study of human diseases from respondents to the Consultation

'To make any real progress in biological research there is no alternative but to use animals.'
Professor Julian Blow

'The fact that animal research provides essential information that is of benefit to both humans and animals is well proven and current available vaccines, surgical procedures and treatments available…support this argument. In many instances this information could not have been provided by any other method…'
Institute for Animal Health, Compton Laboratory

'Animals are important as biological processes are complex and cannot be replaced or simulated properly by computers.'
Mr Kedarraja Kistnareddy

'Research using whole animals has been fundamental to our understanding of whole-animal and whole-organ physiology for decades and will remain so indefinitely.'
Dr RM Ridley and Dr HF Baker

'It is not proved that animal research is a superior route to information. Transference of results can, and has, proved misleading.'
International Primate Protection League UK

'Whether rodents are the best animal to study for research related to human disease is debatable but practicalities dictate that these are often used and related results in the scientific literature are likely to focus on rodents.'
Professor Bernie Hannigan

'The only reliable model for a human is a human.'
Anonymous

New therapeutic strategies for rheumatoid arthritis

6.4 RA is one of the most common human autoimmune diseases, affecting up to 600,000 individuals in the UK. It is a crippling disease resulting primarily in a chronic inflammation of joints of the hands, feet, knees, vertebrae or hips. It typically leads to progressive degeneration of the joint tissues with consequent disability and premature death. Although the exact cause of RA is unknown, in the last ten years there have been very considerable advances in the understanding of the molecular and cellular basis of the disease process. Animal models of arthritis have been used to study these processes and to devise and test new treatments. A successful treatment for RA has been developed, which has also led to therapeutic interventions for other chronic inflammatory conditions (see paragraph 6.10).[3]

6.5 There has been some debate about the relative relevance and contributions of *in vitro* and *in vivo* animal work to the study of RA. A review of the literature reveals that both animal models of arthritis and *in vitro* studies with human RA joint tissue have been used simultaneously and often by the same researchers. It would therefore be wrong to describe particular significant steps in the understanding of RA as having relied only on *in vitro* or *in vivo* methods. Experiments using both approaches relied on the results of previous experiments with animal and human tissue, live animals and human volunteers.

6.6 RA in humans is characterised by a chronic inflammation of the lining of the joint capsule (synovium). Inflammatory cells invade the synovial membrane of the joint, and there is excessive local secretion of molecules (cytokines) that produce inflammation. In the late 1980s, several groups of researchers started to examine the possible role of these molecules in RA after various cytokines were detected in the synovial fluid of patients.[4] It became clear by the early 1990s from studies on human tissue[5] and, later, in animal models of arthritis that the inflammatory process depends on a cytokine known as tumour necrosis factor alpha

3 Vilcek and Feldmann M (2004) Historical review: cytokines as therapeutics and targets of therapeutics *Trends Pharmacol Sci* **25**: 201–9.

4 For example, see Hopkins SJ and Meager A (1988) Cytokines in synovial fluid: II The presence of tumour necrosis factor and interferon *Clin Exp Immunol* **73**: 88–92.

5 A seminal discovery was made by Brennan FM, Chantry D, Jackson A, Maini R and Feldmann M (1989) Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis *Lancet* **2**: 244–7, who concluded that TNF plays a pivotal role in arthritis using inflamed tissues from patients of the disease; described later in Higgs G (2004) Molecular genetics: the Emperor's clothes of drug discovery? *Drug Discov Today* **9**: 727–9.

(TNFα).[6] Enhanced TNF production in the affected joints results in release of other cytokines and of growth factors that cause abnormal growth of new blood vessels, increased blood flow and destruction of cartilage. Once the crucial role of TNF became clear, it was proposed that neutralising TNF or switching off its production in the joint might reverse joint inflammation. Researchers were able to test the usefulness of neutralising TNF with anti-TNF antibodies,[7] both in *in vitro* studies with human joint tissue and in an arthritis model in rodents. In both cases, the antibodies reduced inflammation in joint tissue by binding specifically to the TNF molecules.[8] Thus, researchers used *in vivo* studies of rodent arthritis models to complement *in vitro* studies of human RA joint tissue to understand the pathogenesis of immune arthritis.

The rodent model for arthritis

6.7 The rodent arthritis model is produced by the injection of bovine or chicken collagen,[9] together with a chemical that increases the resulting immune reaction, into inbred strains of mice or rats. Swollen joints and arthritis appear within 20–40 days. Although collagen-induced arthritis in the mouse does not exactly mimic RA in humans, it has a number of similarities. For example, the model allowed the primary role of TNF in joint inflammation to be examined, as it is common to both forms of arthritis. The mouse model for arthritis played a significant role in the development of the current and successful therapeutic intervention of blocking TNF to alleviate RA in humans.

6.8 Once arthritis develops, a painful swelling of the paws occurs, accompanied by erosions of the joint cartilage. In humans, painful swelling is accompanied by pain in the extremities. Similar effects resulting from the inflammation occur in mice, which may affect the welfare of the animal considerably since rodents use their front feet extensively for grooming, holding food, eating and moving around. Severely affected animals are usually euthanised before the end of the experiments.

Human clinical trials

6.9 It had been demonstrated *in vitro* that antibodies against TNF (anti-TNF) reduced the production of other cytokines involved in the inflammatory response.[10] Subsequent animal experiments established that anti-TNF could be used to reduce the symptoms of inflammatory joint disease without seriously impairing the function of other tissues and organs. Clinical trials to assess the effect of anti-TNF reagents in humans began in 1992. Infliximab, a monoclonal antibody against human TNF, was used in a series of trials in patients to test the safety, efficacy and pharmocokinetics of anti-TNF therapy. The therapeutic dose used for the human trials was based on the mouse studies.[11] The clinical results in RA patients treated with infliximab demonstrated substantial benefits: patients reported alleviation of symptoms such as swelling, pain, stiffness, tiredness and lethargy

6 The abnormal synthesis of TNF by cells invading the joint capsule amplifies the inflammatory cell cascade, triggering the release of other inflammatory cytokines which cause tissue damage when present in excess.

7 Anti-TNF antibodies bind specifically to TNF molecules. For a description of the function of antibodies, see paragraphs 5.24-5.25.

8 Williams RO, Feldmann M and Maini RN (1992) Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis *Proc Natl Acad Sci USA* **89**: 9784–8. For a review and references to simultaneous work, see Vilcek and Feldmann M (2004) Historical review: cytokines as therapeutics and targets of therapeutics *Trends Pharmacol Sci* **25**: 201–9.

9 Collagen is a tough, fibrous protein that forms a major component of skin, tendons, bones, cartilage and other connective tissues. It helps to hold cells and tissues together.

10 Brennan FM, Chantry D, Jackson A, Maini R and Feldmann M (1989) Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis *Lancet* **2**: 244–7.

11 Vilcek and Feldmann M (2004) Historical review: cytokines as therapeutics and targets of therapeutics *Trends Pharmacol Sci* **25**: 201–9.

a short time after being treated with the medicine. The first study was carried out in RA patients in which all other available therapies had failed. Following the success of this initial trial, larger studies were performed at four European centres.[12] These were followed by successful repeated-dose studies, which showed a long-term therapeutic benefit of the treatment.

6.10 Several types of anti-TNF treatments have now been approved by regulatory authorities in the USA and Europe and represent a major advance in the treatment of RA.[13] So far, more than 200,000 patients have been successfully treated, with marked improvement in their physical activity and quality of life. Anti-TNF therapy has now been adapted successfully to treat other chronic inflammatory conditions including inflammatory bowel disease (Crohn's disease), the rheumatic disease ankylosing spondylitis, psoriasis and psoriatic arthritis.[14]

The transmissible spongiform encephalopathies

6.11 The TSEs are a cluster of degenerative brain diseases. The prototype TSE is scrapie in sheep, but a range of TSE diseases affect different species, including humans. Kuru is a human TSE that was once endemic in New Guinea. It was transmitted by ritualistic cannibalism, which involved eating the brain tissue of other people. The most common TSE in humans is Creutzfeld–Jakob disease (CJD), which occurs sporadically in the human population, with an annual incidence of about one person per million.[15] Kuru was the first human TSE that was shown to be transmissible and this was achieved by injecting brain material from patients into chimpanzees. A similar approach showed that CJD could be caused by a transmissible agent, whereas most other neurodegenerative diseases, such as Alzheimer's disease or Parkinson's disease, are not transmissible.

6.12 In 1986, a new TSE disease, BSE, was recognised in cattle. It reached epidemic proportions in the UK in the following few years, leading to over 180,000 cases. The origins of BSE have never been established, but it is thought that the epidemic was caused by the now-prohibited practice of feeding ruminant-derived meat and bone meal (MBM) to ruminants as a protein supplement. Evidence of infection with the BSE agent also appeared in zoo animals that had been fed MBM or bovine carcasses, and in domestic cats, which had presumably consumed bovine products in cat food and developed a feline form of BSE.

6.13 In 1996, the first human cases of a new type of TSE, known as vCJD, were observed in young people in the UK. The causative agent of vCJD was shown to be indistinguishable from the BSE agent and infection was presumed to have been caused by eating BSE-contaminated food. By April 2005, 155 cases of definite or probable vCJD had been confirmed in the UK with an average age of onset of clinical symptoms of 29 years of age, and median duration

12 Vilcek and Feldmann M (2004) Historical review: cytokines as therapeutics and targets of therapeutics *Trends Pharmacol Sci* **25**: 201–9.

13 In a further series of experiments involving the mouse collagen arthritis model, it was shown that the severity of chronic arthritis could be reduced with a combination of anti-TNF antibodies and antibodies against T cells. There later followed a Phase III clinical trial combining anti-TNF treatment with a conventional immunosuppressive treatment to inactivate T cells in the joint lesions. As in the case of the studies in mice, this refinement of anti-TNF therapy proved successful in halting the progressive degenerative changes in the joint cartilage and bone in affected joints in patients who were resistant to conventional drug-based treatment.

14 Vilcek and Feldmann M (2004) Historical review: cytokines as therapeutics and targets of therapeutics *Trends Pharmacol Sci* **25**: 201–9.

15 Three forms of the disease had been recognised prior to 1986, sporadic, inherited and iatrogenic (acquired through medical intervention). See The BSE Enquiry (2000) *Report of the BSE Enquiry*, Volume 2, Chapter 2, available at: http://www.bseinquiry.gov.uk/report/volume2/chaptea2.htm#817773. Accessed on: 12 Apr 2005.

of illness of 14 months, leading to death.[16] As the incubation period of TSEs can last for many years, the extent of human infection with the vCJD agent is unknown. For Kuru, the average incubation period was approximately ten years, but in some cases it exceeded 40 years. Thus, human cases of vCJD may continue to appear well into the 21st century. The BSE epidemic in cattle and the sudden appearance of previously unrecognised TSEs in humans and other species led to an unprecedented focus on experimental animal models of these diseases.

The prion hypothesis

6.14 For many years, the nature of the agent that caused TSEs was unknown. Research showed that they were not caused by classical infectious agents, such as viruses or bacteria. Lack of evidence that any form of DNA or RNA was involved led to the development of the *prion hypothesis*. According to this theory, TSEs were caused by a replicating abnormal form of a protein (a prion), which imprinted its configuration on normal molecules. This would allow prions to be transmitted between animals or humans, causing the disease. This novel hypothesis has subsequently been supported by a large number of experiments, most of which involved inducing TSE in animals.[17]

Animal models for TSEs: understanding the disease process

6.15 The pathogenesis of TSE diseases is complex and involves transfer and replication of the infectious agent (a prion), which spreads to the CNS via the blood or nerves. Prions do not induce an immune response. The pathology involves the accumulation of abnormal prion proteins in the brain and lymphoid tissues, and the degeneration of nerve cells (spongiosis). The pathogenesis of these diseases cannot be studied *in vitro* as they involve various physiological systems such as the alimentary tract, lymphoid tissue, nerve routes, peripheral ganglia and various brain regions.

6.16 One of the major steps in the study of the pathogenesis of TSEs was the development of experimental mouse models for the sheep disease scrapie, which had long been recognised as being transmissible between sheep. Transmission of the scrapie agent to mice (by injection of an extract of infected brain tissue from affected sheep into the brain) led to the development of a series of mouse models for scrapie. They were used to identify significant stages in the development of this disease and in defining the different strains of the infectious agent. These studies established that the agent was an abnormal form (PrPsc) of a normal protein (PrP). GM mice in which the PrP gene had been knocked out (see paragraph 5.19) were found to be completely resistant to scrapie, as there is no PrP protein for the PrPsc protein to convert to prions. With regard to welfare implications, mice involved in research on the developmental stages of scrapie typically experienced progressive neurological dysfunction, behavioural and gait abnormalities as well as weight loss. Researchers aimed to limit suffering by euthanising animals at the stage when they were unable to eat or drink without assistance. In some cases, animals were euthanised when they reached certain stages that were known to precede the experimentally induced terminal disease.[18] Other welfare implications may arise from the

16 The National Creutzfeldt–Jakob Disease Surveillance Unit (2005) *CJD Statistics*, available at: http://www.cjd.ed.ac.uk/figures.htm. Accessed on: 12 Apr 2005; World Health Organization (2002) Fact sheet: *Variant Creutzfeldt–Jakob disease*, available at: http://www.who.int/mediacentre/factsheets/fs180/en/. Accessed on: 12 Apr 2005.

17 For a description of the identification of BSE as a TSE, see The BSE Enquiry (2000) *Report of the BSE Enquiry*, Volume 2, Chapter 2, available at: http://www.bseinquiry.gov.uk/report/volume2/chaptea2.htm#817773. Accessed on: 12 Apr 2005.

18 As defined in, for example, Dickinson AG, Meikle VM and Fraser HJ (1968) Identification of a gene which controls the incubation period of some strains of scrapie agent in mice *Comp Pathol* **78**: 293–9; Thackray AM, Klein MA, Aguzzi A and Bujdoso R (2002) Chronic subclinical prion disease induced by low-dose inoculum *J Virol* **76**: 2510–7.

fact that some mice used in this type of research are allowed to age. They may therefore show signs related to old age, such as abscesses, starey coats (not lying flat) or holding their tails abnormally.

6.17 Similar experimental studies have demonstrated the transmissibility of BSE between cattle, sheep and primates. Transmission of BSE to monkeys by injection of bovine prions into the brains of macaques was the first demonstration that BSE was able to cross the species barrier from ruminants to primates. These experiments, undertaken in 1996 in the UK and France, were a forewarning that BSE might be transmissible to humans.

6.18 As there is no immune response to prion infection, it has not yet been possible to develop diagnostic tests that demonstrate the presence of the disease before symptoms occur. Although there is now a range of biochemical markers for detecting the abnormal protein in potentially affected tissues, infection of mice remains the accepted standard for diagnosing prion diseases.

6.19 In view of the potential number of human cases, it is important to develop intervention strategies aimed at slowing down or preventing the spread of prions. This may eventually be achieved by treatment with medicines or through the development of a vaccine. A vaccine could theoretically stimulate the production of antibodies to PrPsc, thus preventing prion proteins from spreading *in vivo*. Scientists using animals in research with this aim assert that the development of effective therapeutic strategies is likely to depend on continued research on animals.

The contribution of animal models for TSEs to public health policy

6.20 The *in vivo* models for the pathogenesis of TSEs have had decisive influence on the development of policies for public health aimed at controlling these diseases in cattle and sheep, and to protect humans from further exposure to agents of animal TSEs.[19] The current public health measures are based on evidence obtained from experiments on the pathogenesis of TSEs in cattle, sheep, pigs and chickens. Without these studies, it would have been difficult to know how to devise and implement public health measures, other than to prohibit the eating of any animal products, since, at the time, researchers were not able to undertake the research by alternative, non-animal methods.

BSE pathogenesis and public health measures

6.21 In several large-scale studies on the pathogenesis of BSE, scientists infected calves by feeding them with an extract of cow brain taken from an animal with the disease. The spread of infectivity was then monitored in various tissues. Infectivity was determined by administering extracts of tissue to mice and assessing if they develop the disease (see paragraph 6.18). Several hundred cattle and several thousand mice were used in these experiments. These studies established unequivocally that BSE replicates early on in the gut lymphoid tissues and then spreads to other lymphoid tissue and via major nerves to the CNS. The highest levels of infectivity were found in gut-associated lymphoid tissue, major nerves in the head and neck, brain, spinal cord and collections of nerve cells embedded in the vertebral column known as the dorsal root ganglia. Little infectivity was detected in skeletal muscles.[20]

19 Experimental transmission studies in pigs and chickens, for example, showed that these animals are not susceptible to BSE when fed infected tissue, thus allaying fears that pigs and poultry, which were also exposed to infective MBM, could be infectious for humans through the food chain.

20 The BSE Enquiry (2000) *Report of the BSE Enquiry*, Volume 2, Chapter 3, available at: http://www.bseinquiry.gov.uk/report/volume2/chaptea8.htm#821257. Accessed on: 26 Apr 2005.

6.22 The results from these and many similar studies on the pathogenesis and transmission of TSE between animals have been used to develop policies for public health to prevent the transmission of BSE from cattle through the human food chain. Specifically, they led to the banning of bovine offal for human consumption, the removal of brain, spinal cord and dorsal root ganglia, and the deboning of beef intended for public consumption. Based on knowledge of the dynamics of the spread of prions *in vivo*, the pathogenesis studies also provided the evidence for the development of the initial *Over Thirty Month Scheme* (OTMS), whereby the UK Government was able to purchase, for slaughter and ultimate destruction, cattle which were over 30 months of age. This implemented EU Regulations that ordered the prevention of the sale of beef from cattle over this age for human consumption in the UK. The OTMS was a crucial element of legislation for public health, and it may well have averted a larger number of vCJD cases than experienced so far.[21]

BSE pathogenesis studies in sheep – a model for vCJD

6.23 Sheep are susceptible to infection with the BSE agent, and the dynamics of infection and spread of prions in peripheral tissues is similar to that of vCJD in humans. Thus sheep are commonly held to be a useful model for vCJD. Studies of scrapie in sheep were the first to show that prions could accumulate in the tonsils, and this was shown subsequently to be the case for vCJD. There followed an analysis of the prevalence of vCJD in the human population through retrospective studies on tonsils, and later appendices. The results provided the first information on the number of people that could be incubating the disease.

6.24 BSE pathogenesis studies in sheep also showed that blood can be infectious. BSE can be transmitted between sheep by blood transfusion and current experiments are aimed at identifying the blood fraction that contains infectivity. Scientists conducting these experiments are also interested in exploring the implications of human-to-human transmission of vCJD through blood and have guided UK policy for public health by limiting the potential for this type of spread of vCJD. In 2003, it was found likely that two people who died of vCJD were infected by blood transfusions. As a result, the Department of Health announced in 2004 that anyone who had received a blood transfusion in the UK since 1980 would no longer be able to donate blood themselves.[22]

The discovery of the hepatitis C virus using the chimpanzee

6.25 We now consider a more historic example of animal research for the study of disease. The existence of a blood-borne hepatitis virus that was neither type A nor B was described in the 1970s, following the identification of both these types. Throughout the 1980s, assays were developed to try and identify the cause of what was then termed non-A, non-B (NANB) hepatitis. However, none of the tests were sufficiently reproducible or specific.[23] Therefore an experimental chimpanzee model was developed, as this species was the only non-human animal that could be infected with the NANB hepatitis agent, which is still not able to be propagated *in vitro*. The chimpanzee model was used to demonstrate that NANB hepatitis was indeed transmissible, and allowed the isolation and characterisation of the virus.[24]

21 In 2004–5, the UK Government is implementing a transition towards replacing the OTMS with testing for BSE in cattle of over thirty months of age. See Department for Environment, Food and Rural Affairs (2005) *BSE: Public health issues – Over Thirty Month cattle – FSA review of the OTM rule*, available at: http://www.defra.gov.uk/animalh/bse/publichealth/otm/review/index.html. Accessed on: 26 Apr 2005.

22 National Blood Service (2004) *Variant CJD and blood donation*, available at: http://www.blood.co.uk/pdfdocs/vcjd.pdf. Accessed on: 26 Apr 2005.

23 Farci P (2002) A commentary on the original *Science* paper (Choo QL, Kuo G, Weiner AJ *et al*. (1989) Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome *Science* **244**: 359–62) *J Hepatol* **36**: 582–5.

24 Farci P (2002) A commentary on the original *Science* paper (Choo QL, Kuo G, Weiner AJ *et al*. (1989) Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome *Science* **244**: 359–62) *J Hepatol* **36**: 582–5.

Researchers used large volumes of blood from an infected chimpanzee with a high level of infection to isolate the virus. Proteins in the chimpanzee blood were then screened against serum from a NANB hepatitis patient, which was expected to contain anti-NANB hepatitis antibodies. Eventually a NANB hepatitis viral protein in the chimpanzee blood was found to react with antibodies from the human patient, possibly due to the high levels of viral particles in the chimpanzee blood. With the genome available, it was possible to develop reliable diagnostic tests for what was subsequently termed hepatitis C. Treatment strategies have also been developed in animals although a vaccine does not yet exist.

6.26 The animals in the study described above could be expected to suffer symptoms similar to those experienced by humans, especially at high infection doses. According to the US National Center for Infectious Diseases, 80 percent of people with hepatitis C have little or no signs or symptoms, whereas others may experience jaundice, fatigue, dark urine, abdominal pain, loss of appetite, nausea and eventually chronic liver disease.[25] Additional implications for welfare relate to the long-term husbandry of the infected animals as they may be infectious to other animals and to humans, and must therefore be kept in single housing.

6.27 The major cause of hepatitis C infection was formerly blood transfusion.[26] It is now routine to screen donated blood for hepatitis C, which has vastly reduced transfusion-mediated infection in industrialised countries. The discovery and characterisation of the virus, its role as the etiological agent and the mechanisms whereby it produced disease in chimpanzees led to an understanding of the primary role of the virus in post-transfusion hepatitis and its tendency to induce persistent infection and chronic liver disease. Approximately 170 million people worldwide are chronically infected with hepatitis C,[27] many of whom will develop cirrhosis and liver cancer.

6.28 Because of the long asymptomatic period (up to 20 years), most infected people are unaware that they carry the virus and continue to be a source of new infections. Diagnostic assays to detect the virus are therefore essential to identify these patients. Current work on chimpanzees is not permitted in the UK, as the Home Office does not grant licences for research involving the great apes (see paragraph 13.6). Without the research described above, very little would be known about hepatitis C, and diagnostic tests would not be available. Many scientists believe that the lack of a reliable animal model other than the chimpanzee is the single greatest barrier blocking the development of a safe and effective vaccine.

Study of polio and the development of polio vaccine

6.29 Animal disease models have been used in the study of poliomyelitis (polio), enabling an understanding of the disease process at the cellular level and facilitating the subsequent development of an effective vaccine. The polio virus enters the body through the mouth, from where it can travel to the digestive system and enter the bloodstream. The virus invades the CNS and destroys motor nerve cells, leading to paralysis and sometimes death. Before vaccines were introduced in developed countries in the late 1950s and early 1960s, polio was a common disease, estimated to be responsible for crippling more than half a

25 National Center for Infectious Diseases (2005) *Viral Hepatitis C*, available at: http://www.cdc.gov/ncidod/diseases/hepatitis/c/fact.htm. Accessed on: 26 Apr 2005.

26 Alter HJ, Purcell RH, Shih JW *et al*. (1989) Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis *N Engl J Med* **321**: 1494–500.

27 World Health Organization (2000) Fact sheet No. 164 *Hepatitis C,* available at: http://www.who.int/mediacentre/factsheets/fs164/en/. Accessed on: 26 Apr 2005.

million people around the world per year.[28] Since the introduction of vaccines, polio has largely been eliminated from industrialised countries.[29]

6.30 It had long been thought that polio was infectious, and in 1908 two researchers aimed to induce polio in several animals by injecting them with extracts of spinal cord material from a boy who had died of the disease. While the extracts did not cause polio-like disease in rabbits, guinea pigs or mice, the disease manifested itself quickly in Old World monkeys. Later, researchers were able to transmit polio from monkey to monkey by the injection of extracts of diseased spinal cord. Thus the virus could be propagated and an animal model of the disease was created. Use of this animal model in further studies led to the identification of the polio virus. Welfare implications for animals used in this early research extended over a broad range, but could be expected to resemble some of the symptoms experienced by humans.

6.31 In 1939, researchers were able to adapt one of the strains of the polio virus to make it infectious to mice, thus creating a more convenient rodent model for the disease. In the 1940s, researchers who were subsequently awarded a Nobel Prize demonstrated that the polio virus could be grown in cultured human cells, a property essential for future research on the virus. It was still not possible to observe the virus under the microscope at that time. Therefore, in order to confirm that the virus did propagate in cultured tissue, fluid was injected from the cultures into animals to observe if the disease developed.[30] In 1949, research on rodent models showed that there are in fact three types of polio virus.[31] In the 1950s Dr Jonas Salk used cultured monkey kidney cells to grow the virus. He then used the virus particles to produce the first vaccine which was found to be very effective at preventing the disease in humans, although people could still carry and spread the virus if it invaded their intestinal tract. In the 1960s, a new oral vaccine against the disease was developed. This vaccine contained live virus which had been 'attenuated', or weakened, by repeatedly growing it in cultured monkey cells. The vaccine produced an adequate immune response without causing an infection. The live attenuated virus, however, can sometimes revert to a virulent form and cause infection. Animals are currently used to test the potential virulence of each batch of vaccine that is produced to overcome the problem of occasional vaccine-associated poliomyelitis (see Box 8.5).

6.32 Mice and monkeys were used during important stages of the study of polio and the subsequent development of the vaccine. However, the initial development of the polio vaccine is regarded by some as an example which shows that animal research is misleading.[32] The early research was controversial because, in the first half of the 20th century, the dominant scientific theory was that the polio virus entered the body through the olfactory nerves of the nose, as indicated by experiments in monkeys. Scientists, particularly in the USA and Canada, inferred from these observations that it would be useful to develop prophylactic nasal sprays. The sprays were tested on animals and then on humans. In one large-scale trial in Toronto in 1937, the spray was tested on 5,000 children. The trial results soon revealed that the spray was ineffective as a preventative for infection by the virus and,

28 Eggers HJ (1999) Milestones in early poliomyelitis research (1840 to 1949) *J Virol* **73**: 4533–5.

29 The World Health Organization has recently estimated that polio would be eliminated during 2004–5, although similar statements have been made before. In 2003 there were 784 confirmed cases of the virus, mostly occurring in Africa and south-east Asia, particularly in Nigeria, India and Pakistan. See World Health Organization *Polio Eradication*, available at: http://www.polioeradication.org/; *Polio Case Count*, available at: http://www.who.int/vaccines/casecount/case_count.cfm. Accessed on: 26 Apr 2005.

30 For a mini-review of early polio research see Eggers HJ (1999) Milestones in early poliomyelitis research (1840 to 1949) *J Virol* **73**: 4533–5.

31 Current vaccines contain a mixture of the three types which together confer immunity.

32 See Paul JR (1971) *A history of poliomyelitis* (New Haven and London: Yale University Press).

furthermore, that the spray caused adverse reactions.[33] It was then discovered that humans are in fact primarily infected via the digestive system and not through the nose. The researchers had not fully understood the pathogenesis of the disease and wrongly assumed that viral entry was via the nose. Thus, this error does not support the claim that polio is an example showing that, in principle, animals are unsuitable models for the disease. Rather, it indicates that failures in this case resulted from a false hypothesis made by the researchers.

Diseases for which treatments and cures have been difficult to develop

HIV/AIDS

6.33 Mounting epidemiological evidence led to the recognition of the infectious nature of the HIV/AIDS disease in the early 1980s. Shortly after, it was demonstrated through studies with chimpanzees that the primary disease-causing virus, HIV-1, was transmitted in blood and blood products and body fluids. These findings revealed that national blood banks were at high risk of providing contaminated transfusions and transfusion products to patients. Widespread screening of blood supplies was quickly initiated. Two major groups of HIV viruses, termed HIV-1 and HIV-2, were identified, each consisting of a complex range of variants. As the virus replicates in infected people and populations, it generates natural variants that continuously escape and evade the human immune system. In addition, the complexity of the virus within each person depends on their own genetic makeup so that within a population of infected people there develops a large variety of different types of HIV. This rapidly evolving virus population is a 'moving target', and has become one of the major scientific obstacles facing the medical research community. The virus also has complex interactions with a number of different types of cells within the body, particularly those that have a primary role in the immune system. For these reasons it has not yet been possible to develop a vaccine or effective means of ridding the body of the virus.

6.34 An ideal animal model for HIV-1/HIV-2 infection would have the following features: practicalities such as ease of handling and housing of the animals, a well-characterised physiology and immunology, and readily available species-specific reagents. It would also need to be susceptible to the form of HIV-1 that causes HIV/AIDS in humans or a very closely related virus. The model would require similar routes of infection and target cells, and should develop similar symptoms to those of the human disease.[34]

6.35 However, no single ideal animal model perfectly reproduces the symptoms of HIV-1 infection and development of the disease in the diverse human population. The primate models that are currently available have inherent limitations.[35] Despite the fact that chimpanzees are naturally infected with the virus SIVcpz, which is the most likely forebear of HIV-1 in humans, they are resistant to AIDS. Some macaque species are infected by an HIV-2-related lentivirus called SIVsm, which causes a form of AIDS that closely resembles the human disease. In addition, rhesus macaques are outbred like the human population, and have a similar spectrum of disease outcomes. But while similarities with humans and cross-reactive immunological reagents exist, the current human epidemic is predominantly caused by HIV-1 and therefore the model does not provide all the features needed. More recently, GM rodents engineered to express human receptors on their cells have provided replacements for primates in certain experiments.[36]

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33 Rutty CJ (1996) The Middle-Class Plague: Epidemic Polio and the Canadian State, 1936-1937 *Can Bull Med Hist* **13**: 277–314.

34 Adapted from Lewis AD and Johnson PR (1995) Developing animal models for AIDS research – progress and problems *Trends Biotechnol* **13**: 142–50.

35 Lewis AD and Johnson PR (1995) Developing animal models for AIDS research – progress and problems *Trends Biotechnol* **13**: 142–50.

36 For example, see the description of research at the Biomedical Primate Research Centre, available at: http://www.bprc.nl/BPRCE/L4/AltRep.html. Accessed on: 27 Apr 2005; Van Maanen M and Sutton RE (2003) Rodent models for HIV-1 infection and disease *Curr HIV Res* **1**: 121–30.

6.36 Scientists have developed a hybrid virus SIV/HIV-1, termed SHIV, which infects rhesus macaques. This allowed the replacement of chimpanzees with a new model for the research into the HIV-1 disease and potential vaccines. Although some progress has been made in understanding the disease, the HIV/AIDS disease is rapidly changing. The viral variants that are engineered and used in the laboratory are often outdated before they are evaluated against new vaccine candidates.[37]

6.37 The first two Phase III clinical trials of vaccines in humans have recently failed.[38] The strategy pursued was one that had originally seemed effective in a laboratory setting using chimpanzees in the late 1980s and early 1990s. While it is important to consider this example as a possible failure of an animal model to predict the outcome in humans, scientists also assert that it is imperative to closely examine the data and the interpretations made from these studies. It proved possible to protect chimpanzees vaccinated with HIV-1 vaccine strains from closely related viral variants. But when tested in humans, the vaccines were exposed to an extremely wide variety of HIV-1 variants circulating in the population.[39] It could therefore be concluded that the failure was primarily a result of invalid extrapolation of data and/or the use of an untested hypothesis by the investigators before proceeding to Phase III clinical trials.[40]

Cancer

6.38 Cancer encompasses a wide range of complex and different diseases of many different cell types and organ systems, characterised by uncontrolled cell division and abnormal tissue growth. Some forms of cancer are genetically inherited, others are caused by the environment, viral infections or chronic inflammation. Some affect the young whereas others more commonly emerge late in life. Animal research has contributed to many advances in the treatment of cancers, and in contrast to the situation 25 years ago, some cancer types are now largely curable diseases. Nevertheless, cancer remains a leading cause of death in developed countries, and it has been observed that research progress has been slow despite the extensive use of animal models.

6.39 Many animal models in cancer are provided by various strains of rodents. There have been difficulties in translating cancer treatments that are effective in rodents (mostly mice) to humans. This is commonly due to genetic, physiological and immunological differences between the mouse and humans. Primate models of cancer are rare, expensive and the animals are difficult to handle and house. Thus, there is a large gap between 'proof of concept' studies in mice and an effective therapy in humans. With a lack of primate models, the genetic differences which remain between humans and mice mean that therapies developed in mice cannot be moved with any confidence to the clinic. The translation of observations from basic research in the laboratory to human cancer trials has often been a slow and disappointing process. Nonetheless, there have been some notable successes such

37 In addition, evidence now indicates that the HIV-1 epidemic is having an impact on the genetics of the human population that is most heavily affected by the epidemic, thus further increasing the complexity.

38 Cohen J (2003) AIDS Vaccine Trial Produces Disappointment and Confusion *Science* **299**: 1290–1; Cohen J (2003) AIDS Vaccine Still Alive as Booster After Second Failure in Thailand *Science* **302**: 1309–10.

39 Klausner RD, Fauci AS, Corey L *et al*. (2003) Enhanced: The Need for a Global HIV Vaccine Enterprise *Science* **300**: 2036–9.

40 See also Lemon R, Dunnett SB (2005) Editorial: Surveying the literature from animal experiments BMJ 330: 977-978. The authors comment on reviews which claim that animal research frequently fails to prevent problems which arise in later trials in humans, or once a medicine has been marketed. They refer to a case given to support this view, in which problems arose in human trials of a post-stroke treatment involving the calcium channel blocker nimodipine. They observe that the example is not suited to support a lack of scientific validity of animal research in this area, as the researchers conducting the nimodipine trials failed to take into account publications which showed that the medicine had deleterious effects in animal experiments. The authors highlight the importance of ensuring that all relevant results from animal research are reviewed before commencing a clinical trial of a new treatment, and that care needs to be taken to avoid that scientific, commercial or personal pressures lead to an inappropriately narrow selection of evidence.

as tamoxifen for the treatment of breast cancer and goserelin for prostate cancer, both developed using experiments in rats and mice.

Summary

6.40 Certain animal models have played significant roles in the study of particular diseases and have led to the development of effective interventions. For RA, polio and hepatitis C successful treatments. In the case of TSEs, animal models have been essential for increasing our understanding of the nature of the diseases and in the development of public health measures to limit their spread. The animals involved in this type of research usually suffer from the characteristic symptoms of diseases such as hepatitis C, RA or scrapie. Where possible, animals are euthanised at humane endpoints, although this may not always be the case if the long-term implications of the disease are under study.

6.41 We have also noted that certain animal models of human disease have their limitations, and that there are examples where treatments that are effective in animal models fail to have the same effect in humans. This is primarily because of the complex pathogenesis of diseases such as HIV/AIDS and cancer which have many different sub-types in humans and animals. Scientists involved in this type of research believe that further refinement of models that are more closely related to humans, especially primate or GM animal models, may accelerate the process.

6.42 The research summarised here has provided significant knowledge about disease processes and helped to identify strategies for interventions. Although the development of treatments for some cancers has been slow, there have also been successes in the case of breast and prostate cancer. Knowledge about basic biological processes in other forms of the disease has increased. Such insights are likely to improve understanding of similarities and differences in disease processes in humans and animals which may contribute to increasing knowledge about the development of preventatives and cures. Similarly, the failure to develop a fully effective cure or treatment for specific diseases, especially for complex multisystem diseases such as AIDS, does not by itself imply that existing animal models are generally invalid. Rather, these observations should invite reflections on how research methodology and existing animal models can be improved.