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➢ Defining the basis of breast cancer heterogeneity

This project will determine how different types of breast cancer are formed as a result of combinations of different genetic mutations occurring in different cell types within the breast. There are two major barriers to effective breast cancer therapy. The first is the difference between tumours, which means there is not a single standard breast cancer treatment. The second is the presence of different cell types within tumours, each of which may respond differently to therapy, in particular Cancer Stem Cells (thought to be responsible for resistance to tumour therapy). Understanding the biological basis for these differences will not only be a fundamental advance in our understanding of breast cancer biology, it will enable current treatments to be directed towards patients more likely to respond as well as identifying new therapeutic targets which will be specific for distinct breast cancer types and distinct cell populations, including CSCs.

The project will cross existing mouse strains, as well as creating new genetically engineered mouse models, to establish new mouse models of breast cancer in which genetic mutations are targeted to specific cell populations within the mouse breast. Breast cancers which develop will be analysed to identify those which most accurately resemble the human disease, telling us that a combination of a particular gene mutation occurring in a particular cell of origin is likely to be important development of a specific breast cancer subtype. Genes switched on in these new subtype-specific mouse models will be compared with genes switched on in human breast cancer and inhibited using either available therapeutics or a genetic trick which switches off gene expression. The effects on growth of the breast cancer subtype will be assessed indicating whether the gene identified is a potential therapy in the human disease.

Mouse models are the most relevant way of modelling breast cancer because they reflect the complexity of the various influences the whole body has on tumour growth. The latest generation of mouse models resembles much more closely human breast cancer and only with mouse models can we achieve our goal of targeting cancer initiation to particular cells of origin. However, mouse model work is strongly complimented by analysis of functions of genes of interest in cell culture and we will pursue this in parallel.

The project will also test whether genes we have previously identified as switched on in normal breast stem cells are important for their biology. We will isolate normal mouse breast cells and in cell culture force these genes to be switched either on or off to determine how this affects the behaviour of the cells. We will also transplant, by injection, these manipulated cells back into a mouse breast to determine whether their stem cell potential is enhanced or blocked. If these genes are important for the biology of normal cells they may be important for the biology of breast cancer stem cells and thus potential therapeutic targets. To test this, we will manipulate cancer cells from mouse breast cancer models in a similar way and determine whether this enhances or blocks tumour growth.

We have determined the minimum numbers of mice to be used in our studies which will give a statistically significant result using mathematical modelling based our previous findings. Typically, a group of mice with a specific genetic background expected to develop tumours will include 20 animals. In experiments in which normal breast cells are transplanted, each test group will include up to 25 animals. In experiments in which cancer cells are manipulated and then transplanted, each test group will typically include 6 animals. Over the lifetime of the project (five years) we expected to use approximately 2700 animals each year. However, most of these will be in our breeding programme.

We will end experiments at the first possible humane endpoint which enables the object of the experiments to be achieved with the least possible suffering. We will use suitable anaesthesia and analgesia under veterinary guidance.

Our studies will increase our understanding of the biology of breast cancer and of the earliest stages in its development. They will highlight the basis for the differences between breast cancers and will identify new therapies for specific breast cancer types as well as informing which types are likely to be most responsive to current therapies.

Regulation of Peripheral Biogenic Amine Receptors

Biogenic amines are naturally occurring substances released in the body or obtained in our diet. They control the peripheral organs such as the heart, vasculature and airways by interacting with receptors located on these organs. The sensitivity of the lungs and cardiovascular system to biogenic amines is altered in disease and by drug treatments. There is a fine homeostatic control of the sensitivity of these organs to biogenic amines which influences the activity of the peripheral organs in disease and during treatment with drugs. The diseases of interest are asthma, chronic obstructive pulmonary disease (COPD) and hypertension. The work will develop animal models of asthma and COPD and of exacerbations of the disease associated with respiratory infection. Corticosteroids are the mainstay treatment of asthma and COPD exacerbations but there is resistance, the mechanisms for which will be studied.

Since the changes in reactivity occur in the intact individual, a holistic approach is necessary involving the whole animal. Therefore non-animal alternatives are not relevant. However, in vitro studies will play a part in parallel studies. For example, characterization of viruses to be used for inoculation of animals in exacerbation studies and for preliminary studies of antiviral activities of drugs (e.g. corticosteroids) will be undertaken *in vitro* using viral growth in culture. Isolated tissues will also be used from laboratory animals, abattoir sources and from humans for receptor characterization and mechanism studies *in vitro*.

The numbers of animals will be kept to the minimum by designing the experiments so that the reactivity to a number of biogenic amines can be tested in the same animal. We will also take multiple measurements from the same group of animals. After termination of an experiment, tissues will be stored for retrospective measurements of biochemical markers. From the variance of the parameters to be measured we know the minimum number of animals in each group to provide statistically meaningful results.

Rats (total 850), mice (3,600) and guinea-pigs (4,330) have been selected because they have the lowest level of sentience that is appropriate for *in vivo* measurements of respiratory and cardiovascular parameters. An element of training of mice and guinea-pigs for these measurements is possible. Each species has its relative merits; guinea-pigs are closest to humans in their respiratory physiology and pharmacological responses, while mice offer more opportunities for determination of biochemical markers because of the availability of assay kits. Rats provide stable cardiovascular parameters. Rabbits (20) provide abundant blood and chicken embryos (50) provide high yield influenza virus growth.

Respiratory measurements will be made in conscious animals either lightly restrained (guinea-pigs, rats) or unrestrained (immature guinea-pigs, mice) in a chamber. Any distress from restraint will be minimal because of prior familiarization, training and handling of animals. Drugs will be administered by injection (intraperitoneal, subcutaneous, intramuscular), by inhalation, and orally. Virus will be administered intranasally or into the trachea under light anaesthesia. Short term discomfort might be expected during injections, but stress will be minimized by careful handling and assistance from a handler. Avoidance of puncturing the gut with ip injections will be by use of optimum gauge and length of needle and injection away from liver and stomach. Irritation caused by repeated injection will be avoided as much as possible by injecting small volumes and using different sites for repeated injections. Infection will be avoided by the use of aseptic techniques. Body weight will be monitored daily during repeated administration schedules

Science will advance from this project because of the knowledge gained on how sensitivity to biogenic amines in our body or diet of peripheral organs are regulated. This can occur in disease or after continuous or repeated exposure to the amines or other drugs that interfere with their actions. Thus, this study will help to explain the changes in sensitivity that occurs in

diseases such as asthma, COPD, lung infection and hypertension. The information will, however, have much wider application to other organs and diseases. The information will help in developing new and improved treatments for these diseases. In particular, it will attempt to develop approaches to overcoming steroid resistance in the airways.

Manufacture and Development of Paracox Coccidiosis Vaccines

This project involves the production and potency testing of the Paracox® vaccines which are a vaccine inducing active immunity against coccidiosis through the addition of the vaccine to the drinking water, feed or spray of chickens between 1 to 9 days of age.

Paracox® vaccines are anti-coccidial vaccines with a pan-European Marketing Authorisation. Paracox® provides protection against all seven species of *Eimeria* known to parasitise the domestic chicken and is particularly designed for use in broiler breeder and table-egg layer. Paracox®-5 contains four *Eimeria* species and is tailored for use in broiler chickens.

Coccidiosis infections of domestic fowl are ubiquitous and a major cause of disease and production losses throughout the world. A commonly used method for the control of coccidiosis is the use of chemotherapeutic agents which are increasingly being banned from use in some countries. Resistance of coccidia has developed to all of the anti-coccidial drugs introduced so far (Chapman, 1997a); there are concerns about drug residues in poultry products (Mc Envoy, 2001; Young & Craig, 2001); and there are strong desires of consumers to ban drugs from animal feeds (Young & Craig, 2001).

Welfare benefits of using vaccination include the birds remain protected for entire broiler cycle after a single administration and reduce the risk of late outbreaks. Other welfare benefits include the eliminatation of undesirable side effects caused by the use of coccidiostats and meat residue concerns for consumers.

This project will be undertaken as part of the EU Batch release process which ensures that a product was made under GMP and in accordance to the marketing authorisation issued to the company in compliance to the EU Directive 2001/82/EC as amended by 2004/28/EC Article 81. European Pharmacopoeia monograph 01/2005:0062 requires that batch release safety testing be carried out. Therefore, as the potency tests are a regulatory requirement which must be carried out in the target species, chickens will be used in this project instead of alternative in-vitro tests such as tissue studies or computer simulations.

Inositol Metabolism in Euryhaline Teleost Fish

Bony fish such as the European eel and the Atlantic salmon, exhibit the genetic plasticity to enable survival in both freshwater (FW) and seawater (SW) with only minimal changes in the osmolality and ionic composition of their body fluids. Recent evidence has suggested that the simple organic alcohol, inositol, is central to the ability of eels to adapt to SW environments. This polyol, which is synthesised and accumulated in a variety of tissues such as the gill, skin and fins, acts in many hormonal signalling pathways and also as an organic osmolyte, preventing the osmotic loss of water and the subsequent desiccation of fish when in SW. Body surface epithelial cells, which accumulate inositol can then act as a barrier between the hypo-osmotic internal environment of the fish and the hyperosmotic external environment.

This project will investigate the expression and function of genes involved in inositol production and distribution in the eel and the salmon, to determine the roles of this osmolyte in both FW- and SW-adapted teleosts. Although sexually immature FW "yellow" eels can successfully acclimate to acute transfers to SW, the sexually immature FW salmon parr must first go through an endocrine-induced maturation process called smoltification before fish can survive in SW. The ability of mature and immature life stages of both eels and salmon to regulate inositol production in response to increased environmental salinities will be investigated as will the effects of cortisol, a hormone known to be involved in sexual maturation and salinity adaptation in fish.

In addition we have recently discovered that an essential enzyme responsible for the cellular production of inositol, inositol monophosphatase (IMPA), can be inhibited or in some cases stimulated by low concentrations of a wide range of organic toxins e.g.etrachlorvinphos, phenthoate, diquat dibromide known to be found in FW. Any perturbations in the activity of this key enzyme by any environmental toxins are likely to have profound effects on the subsequent ability of fish to osmoregulate. Potential deleterious perturbations would certainly compromise the ability of fish to osmoregulate and this is likely to have severe implications with respect to fish migration and the overall fecundity of the species. Over the last 30 years there have been dramatic declines in both salmon and in eel populations. Although the reasons for the decrease in the populations of both species are undetermined, exposures of fish to a variety of anthropogenic toxins have been implicated in a number of studies.

This project will determine if any of the major persistent environmental toxins have any effects on the enzymes responsible for the production and tissue distribution of this essential organic osmolyte and signalling molecule. In addition we will assess the possibility of developing non-destructive tissue sampling techniques to assess if inositol-related gene expression and osmolyte status in eels and salmon could act as potential biomarkers for normal changes in development (eg. parr-smolt; yellow/silver transformations) or toxin exposure.

Development, function and regulation of the immune system

T lymphocytes (“T cells”) are a subpopulation of white blood cells that control the immune system. When T lymphocytes fail to function correctly in people, the immune system fails, usually resulting in death. The present projects will characterise molecules that act as messengers inside T cells to regulate their behaviour or function. The control of T cells is critical for immune responses. Correct functioning of T cells is thus required if the various components of the immune system are to fight infection whilst not attacking normal tissues and structures (so-called 'auto-immune' disease such as type 1 diabetes, arthritis, multiple sclerosis, lupus). Not only must the right numbers of T cells be produced, but they must also move to the places in the body where they are needed and, once there, have the correct effect (boosting or damping down the activities of other cells and/or having direct effects on infected cells or on invading bacteria, viruses or fungi).

Our studies of the immune system include experiments on T cells in tissue culture models. We aim to selectively eliminate genes that we think might control T cells and then assess the impact of these changes on the immune response to viruses and bacteria. The immune system is very complex and it is not possible to fully understand the importance of different molecules without doing animal experiments.

We use the mouse as the animal of choice, as its immune system is very similar to that of humans. We can also thereby make use of the large number of mouse lines in which specific genes (encoding known or suspected ‘key players’ in the control of T cell function) have been altered.

The experiments we do to modify the immune system of mice do not cause any significant harm to the animals. Because we sometimes need to assemble complex combinations of genetic alterations, most of the mice will be used in breeding programmes (up to 25,000 mice over the course of five years). In the great majority of cases, we then achieve the scientific benefit with the need for further intervention, that is the animals are killed humanely and we undertake detailed analyses of the tissues and cells of the immune system. One of the possible consequences of the immune system going wrong is immune-deficiency, but we guard against the possibility of infection having serious effects by keeping all our animals in extremely clean conditions. We also monitor all our animals very closely for any signs of auto-immune disease or tumour formation. Scientific interventions that are undertaken in-life (about 5000 of the animals that are bred) will include standard immunisations and other challenges to the immune system, and experiments in which cells harvested from one animal are transferred to another to understand how they move through the body to their particular sites of action. We do not expect any animal to develop significant clinical signs of ill-health.

When studying how T cells determine where to go in the body, we often inject cells derived from one mouse into another. Occasionally, these experiments require the recipient’s immune system to be suppressed, in order for the injected cells to survive and have their desired function. We use standard procedures of gamma irradiation to cause this suppression. Up to 500 mice (of the 5000 animals in which scientific interventions occur in-life) may receive this irradiation.

One way in which the immune system can fail to operate normally is that tumours (leukaemias and lymphomas) can arise. Some of the genetic alterations we study may have the effect of making this more likely and, very importantly, some drugs known to affect the activity of the various control mechanisms in the immune system may inhibit the formation and/or subsequent growth of these tumours. If this can be demonstrated in mice, then these compounds are potentially important anti-cancer agents in humans. we expect to use up to 500 mice (again from the 5000) to investigate such compounds. Tumours are not expected to have significant outward effects on animal welfare during these studies but, should any be observed, the affected mice will be killed immediately and humanely.

In summary, understanding the messengers inside white blood cells that control the immune system, is essential to identify new targets for drugs that can be used to treat autoimmune diseases, immune deficiencies and cancers of the immune system.

New treatments for inflammatory skin diseases

We wish to test potential new treatments for the skin disease psoriasis in a mouse model system. We also wish to examine the molecular mechanisms by which these treatments, and some more conventional ones, exert their activity.

Psoriasis can be a serious human health problem. In addition, because it results in an unsightly appearance to the skin, it can cause psychological distress to the sufferer. Existing treatments do not always work very well, particularly over long periods of time.

We have developed a genetically altered mouse line that appears to recapitulate the course of human psoriasis. A number of novel compounds that show activities in cell cultures consistent with their being anti-inflammatory in the intact organism have been developed. We now wish to test them, predominantly as creams applied to the skin, in this model system, in order to demonstrate whether further development testing in humans would be justified. The skin is a complex multi-layered organ, which cannot yet be modelled fully in laboratory culture. In particular, we believe that blood-borne cells and molecules contribute to psoriasis and therefore the blood supply is essential for the model to be realistic.

Our mouse line develops psoriasis-like patches on the skin only when these are specifically elicited and the extent of the disease can be controlled so that it does not cause undue distress or harm to the animals. Importantly, we can breed the line under in the absence of the inducing substance, so that there are no welfare issues during this process. We expect to need about 2000 mice over the course of the project to generate sufficient numbers of animals with the correct genetic make-up for use in our studies.

The studies will involve groups of about five mice being treated with cream containing a specific dose of the test compound. We normally expect to test about three different doses of each compound, plus a control where the cream in which the compounds are normally suspended is tested in its own. Our estimate of the number of animals needed to test each compound is therefore 20. Over the five years of the project licence we might test up to 20 compounds. It is, of course, very important than the compounds do not themselves cause any skin irritation. In the event that a compound is strongly predicted to be anti-inflammatory in the initial laboratory studies but there are no existing data on its effects on the skin, we will conduct a very small trial in normal mice, starting with a low dose and increasing it only if there no skin irritation is seen. This will allow us to determine the maximum dose to be used in the anti-psoriasis tests.

Only if there is no information in the scientific literature on this, and maintain the genetic alteration in a breeding colony without any harm being experienced by the animals, as the disease only appears after the administration of an “inducing” substance.

Human psoriasis is often a chronic condition and current treatments are not always effective or may, after a period of apparent success, fail to prevent the disease recurring. We therefore need to understand more about how it develops and to develop better human medicines.

Development of therapies for neurodegenerative diseases

Alzheimer's disease and Parkinson's disease are chronic neurodegenerative conditions for which there is a huge unmet medical need. There are no disease modifying medications for these conditions, and the medications that exist only serve to provide some symptomatic relief in the early stages of the disease but become less effective as the neurodegeneration progresses. We will seek to develop molecules that primarily have disease modifying actions by either providing neuroprotection (preventing neuronal loss) or neurorestoration (stimulating endogenous recovery or repair of the brain). However, therapeutic opportunities will still be explored that provide symptomatic relief through a novel mechanism of action, either alone or in combination with disease modification.

In most cases the project takes novel chemical entities that have been shown in test-tube situations to have a specific set of biological properties (such as inhibiting/activating receptors or inhibiting kinases/enzymes) that are thought to play a role in cell death and neurotoxicity. Selected optimized compounds (the most effective in the test-tube tests and that are predicted or are shown to cross the blood brain barrier) will be tested in various rodent pathological systems that model aspects of the disease. As each neurological disease is different we will utilize various animal models in the project.

Injection of neurotoxins can destroy or damage cell bodies in brain regions that are linked to a particular neurotransmitter pathway or disease state. It is thought that some brain disorders occur – or can at least be controlled by – changes in the electrical circuits generated by the nerve cells (neurons) communicating with each other by release of chemical messengers. Each of those messengers has a very specific and elaborate control system. For example, dopamine neurons in the hindbrain make projections to the front of the brain where they release dopamine to allow movement to occur. Neurotoxins such as 6-hydroxydopamine or 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) that are taken up or injected into the dopaminergic cells bodies (substantia nigra) or terminals (striatum) can produce selective patterns of neuronal loss in the nigrostriatal pathway and associated neurochemical and behavioural deficits that are similar to that observed in human Parkinson's disease.

Other pathological models that will be used involve the over-expression or removal (knock-out) of a gene/gene product that is likely to produce changes in the brain similar to those reported in the human brain of Alzheimer's disease (AD) and Parkinson's disease (PD) patients. Genetic studies have shown that altering (mutating) certain genes increases the risk of AD and PD. For, example studies on post-mortem AD brain have shown the presence of extracellular amyloid deposits called plaques and intracellular accumulation of Tau protein in tangles. These deposits occur primarily in the areas of the brain that control learning and memory and the accumulation of these abnormal proteins is thought to cause the memory disruptions, brain shrinkage (neuronal loss) and pathology in AD. In is now possible to over-express amyloid and/or Tau mutations in mice and thereby reproduce the accumulation of these deposits in the mouse brain. In this project we would then examine if compounds/drugs that inhibit the biological processes that lead to the pathology can prevent these deposits occurring and the associated neurochemical (and behavioural) deficits.

Although mutations are known to underlie some of the rare, inheritable forms of the disease, the aetiology of the more common sporadic cases remains unknown and likely involves complex interactions between various genetic and environmental factors. Neuroendocrine malfunctions may also be involved in the disease process, particularly because it is established that stress hormones can negatively affect neuronal survival. Epidemiological evidence further supports a role for stress as a risk factor for AD because elderly individuals prone to psychological stress are more likely to develop the disorder than age-matched, non-stressed individuals. Therefore, on occasions animals may be exposed to a mild stressor to

measure the impact on biochemical processes that are involved, or may underlie, the pathological changes that occur in the disease.

The medicinal chemist now has many ways of knowing the basic properties of the molecules he makes from experiments in test tubes using cultured neurons or neuronal membranes, for example, how many molecules are needed to link to a receptor, how many receptors it links to at that concentration, how it might be absorbed and metabolised by the liver and how easily it might cross the blood-brain barrier. However, the effects the molecules might have on the sorts of complex systems that control the functions that are most disturbed in brain disorders like Alzheimer's and Parkinson's disease can only be tested in a living animal.

Many of the models used in the project will involve surgery and when animals are subjected to surgical techniques, these are always carried out under general anaesthesia with post-operative analgesia and observation. Should any adverse effect occur the animal will be immediately and humanely killed. Where adverse effects are seen after drugs/new compounds are administered, most likely because of unpredictable interactions, the animals will be immediately and humanely killed. The numbers of animals used will be kept to a minimum by the use of good experimental design and statistics principles. In most experiments we will use ex vivo and in vivo neurochemical techniques to measure neuronal function in the disease model to assess the extent of neuronal loss and the response to drug treatment. In some cases the experiment may be chronic to model the slow nature of the degenerative disorder. In these cases the animals will be monitored regularly by the scientist, the NACWO and the veterinary surgeon and any mild health problems dealt with accordingly. In the event of a severe adverse reaction, such as a seizure, the animal will be humanely killed. It is estimated that about 1000 rats and 1000 mice will be used per year over the course of the project.

It is anticipated that the experimentation described in this licence will contribute to the identification of at least two novel chemical entities being chosen for further pharmaceutical development for the treatment of neurodegenerative diseases. In addition, basic knowledge of the role of different molecular targets in controlling neuronal function in intact animals and in animal models of neurological diseases will be gained using pharmacological tools not previously available. Using this information will allow identification of appropriate molecular mechanisms that will hopefully lead to therapies that can prevent or slow the progression of devastating diseases like AD and PD.

The control of liquid transport in the lung

We wish to discover how the volume, composition and thickness of the very thin layer of liquid in the gas exchange part of the lung (the alveoli) is controlled Too much liquid results in alveolar oedema as seen in heart failure, asthma and premature baby lung disease; too little leads to inadequate removal of mucus and infection as in cystic fibrosis. Elevation of glucose in this layer can promote bacterial infection. Understanding the mechanisms involved will advance knowledge and allow development of managements and medications to alleviate these conditions.

We plan to use rats and mice because of their availability and we have experience of experiments in these species so reducing losses through inexperience. Cardiac bypass surgery will be performed under anaesthesia so that the lungs can be artificially perfused with oxygenated blood substitute. This will keep the lungs alive when liquid is put into the lung air spaces. We can make precise measurements of the volume of the liquid and thus know how fast it is being absorbed or secreted over a few hours. Various compounds - natural hormones, drugs and inhibitors of known cell functions - can be administered either through the perfusate or via the liquid in the lung and their effect on liquid volume calculated. The animals receive only one injection into the abdomen and die at the point of cardiac bypass under anaesthesia. The number of animals used will be about 400.

In a separate group of rodents, to establish whether the hormones which we know develop the lungs of fetuses just before birth have a continuing effect after birth, we will remove the glands which secrete thyroid hormone (thyroid) and cortisol (adrenal) under anaesthesia. After recovery (up to several weeks later) we will test the ability of their lungs to handle liquid. To prove the glands are necessary we will replace the “missing” hormones in a sub group of animals. We will design the sequence of experiments to keep the number of animals used to a minimum (about 40). The rodents will be given analgesia postoperatively and any animal seen to be suffering will be humanely killed.

The reason we wish to examine whole lungs is because the cells responsible for liquid transport are in their natural state. Lungs cells grown out of the body, even for a short time, and immortalised cell lines derived from lung lining cells, have altered function and do not behave in the same way as the whole lung. However, we are also using cell lines in culture and these results will give us leads about compounds to use and effective concentrations thus reduce further the number of animals needed.

Nutritional requirements of dogs and cats

We are primarily interested in the interaction between nutrients and/or foodstuffs and the health and wellbeing of dogs and cats. In this sense we investigate individual or multiple body systems, initially to understand the fundamental principles of nutrition and health and subsequently to determine the impact of varying nutrients and foodstuffs on maintaining health or preventing disease in these species. We are interested in all life stages and the life-time experience of nutrition on the animals involved in the studies.

Whilst research has been carried out over the years to establish nutrient requirements for dogs and cats in health and disease, there are still significant gaps which need to be filled. Studies will be carried out to determine effects of feeding diets containing different levels of the nutrient(s) of interest to groups of dogs or cats over time. Some measures of health can be determined without the need for regulated procedures e.g. through collection of faeces or urine, whilst others such as blood sampling will require the use of regulated techniques in order to obtain data.

For some aspects of this work it is possible to use an *in vitro* approach and where possible this route will be followed e.g. the study of metabolic energy expenditure in cell cultures; the development of an *in vitro* model of plaque and calculus development; computer modelling of taste receptor and tastants interactions. However, the ultimate aims are to understand the impact of nutrition on dogs and cats and establish whether health benefits are associated with intake of particular levels of nutrients and, therefore, the studies need to be carried out in these particular species of animals.

Power analysis calculations will be performed to determine the number of animals required for each study. An adaptive experimental design may be employed, whereby the animals are recruited in stages. Progressive stages of data collection are then used to inform and amend the final powering of cohort numbers such that only the minimum number of animals required for significance determination are included in the study. Experimental design and statistical analysis is supported by in-house and external statistical expertise.

Most procedures are performed in conscious animals that have undergone extensive habituation to the procedure and by highly trained and experienced people. All procedures performed individually, or cumulatively, are considered to be no more than mild in severity. Approximately 200 dogs and 300 cats will be used in these studies and each will be homed to private households when their involvement in the studies is completed.

The studies performed will ensure that there is robust scientific evidence to support values for recommended allowances and safe upper limits for different nutrients such that nutritional deficiencies or excesses are avoided. Equally, understanding oral health disease in dogs and cats and developing appropriate solutions will help reduce the significance of this widely encountered and painful disease. It is our intention to publish these data in the scientific literature, where feasible, to maximise dissemination of this knowledge.

Neuroinflammation, Demyelination and Degeneration

This project aims to increase our understanding of how inflammation and demyelination (loss of the insulation from around nerve fibres) affect the brain, spinal cord and peripheral nerves. Inflammation plays an important role in many neurological disorders, including Alzheimer's disease, Parkinson's disease, motor neuron disease and, especially multiple sclerosis, where demyelination is also important. The main target of the project is multiple sclerosis, but the findings will be relevant to the other diseases as well. There is no known cure for multiple sclerosis, and cost-effective therapies are few, and can have severe side effects. There is no known way of preventing the slow advance of the disease, which is what eventually often confines patients to wheel chairs.

In multiple sclerosis the evidence is that inflammation can cause symptoms such as blindness, paralysis and numbness: and these symptoms can also arise from demyelination. Although inflammation is clearly important, we understand relatively little about it in the brain, but brain tissue is remarkably sensitive to inflammation. Some research suggests that a chemical called nitric oxide is involved, and this is produced in high concentrations within inflammatory lesions, but our evidence is that other factors are also involved, and we plan experiments to find out what they are.

It is impossible to study the myriad effects of a true inflammatory reaction under tissue culture conditions, and we simply do not know what factors to put into computer simulations, so it is necessary to study animals: the proposed research involves rats and mice. These animals have been much studied in the past and we know that their nervous system responds in the same way as humans, certainly for the studies that are planned. Fortunately, in most experiments the lesions in animals can be induced in parts of the nervous system that do not result in any detectable deficits at all, but in other experiments some lesions can occur in parts that cause symptoms such as hind limb and tail weakness.

One of our aims is to scan the experimental lesions using the most modern methods as are used in patients – magnetic resonance imaging (MRI), positron emission tomography (PET) and spectroscopy. These imaging methods have transformed the clinical care of patients, and our understanding of the disease processes, but their value is currently limited by the fact that we do not know what some of the different signals mean. By scanning model lesions we can learn to relate the different signals to particular types of pathology, and so gain much more information from the scans in the clinic.

Another major aim is to develop new strategies to protect the nervous system from damage due to the inflammation. We have already successfully identified a medicine that can protect the nerve cells, and this medicine is currently being tested in clinical trial. We are now anxious to extend this line of research to identify other mechanisms of potential therapy.

Sleep and general anaesthesia: basic mechanisms

This project seeks to understand how general anaesthetics and sleep-inducing drugs act with the aim of developing safer, more efficient or more selective agents.

Modern surgery would be impossible without general anaesthetics, yet the underlying mechanisms by which they produce unconsciousness and pain relief are only just beginning to be discovered. Understanding the molecular actions and neuronal pathways involved presents a major intellectual challenge for basic neuroscience, and research into anaesthetic mechanisms can be expected to provide fundamental information on neuronal excitability which has broader applicability. It is widely recognised that the anaesthetic drugs presently used in clinical practice are far from satisfactory. Currently used anaesthetics are relatively "non-specific", mostly act at high concentrations, and affect many targets. Consequently, many patients suffer from undesirable side-effects from the anaesthetic and analgesic drugs used for their perioperative care. Serious morbidity (*e.g.* cardiovascular side effects) can be provoked in already compromised patients, which is an issue of increasing concern in an ageing population. Our Programme seeks to understand which molecular targets are responsible for the desirable effects of the anaesthetics and the neuronal pathways that are involved.

In work which seeks to understand how general anaesthetics and sleep-inducing drugs cause unconsciousness, the use of animals is unavoidable. Studies on cultured cells are used to define which anaesthetic targets are important and which are not, but the use of animals is essential to understand how effects on these specific targets combine to produce the end result – a loss of feeling/consciousness/sleep.

Animal suffering is minimal in our experiments because the drugs we study are anaesthetics. By their nature, thee drugs render animals insensible to pain. In some experiments where we investigate specific neuronal pathways, and where anaesthetics or sleep-inducing drugs are applied to specific parts of the nervous system, animals are closely monitored; the experiment is terminated, if suffering is evident. Such events are rare, however.

We use rats and mice. For rats, much is known about their physiology and neuroanatomy. We use mice because genetic engineering allows particular mice to be bred which carry putative anaesthetic targets which have been modified genetically. Thus we can investigate the roles of individual anaesthetic and sleep drug targets in the response on the whole animal.

We use, typically, 3000 mice and 200 rats. Approximately 200 mice and 50 rats are killed humanely and their brain tissue used immediately. The remainder of the animals are used in procedures that last only a few hours for individual experiments. Animals are occasionally re-used after a period of about one week. After this, all animals are humanely killed.

Our research aims to understand how general anaesthetics and sleep-inducing drugs work. We hope this knowledge will both advance our understanding of how the brain maintains normal consciousness, but also provide information on how to develop safer and more selective anaesthetics with fewer undesirable side-effects (such as nausea and vomiting) or sleep drugs which no not cause side effects.

Gene Function and Regulation in Sexual Development and Cancer

The overall goal of our studies is to understand the cellular and molecular mechanisms underlying the development and function of the prostate, gonad and adrenal how these mechanisms are deregulated in pathological conditions such as cancer.

Understanding the process of sexual development will provide novel information on how organs develop during embryogenesis and why this process sometimes fail to give rise to malformations or pathways are inappropriately activated and induce tumourigenesis.

We will analyse the phenotype of mice that have been genetically modified to inactivate or misexpress factors in the various cell types of the gonad, adrenal and prostate in an effort to identify drivers and gene networks that are important in human disease. The interactions with known cell signalling pathways will also be investigated by using substances that are known to modify these pathways and by using genetic modification to specifically mark cells such that their fate can be followed in vivo.

The study of the complex interactions of tissues that are required for organ development during embryogenesis and during the process of carcinogenesis can only satisfactorily be done using in vivo systems. Nevertheless, we are continually developing *in vitro* cell culture and three dimensional tissue assays and comparing them to the *in vivo* models in an effort to establish animal replacements. We have set up in vitro organ culture systems for the embryonic gonad, adrenal gland and prostate to study aspects of the cellular interactions important in organogenesis. We are also developing methods to introduce genetic modifications in cells and tissue such that we avoid complex genetic breeding and minimise the number of animals generated. We use the mouse as a model system because of the available techniques that allow genetic manipulation and the vast background knowledge we have of its embryology, cell biology and pathogenesis.

Most of our studies involved breeding of genetically modified mice and the phenotypic analysis of the different strains. In most cases these mutations do not cause discomfort or distress and the analysis takes place before any severe phenotypes are observed. For animals that develop neoplastic disease, the tumour burdens are kept within acceptable limits to minimise suffering.

The data from our studies will aid in the diagnosis and treatment of patients with disorders of gonad and adrenal development and in the search for novel biomarkers of tumour progression and possible targets for drug development as well as providing mouse models for preclinical studies.

Development and evaluation of new cancer therapies

Although significant progress has been made, there is still a need to develop more effective cancer therapies: notably, there is now the opportunity to develop personalised 'targeted' treatments with fewer side-effects than with conventional cytotoxic treatments. Our main strategy is to develop drugs that act on the molecular mechanisms linked to cancer development. The second approach is a form of gene therapy that only activates a drug at the tumour site, hence minimising damage to normal tissues.

Initial studies use computer-based design and molecular methods together with extensive testing in human cancer cells in culture. Despite the optimal use of such assays, it is essential (and a legal requirement) that drug development includes intact animals. This is because agents that will be given to cancer patients have to be taken up into the body (for example from a tablet) and into the cancer cells. They may also be broken down in the body or attach themselves to blood proteins. In addition, solid cancers require an intact blood supply to grow and are able to spread throughout the body. Only animal models provide the means to evaluate how new agents will behave with respect to these key features of human cancers.

Most drug development utilises rodents, so we have a considerable amount of information on existing drugs for comparison with our new agents. Immunocompromised mice that allow the growth of human tumours are generally used, or genetically-modified mice which develop tumours with the same mutations as human cancers. These models predict quite well the activity of drugs in the clinic. Mouse numbers are calculated to minimise usage while allowing robust and statistically significant results. Potential new treatments are evaluated in a 'test cascade'. Agents progress to the next stage only if satisfactory, or else are further improved. We first calculate from extensive laboratory experiments the levels of an agent that are needed for efficacy. Then we test that this is tolerated in animals and will give the concentrations and exposure required. We next develop molecular markers of response and test that these are inhibited in short-term studies in animals before taking the very best agents into therapy trials.

Although we expect side-effects to be less severe, these can occur and are mostly evident in early stages, before the agent has been fully optimised. Possible symptoms include loss of condition, weight loss and effects on organs, e.g. skin or liver. Mice are checked frequently for signs of ill health and specific score sheets (e.g for tumour ulceration or early/late side-effects) provide objective records and defined endpoints. Tumours are generally grown just below the skin, where they are easily measured, and experiments terminated before defined limits are reached. In some studies we inject tumour cells into the blood or into a hind limb bone, mimicking disease which has spread. Here we use sophisticated imaging techniques to evaluate responses to therapy throughout tumour development, enabling earlier discrimination of effective agents. Many of these are translatable to the clinic, as are biomarkers of response.

We will deliver novel anticancer agents for clinical trials during the lifetime of this project.

Cellular and Molecular Basis of Hearing and Deafness

The project aims to understand some of the many different causes of deafness, and to develop methods for preventing or reversing hearing loss caused by aging or certain medicines.

There are several reasons for doing this project. First, although mutations in many of our genes are known to cause hereditary deafness, a form of deafness that is passed on from generation to generation, the exact nature of the defects in the ear caused by these mutations are often unknown. Without knowing what is wrong with the ear it is certainly not possible to fix it, or to advise on the best type of treatment. Second, commonly used medicines and loud sounds can also cause hearing loss, and the effects of noise, medicine or aging are often made worse by mutations we carry in our genes. Quite why there are these unfortunate interactions between, for example, medicines and mutations remains unknown, and we need to understand why this is so if we are to stop it from happening. Finally, there is evidence that the hair cells in the inner ear that enable us to hear can, in some types of animals, regenerate if they are lost. Although regeneration does not occur in mammals like man, hair cells can repair themselves if damaged, and it may also be possible to re-awaken the regeneration process that is normally dormant.

The plan is therefore to (i) make animals that will allow us to tell how mutations in different genes cause deafness in humans, (ii) use these animals to understand why mutations make us more susceptible to deafness caused by medications, noise and ageing. We will also search for compounds that might prevent medications from causing deafness and explore ways of improving the repair process or arousing regeneration.

The inner ear is a very complicated organ. It contains very many different cells types and, as yet, there is no substitute for using animals in this research. Where possible will use cells growing in a dish, but these can only be used for preliminary studies. We will use the minimum number of animals possible, the numbers required to obtain results that are mathematically significant. The project will use ~3000 mice and 10000 zebrafish larvae per year.

The protocols should involve the least suffering possible due to the use of anaesthetics and analgesics. Where a procedure does has the potential to cause suffering we have taken every step possible to reduce the possibility this happens. We use mice because they have genes and ears that are very similar to those of humans. We use fish because the hair cells on their surface that detect water flow are very similar to the cells in our ears that enable us to hear.

The procedures in mice involve introducing genetic mutations into the animals and then exposing them to medications or noise, or they involve expressing genes in the ear that may help regeneration. In fish, we will look for compounds that prevent medicines from killing hair cells. The likely adverse effects include deafness and audiogenic seizures - short epileptic fits caused by noise from which the animals rapidly recover.

The project should advance our understanding of how noise, certain medications and mutation in our genes cause deafness, and may lead to the discovery of drugs or strategies for stopping, reversing or repairing various forms of hearing loss.

Physiological and Pathogenic studies of diabetes

We are addressing 4 main issues here. 1) There is a shortage of donor pancreas tissues needed to treat diabetes and we want to enlarge the donor pool by preserving the margin donor organs. 2) We want to improve the current human islet transplantation efficacy. 3) We want to test technology to prevent cells being rejected by the host immune system. 4) We want to assess toxicity in materials that are intended to apply to humans for the treatment of diabetes.

The findings from our studies will be translated into clinical human islet cell transplantation to treat diabetes patients with difficult conditions. We hope that the translations will give the patients a long term and better outcome with fewer side effects.

All these work will be done in normal mice or rats or in immune deficient mice or rats or in immune deficient mice or rats that have been equipped with human immune cells, such animals are called humanised mice or humanised rats. The cells will be transplanted into kidneys, liver, spleen.

The purpose of this research program is to see whether the cells that we are working with have the capacity to correct hyperglycaemia in diabetic animals to gain information for further improvement or the cells/materials can effetely protect the transplant cells from rejection by the host immunity system have any undesired toxicity to animals, before we can apply them in humans.

Rodent Acute, Sensitization and Microbial Studies

The project aim is the determination of scientific and/or regulatory endpoints in rodent acute (single dose) toxicity, sensitization, tolerance and microbial studies for submission to regulatory authorities and/or for safety assessment purposes.

Governments require and the public expects that substances (e.g. agrochemicals, industrial chemicals, pharmaceuticals, medical devices, microbes used as pest control agents (MPCAs) and oncolytic viruses used to kill cancer cells) that we are potentially, or actually, exposed to are safe or their hazards are well understood.

The acute toxicity, local lymph node assay for skin sensitization potential, local toxicity/tolerance, and MPCA and oncolytic virus studies in this project are designed determine specific toxicity or regulatory endpoints and/or for safety evaluation.

There is currently no regulatory or scientifically acceptable alternative to the use of animals in these studies. Rodents are used as they are required and accepted by the regulatory authorities for these study types. The regulatory guidance usually indicates the number of animals included in a study; otherwise, the number used is the minimum to achieve the aims of the study.

To prevent unnecessary pain and suffering to animals and refine the studies, a tiered approach to safety testing is generally adopted. All available information will be reviewed to decide whether testing is acceptable. If acceptable, a logical sequence to testing will be determined. The majority of animals on these studies would be expected to experience no effects or those of a mild to moderate severity during the dosing and/or observation phases of the study. However, in order to achieve scientific and regulatory objectives in some acute toxicity studies, some animals may show substantial effects (such as overt clinical signs, effects on bodyweight) and/or mortality.

The procedures performed include the administration of substances by various routes (e.g. oral or injection). In addition to the findings indicated above, occasionally effects may occur which are expected due to the nature of the test material (e.g. pharmaceuticals), but they are not expected to persist for longer than a 24-hour period. Most of the dosing techniques, manipulations or investigations do not cause any lasting adverse effects, but a small number of animals may show temporary moderate distress due, for example, to restraint, confinement and withdrawal of blood.

The information gained from the studies performed under this project can be used by medical, health and safety practitioners, and toxicologists etc. to assess the relative safety of the substances being used, abused or handled and therefore develop appropriate strategies for the treatment or safe handling of the substances. In addition, the information can be used to assist in the selection of dose levels for repeat dose studies in rodents and non-rodents with a higher degree of confidence and therefore minimise animal use and the severity of findings in later studies. Study designs can therefore be developed that cause the least pain, suffering, distress or lasting harm and which have the highest prospect of achieving the desired scientific endpoints.

Investigating mechanisms of tumorigenesis

The development of cancer is regarded as a multi-step process whereby cells acquire multiple mutations, some of which have deleterious effects. Recent research programmes have uncovered the sequence of cancer genomes. These data have detected a variety of mutations in cancer cells but we are yet to explore whether these mutations have any functional significance in the development of tumours. This project will dissect out the importance of some of these very specific genomic defects to specific subsets of cancers.

Cancers often affect the whole body and depend on interactions with the immune system and the surrounding tissues. It is therefore imperative that we mimic this system in our models. The best way to do this, with current technologies, is to produce genetically modified mice which express genes that have been discovered in the cancer cells of patients. First these cancer genes are expressed in cell culture systems to demonstrate that they are able to induce cells to develop into cancer cells. We can then examine how these cells change in response to the cancer gene, but to fully understand the contribution made by other systems within the body, we must confirm the data obtained in the culture systems in our mouse models.

The first step of our project is to express faulty genes in cells in culture to determine if they are able to cause the cultured cells to exhibit properties of cancer cells. If the genes are able to impart survival advantages *in vitro* we will then demonstrate that they are able to cause cancers in genetically modified mice, i.e. mice which are engineered to carry the faulty gene. These mice can then be fully explored for the activity of the faulty gene *in vivo*. In particular, we are interested in discovering how necessary these genes are for the continued growth and survival of tumour cells and secondly, if inactivating the faulty gene in cancer cells causes them to die. These data will have immense clinical application as we can then design drugs which specifically target the faulty gene producing ‘designer therapy’ which will by default have less toxic side effects than current treatments.

The mice will be checked daily for signs of tumour growth by qualified animal welfare staff, fully trained in the husbandry and care of genetically modified mice. Any animals which show clinical signs will be carefully examined and monitored and if necessary euthanized. Clinical diagnostic specialists will assist in the analysis of tumours. We will also monitor the development of cancer in some using non-invasive imaging techniques such as fluorescent imaging. In doing so, we will be able to detect growths before they become invasive and hence reduce suffering.

We will use mouse models in this project as this is the least sentient animal which can be employed for the study of tumour development. The immune system of the mouse mimics that of our own and hence provides an excellent *in vivo* setting for examination of tumours. The genetically modified mice must be bred to obtain complex genetic backgrounds. Some of these mice will therefore be used purely for breeding purposes and genetic engineering. The remaining majority will come from breeding programmes to produce mice which carry the faulty genes and hence will go on to develop tumours for study. The majority of experiments will be performed on tissues isolated from the animals following euthanasia hence minimising animal suffering and advice will be sought from statisticians in order that the least number of animals can be employed.

We anticipate that this research will inform on mechanisms of tumorigenesis as a result of the expression of products of genomic abnormalities in order that we may develop more efficacious, better tolerated treatments which will improve the survival and quality of life of those patients with cancer.