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Project summaries granted during 2013

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Project Titles and key words

- Mechanisms and Interactions for Decision Making
- Insulin replacement in diabetes
- Development and Optimisation of Infection Models
- Molecular Imaging in Models of Inflammation and Infection
- Pre-clinical stroke & experimental MRI research

  Focal cerebral ischaemia models, stem cells, hypothermia, metabolic syndrome, multimodal MRI
- Contribution of Inflammation to Tumour Initiation

  Cancer, zebrafish
- Mechanisms of blood cell development and leukaemogenesis

  intracellular signalling, leukaemic progression, chemoresistance
- Heart function & structure during cardiac disease

  Heart, cardiac function, cardiac structure, myocardial infarction
- Sphingolipid Signalling in Pulmonary Hypertension
- In vivo testing of drug formulation
- Investigations into Spotty Liver Syndrome
- Training in Specialised Therapeutic Procedures

  Minimally invasive, new procedures, surgery, training.
- Investigation of the Role of Nox and Reactive Oxygen Species in the Pathogenesis of Metabolic Disorders and Cardiovascular Diseases

  Cardiovascular diseases; oxidative stress; type 2 diabetes; NADPH oxidase;
- The Physiology of Left Ventricular Hypertrophy
- Reduction of the Sequelae of Ischaemia

Mechanisms and Interactions for Decision Making

The project is concerned with the role of the frontal lobes of the brain in learning and decision making. The frontal lobes consist of many sub-areas and much is unclear about their function and so the first aim is to elucidate their functions. The next aim is to understand the mechanisms by which they operate. A particular focus is on the way in which frontal lobe areas are parts of distributed circuits that include other brain regions. An important aim is to test how connections with other brain areas within these circuits determine the information that each brain area receives and the influence it has over other brain processes and behaviour. An additional aim is the assessment of the welfare impact of the procedures themselves on the animals.

We measure the activity and structure of macaque brains before, after, and while they learn and make simple decisions between objects or actions to obtain food and juice rewards. Measurements are made with magnetic resonance imaging (MRI).

MRI is non-invasive. We use animals because we also want to examine what happens if intervention are made in the brain that disrupt the way small parts of it operates. In many cases we intervene in the brains of human volunteers with a technique called transcranial magnetic stimulation (TMS). TMS can only be used to investigate a limited number of brain regions close to the scalp. To look at other areas and to examine longer term impacts on brain networks and behaviour we use animal models.

We use macaques because they, like humans, possess a prefrontal cortex. Not only are the macaque's brain connections and activity patterns better documented than any other primate species but macaques can perform the simple decision making tasks. Because its brain is approximately 5cm long it is possible to obtain meaningful data about its function using MRI. To obtain reliable results it is rarely sufficient to examine a single animal. Usually data from three macaques can be shown, using statistical procedures, to provide an indication of whether brain signals are reliably correlated with behaviour or whether a brain intervention affects behaviour. In a series of experiments over five years, we expect to use approximately thirty macaques.

In some cases, the animals are restrained while data are collected and there is a risk that this may cause stress. This is not only a problem for the animals' welfare but for the science if it prevents them from engaging in the learning and decision making behaviours we investigate. We therefore train our animals carefully so they are gradually familiarized with the procedures. Brain interventions, such as the making of focal lesions, are carried out under aseptic conditions, anaesthesia and with analgesics. Veterinary guidelines are followed.

To assess the impact of the procedures on the wellbeing of the animals we can take measures of behaviour and physiology. We hope that these measurements can be used to help us identify the least stressful ways to carry out the research.

Insulin replacement in diabetes

Our main purpose is to characterise implants of insulin-secreting cells together with amniotic epithelial cells to determine if the presence of the amnion cells improves survival and the ability of the implant to control blood glucose.

Diabetes mellitus affects about 4% of the UK population. Although some types of diabetes can be treated by diet, exercise and oral drugs, other types require insulin. These treatments relieve the acute symptoms of the diabetes, but they do not reinstate a normal glucose balance within the body. Consequently patients are vulnerable to long-term complications such as diseases of the eyes, kidneys and nerves. Transplanted isolated insulin-secreting 'islets' from the pancreas can assist insulin replacement, but patients need immunosuppressive drugs following transplantation. These cause long-term detrimental effects both to the patient and to the function of the transplanted cells. We need to find alternative methods in making this a more effective treatment.

We have demonstrated in the laboratory that a combination of islet cells and amnion epithelial cells grow well together and have preserved function, in addition to modulating the effect of the immune system. However, diabetes affects most cells and tissues of the body, and there is no single type of cell or tissue that represents the diversity of effects of diabetes on the whole body. Hence animals (mice/rats) with diabetes are included in this study. This study is designed to contribute to the development of these cell implants for the treatment of diabetes.
Within our study, *all* diabetic animals receive treatment for their condition, some using the currently established islet transplant protocol, and some with the potential new islet-amnion construct. In some animals diabetes will be induced with a chemical that kills normal insulin-secreting cells, as sometimes happens to human patients. The animals show features of diabetes similar to humans, but they do not suffer pain, and the studies do not last long enough for them to develop the long term complications of diabetes.

To test the insulin-secreting cell implants, animals with a genetic disturbance in their thymus will be involved as these animals are slow to reject foreign tissue implants, so they do not need immunosuppressive drugs and continue to allow the tissue grafts to survive. For all studies, the minimum numbers of animals will be used consistent with sensible scientific practise. We expect to use an estimate of 800 mice and 100 rats over 5 years. The animals are constantly monitored for 'markers' (e.g. changes in some blood constituents) that indicate the overall effect on disease progression and the likelihood of preventing long term complications.

The primary potential benefit relates to new knowledge about the immunology and viability of islet-amnion constructs. It is anticipated that the presence of amnion cells in addition to the islet cells will prolong survival of the islets and reduce the need for immunosuppression. This could translate clinically to reduce the detrimental immunosuppressive consequences for patients, and contribute to the advancements of transplants to provide a better physiological treatment option for the management of diabetes.

Development and Optimisation of Infection Models

This project licence will allow us to develop and optimise models of infection to use in a contract service licence which together enable us to provide support services to the Pharmaceutical and Biotechnology industries to assist in the development of antimicrobial agents.

Scientific unknowns and clinical service need

There is an acute shortage of novel agents to treat antibiotic resistant organisms and newly-emerging diseases. Development of new antibiotics is of clear benefit to humans and animals.

Animal numbers minimisation

New models of infection will only be developed or optimised following extensive review of the literature and in-house infection disease model database. Small pilot studies will be carried out to assess the suitability of infection model using standard care drugs and only developed further if preliminary data indicates suitability. At all stages of model design power calculations are used to predict the smallest group size required to produce a high likelihood of a significant difference between groups being observed.

Minimising animal suffering

In all cases studies with either mild or moderate endpoints will be used with close observation of clinical deterioration endpoints. Animals are humanely euthanized prior to reaching ethically agreed endpoints to avoid disease progression. Infection models that could result in pain will be given analgesia as part of the study protocol. The duration of experiments will be the minimum possible to allow clinical disease to establish and antimicrobial drugs to act. All animals are housed in HEPA filtered cages and supplied with sterile water to reduce secondary infections. This licence benefits from our expertise of over 15years in working with infected animals and we will introduce procedural refinements resulting in either from in-house research or from the literature.

Explain why the species or types of animals were chosen

Invertebrate mini-hosts are extremely effective in primary identification of active compounds but are not a complete replacement for vertebrates due to differences in immune response, disease localization and PK.

Rodents are chosen because they are predictive of efficacy in humans. In most cases mice will be used. In studies in which multiple blood samples are required rat is the preferred host, since multiple daily blood samples can be collected with little stress. In some cases mice are not suitable hosts either because they are resistant to infection or because the disease progression is unpredictable; in these cases they will be replaced by rats, guinea pigs, hamsters or cotton rats if their infection more closely replicates the microbial disease seen in humans.

Estimate of number of animals and species used

The number of animals used will be dependent on the service requirements of clients and the number of drugs in the development pipeline but will be approximately 5500 Mice, 3000 Rats, 750 hamsters, 650 cotton rats and 400 Guinea pigs.

Molecular Imaging in Models of Inflammation and Infection

There is an urgent need to develop alternative ways to diagnose and understand disease processes in both animal and human health. Currently very few new drugs successfully make it all the way in to clinical practice in particular in the area of lung inflammation and infection. This is in part due to the lack of disease-specific markers. This project aims to develop non-invasive biomarkers that can be used to serially monitor disease in animals and humans to further understand the processes involved in acute and chronic lung disease.

Having designed optical imaging reagents we intend to develop strategies that will permit accurate measurements of biological processes in animals in real time. Taking advantage of existing models of acute and chronic inflammation and infection we will demonstrate that our reagents work and in doing so identify key targets for potential new drugs. This programme of work has already started in the laboratory testing how the reagents work when put in to cell mixtures and subsequently we will check that these reagents provide information in keeping with current accepted methods of assessing lung disease in animals. After this animals will be imaged multiple times so as to assess the dynamic patterns of development of acute and chronic lung injury.

The nature of the project necessitates animal use as only animal models, with intact immune systems allows adequate assessment of inflammation and provides the relevant imaging markers. Numbers of animals used will be kept to an absolute minimum by performing appropriate power calculations for group sizes with standard interventions and sacrifices. The nature of the serial longitudinal imaging results in a dramatic reduction of animal numbers. We have also developed a wide array of *in vitro* and *ex vivo* systems to minimise numbers further. We intend to use 3000 mice and 100 rats although most mice will be required simply for breeding.

The work concentrates almost exclusively on mice because of the vast literature characterising inflammation in this species and the wide number of reagents available. Our project of work has been specifically designed to minimise suffering. Strict anaesthetic protocols are in place and because the project exists specifically to develop new treatments to reduce human suffering associated with acute inflammation, they are designed to involve short experiments.

The work will centre on models of acute and chronic lung injury occurring predominantly as a result of delivering bacteria or sterile agents in to the lung. This will inevitably lead to some clinical signs of ill health (such as diminished activity, hypothermia, and weight loss), but mice will be kept warm and closely monitored to minimise any suffering.

The potential benefits to science will include novel tools to study disease processes without performing biopsies or killing animals. The programme will provide enhanced knowledge of how inflammatory cells interact and how they access inflamed tissues. The ultimate goals are to improve animal and human health by providing better tools to assess disease and novel therapies to treat these conditions

Pre-clinical stroke & experimental MRI research

This project employs rodents to study stroke-induced brain damage, to test new therapies designed to reduce stroke mortality and morbidity, and to develop new MRI techniques for stroke & brain injury diagnosis. Treatment options for stroke are extremely limited (clot-busting thrombolytic drug, rt-PA) with < 5% of ischaemic stroke patients receiving this drug because of increased risk of haemorrhage beyond a 4.5 hour time window from stroke onset. When administered, rt-PA is successful in opening the blocked artery in less than 50% of patients. Therefore, there is an urgent need for better stroke therapies.

The main aims of our research are to find new therapies (alternatives or adjuncts to rt-PA) and improve current diagnostic imaging by developing new MRI techniques to identify the optimal treatment plan for each patient and improve patient selection for clinical trials.
Specific licence protocols are included for rodent models with stroke risk factors and to test drugs, gene & stem cell therapy, and hypothermia (reducing body temperature).
MRI is non-invasive, capable of assessing evolving stroke-induced brain damage and identifying tissue capable of recovery (penumbra, the primary drug target).

Capacity for repeat scanning means that progression of evolving brain damage associated with stroke or brain injury can be mapped over time to study the effectiveness of restoring blood supply or drugs developed to limit brain damage. The ability to repeatedly measure different parameters in the same animal, combine this with tests of motor function and use the brain for complementary biochemical analysis at the end of the experiment also has a huge impact on reducing animal numbers required to test new drugs or hypotheses (3Rs). Preclinical stroke research is carried out using *in vitro* and *in vivo* models. *In vitro* systems have many limitations (no blood flow component, source of cells rarely from adult brain, mix of cell types in culture different from cell symbiosis *in vivo*) and therefore drugs and hypotheses must also be tested in *in vivo* models before translation to the clinic. Good experimental design (power analysis to predict group sizes) and use of MRI will significantly reduce animal numbers required.

Rodents are the best species for *in vivo* research: It is ethically more acceptable to use rodents than higher mammals, anatomy of the blood vessels and brain structures is well characterised, mechanisms of stroke-induced brain damage are similar to man, and models with co-morbidity and stroke risk factors are available. Thrombolysis (dissolving the blood clot) , the only treatment effective in man, is equally effective, and within the same timeframe, in rodent models.

The mildest ischaemic insult which produces a reproducible amount of brain damage or motor deficit (muscle weakness) is used, to minimise suffering, animals are checked regularly for any deterioration in their condition or for adverse effects of therapy, and prompt veterinary advice sought if problems arise. Up to 350 rats and 50 mice will be used in any year. The ultimate benefits arising from the research will be improved diagnosis and treatment of stroke patients.

Contribution of Inflammation to Tumour Initiation

This project will use zebrafish to look at the interaction between cells of the immune system and the earliest stages of cancer development in the fish embryo so as to understand how inflammation can enhance growth of the early cancer cell.

Cancer is a major public health problem worldwide. Although new therapies have decreased the mortality rate by about a fifth in the UK, it is still the No1 killer for all causes of death. Moreover, cancer incidence rates have risen by a third in the UK since 1970s. There is therefore an urgent need for novel ways of preventing cancer at the very earliest stage of its development. However, this important time in cancer development has, so far, not been possible to study in the laboratory.

There are few animal models in which the cancer cell can be visualized, in vivo, at the very beginning and its progress monitored as it grows within the body. Zebrafish are transparent and allow us to see the early cancer cell in its natural setting by looking under the microscope at the live embryo for several days. We are able to make " Genetically Modified GM Fish in which every cancer cell is fluorescent green and every immune cell is fluorescent red. These GM fish now allow us, for the first time, to see in real time how these two cell types interact with each other within the live animal Also, I chose to use zebrafish, which is a lower vertebrate but still provides a realistic experimental model for cancer development in the human.

I have established GM zebrafish as a cancer model, and have used the live imaging of early fish embryos to see the interaction of Cancer cells (Green) and Immune cells (Red) in real time. For the first time I have shown that the cells responsible for inflammation around the Cancer cell promote its growth at this early time It is important to understand how inflammation can be prevented or reduced by drug treatment in the hope of applying any new findings to the treatment and prevention of Cancer in the Human

Most of our studies will be done in larval fish that do not suffer adverse sensations and are not protected by Home Office regulation, although we will use a small number of adult fish for our live imaging studies. Our live imaging protocol is non-invasive, and it involves lightly anaesthetising fish. I will consult statisticians to determine the smallest number of animals that are necessary to, give statistically significant results. According to our estimation, the number of adult zebrafish that we will maintain will be no more than 2000 per year, and majority of these are for breeding purposes. We will use no more than 200 adult fish per year for live imaging purposes. Also, I will ensure that the live imaging of fish is kept to a minimum time so as to ensure the least suffering of animals.

The anticipated outcome of this project will give new information on how the inflammation that occurs in the vicinity of a Cancer cell can be reduced by drug treatment so as to hinder its development from the very beginning.

Mechanisms of blood cell development and leukaemogenesis

While significant advances have been made to delineate the molecular processes that regulate cancer initiation and progression, a great deal remains unknown. This project licence proposes to gain a deeper knowledge of the mechanisms that mediate the development of stem cells into white blood cells (lymphoid or myeloid lineages), and to elucidate mechanisms that regulate the initiation, maintenance and progression of blood cancers (leukaemias), focusing on chronic lymphocytic leukaemia (CLL) and chronic myeloid leukaemia (CML). We will use mouse models to elucidate the biology of lymphocyte development and leukaemia initiation/maintenance, and as pre-clinical models to test established and novel compounds alone or in combination that have the potential to be future therapies. Specifically we will:

A. Eludicate the molecular events that regulate stem cell lineage commitment and maturation;
B. Define the translational applicability of our novel mouse model for CLL;
C. Utilise mouse models to test established and novel therapeutic agents for their ability to arrest leukaemia development;
D. Transplant human leukaemias into mice to enable manipulation of specific genes/proteins in human leukaemic cells.

While laboratory-based studies inform cellular behaviour in a physiological environment (i.e. a mouse), they cannot fully replace them due to the complexity of biological systems, which exists in a mouse. Importantly, substantial work will always be carried out in cell differentiation/microenvironmental models established in our laboratory, which will enable us to rationalise which experiments to carry forward into mice, thus reducing the number of mice used. As active researchers we are exposed to new experimental approaches in our research area and will incorporate them into our future research program if they prove to be as reliable and robust as the proposed animal experiments. In this way we will endeavour to reduce/replace the estimated 8000 mice that are proposed for use in this project licence. The protocols described will gain the maximal amount of scientific information from the minimal amount of mice, and the procedures are chosen to be the least invasive, thus minimising the suffering of the mice. We have chosen mice, as the broad processes of stem cell development are well characterised and a wide range of reagents are available to address the biological properties of cells. Moreover, we have significant experience working with mice.

The development of CML and CLL in mice, through targeted mutation of a specific protein in mouse cells, or transplantation of human CML or CLL cells into mice, replicates important aspects of the human cancers, enabling us to model selected variances of biological responses for specific patient cohorts. Modeling the human disease in a mouse enables the elucidation of CLL and CML cell responses to novel therapies, which in turn informs clinical trials.

CLL and CML patients relapse due to re-emergence of a persistent chemoresistant population of cells that reside in protective lymphoid organs. Defining the molecular events that mediate this persistence, and targeting these processes, will allow the development of potential therapies towards these chemoresistant cells, eradicating the persistent cell population and eliciting a cure.

Heart function & structure during cardiac disease

This programme of work aims to investigate and characterise the role of molecules/proteins in the heart in order to discover new therapeutic targets for the treatment of heart disease.

The leading cause of premature human death in the UK is due to occlusion of the arteries in the heart and subsequent heart damage and pump failure. Effective prevention and therapeutic advances in heart failure cases await a better understanding of the mechanisms underlying both pump dysfunction and abnormal heart rhythms which lead to heart failure and death. Cardiac dysfunction is caused not only by coronary artery occlusion but also infectious agents including parasites.

Rats/mice/rabbits will undergo either surgical occlusion of arteries in the heart to induce a region of heart damage or parasitic infection. The adverse effects of this procedure may include heart failure. Minimally/non-invasive *in vivo* imaging and assessment of heart pump function and heart rhythm disturbances will also be performed but have little adverse effects. Parasitic infections will be applied to a subset of animals which may result in adverse neurological/heart dysfunction. *Ex vivo* assessment will then be performed in order to establish the functional consequences of the intervention and detailed structural and biochemical analysis.

It is difficult to obtain viable human heart muscle including suitable non-disease human heart muscle. There is considerable variation in age, medication and underlying pathology of any obtainable human tissue and there is the likelihood of progressive disease being present. It is also not possible to investigate the processes at well-defined time points after a single incidence of damage.

Substantial prior and continuing *ex vivo* experiments will inform and limit the number of *in vivo* experiments required and where possible as much information from one animal will be obtained. Approximately 2600 mice, 2000/ rats and 340 rabbits per year may be used.

Experienced licensees will perform the surgical techniques under aseptic conditions and appropriate pain relief will always be administered. Best practice post-operative care will be employed to ensure any discomfort is minimised. Regular examination by veterinary surgeons and experienced technicians will ensure that any untoward effects that do develop are detected early and steps are taken to minimise any distress or discomfort.

The flexibility of being able to use mouse/rat/rabbits is aimed at reducing and refining the number of animals used rather then increasing them. Experiments will not be repeated in both species where unnecessary. The decision as to what species is to be used for a particular set of experiments will depend upon a clear decision at that time as to whether the use of the species tissue with the particular technique maximises the signal to noise ratio for each measure and hence decreases the numbers of animals used.

This project will provide information about the basic physiology and pathophysiology that occurs during heart disease. It will also inform future research and may suggest much needed therapeutic approaches.

Sphingolipid Signalling in Pulmonary Hypertension

This project will study the effects of a disease known as pulmonary hypertension. This is a rare but serious disease with unknown causes which can be symptomatically treated by a number of means, including some drugs or having a heart/lung transplant. However, there is an urgent and unmet clinical need for a better understanding of this disease and improved treatments to be developed. Mice which are deficient in an enzyme called sphingosine kinase, and therefore cannot generate a lipid called sphingosin-1-phosphate (S1P) do not develop pulmonary hypertension when exposed to lowered oxygen (hypoxia) for 2 weeks. It can therefore be hypothesised that this enzyme or S1P itself may be involved in the response to hypoxia which leads to the development of pulmonary hypertension.

Consequently, we have synthesised a number of potent and specific inhibitors of this enzyme and we will test the effectiveness of these in preventing pulmonary hypertension in mice. This project will assist in understanding how spingolipids are involved in pulmonary hypertension and if targeting their formation can alleviate the sysmptoms of pulmonary hypertension

The project will first study the biodistribution of these inhibitors after injection to check their suitability for in vivo use and will then, subject to satisfactory results, use an established model of hypoxia-induced pulmonary hypertension (2 weeks hypobaric oxygen) to study efficacy of the these novel compounds.

It is necessary to use animals as no in vitro system can replicate the way the blood vessels in the lung remodel in response to hypoxia. We will minimise animal numbers by extensive in vitro testing of any drugs to optimise the chances of success and limit the risks of serious or unforeseen toxicity. For all studies we undertake power calculations to assess the number of animals required and we are constantly refining and improving our techniques in line with best practice to ensure that the procedures have a low risk of failure and are minimally invasive with a short recovery time. Over the life of the project licence, we estimate that no more than 500 mice will be used. Mice have been selected as they are a suitable model of hypoxia-induced pulmonary hypertension and the availability of genetically modified animals (for example those lacking sphingoisne kinase enzymes or specific S1P receptor subtypes) allows scope to study the effect of the sphingolipid system in GM animals transferred to this licence.

The techniques to be used are well characterised and not associated with severe adverse effects. Under hypoxic conditions animals do not gain the same weight as normal animals and may in fact lose a small amount of weight. Other techniques such as measuring systemic and right ventricular pressure will be performed under terminal anaesthesia by experiences personnel which will minimise the risks of adverse effects.

As a disease with a devastating effect for those unfortunate enough to suffer from it, and with no truly effective therapy to slow disease progression or effect a cure, we believe it is justified to use animals to learn more about the disease and identify the role of the sphingosine kinase enzyme.

In vivo testing of drug formulation

The project aims to screen different formulations in animal models of diabetes and cardiovascular disease for therapeutic efficacy along with pharmacokinetics of the drugs that will help to better understand the in vivo fate of the drugs loaded into advanced drug delivery systems. The distribution of particles into different organs of body is not very well understood. The fate of the particles shall also bear important consequences on the rate and distribution of the drugs incorporated in them. The amount of drug reaching the target site in the body after administration from a particular route is to be understood clearly to predict the effect of the particulate-encapsulated drug. Also, it is not known if a decrease in the amount of drug required to provide a therapeutic benefit is possible. This could possibly decrease the cost of therapy while decreasing the side effects related to the drug. Finally, improving formulations used in diagnostic imaging in order to prevent tissue damage is an important clinical need and this project will address this as well.

The studies envisage developing insights into correlation between the amount of drug in blood and various tissues following treatment with developed formulations and the therapeutic benefit exhibited. We intend to demonstrate the efficacy of the advanced delivery systems against the conventional formulations. The project has several short-term, moderate and long-term benefits catering the needs for healthcare. The short-term includes understanding the pharmacokinetics and the feasibility of novel delivery strategies in preclinical setting; the moderate benefits include identifying the new indications of existing drugs aided by better delivery approaches and finally the long term benefits include better management of human healthcare thereby cost-cuttings to NHS where there is significant spend in diabetes and CVD.
If we get funding we would expect to use up to 2,200 Rats, over the period of 5 years.

The principal adverse effect on the rats comes through creating a disease model. We have estimated that most of the procedures may fall into moderate severity. Diabetic rats will lose weight, body condition & they will drink a lot of water, urinary or blood glucose levels will be monitored daily. They will not be kept for extended periods so the diabetes is not expected to progress to other complications, and they are not allowed to lose more than 20% body weight compared to age matched control animals. Hypertensive rats will have high blood pressure (BP) & will be regularly monitored, the BP will not be allowed to reach dangerous levels that may result in cerebrovascular accidents (e.g., stroke). Hence, we do not expect to see any clinical adverse effects as a result of hypertension. Some rats will be given repeated doses of drugs. The stress to the rats can be ameliorated through good handling & injection technique and frequent monitoring for any adverse effects. Typically at the end of the experiments rats are humanely killed to collect tissue & blood for analysis.

The amount of drug that is available for therapeutic benefit to the body can only be estimated in a whole body system. Also, to assess the effect of drug on a particular disease, only the whole body system can provide such information.

A series of in vitro studies are planned to help minimise the number of animals required. The number of animals estimated for each study was chosen according to the power calculations made for each experiment based on the key biological parameters responsible for the outcome. Pilot studies are planned wherever necessary. Good handling to minimise discomfort and at least daily observation after dosing. Veterinary advice will always be sought where and when necessary.

Investigations into Spotty Liver Syndrome

The aim of the project is to determine whether a newly-discovered bacterium is the causative agent of Spotty Liver Syndrome in chickens, and if so, to understand the disease more fully.

Spotty Liver Syndrome (SLS) is a condition of domestic fowl that causes acute mortality with characteristic gross and microscopic pathology. Significantly, the cause of the disease is as yet unknown. Recently a novel *Campylobacter* has been isolated from five outbreaks of SLS that occurred in four free-range laying flocks in England. This organism has distinctive culture requirements which mean it would typically be overlooked under standard laboratory culture protocols. It is hypothesized that this novel *Campylobacter* is the causative agent for SLS. Demonstrating the role of the bacterium in the disease can only be done in controlled experiments using an appropriate live animal model, and chickens are evidently appropriate.

Since there is no knowledge of the transmission route(s) of the disease, an invasive (intraperitoneal) route of inoculation using a high dose has been chosen for the initial studies. As the incubation period for infection is also unknown, there will be regular and frequent observations and post mortem examinations will be performed at frequent intervals following challenge. Expert opinion and statistical advice have been sought to ensure the minimum number of birds are used to provide statistically valid data. Any findings would then be used to aid the design of future studies aimed at understanding the disease more fully and hopefully developing successful intervention measures.
Up to 300 birds may be used over the duration of the project.

The disease can result in acute liver disease and death. However, such an outcome is unlikely in our proposed studies. Frequent and regular observation by trained carers and the use of clinical score-sheets will ensure the progress of disease is closely monitored. Birds showing symptoms will be humanely killed immediately to avoid full disease progression and undue suffering.

The project has the potential to confirm the identity of the causative agent of a serious poultry disease and thus significantly aid in our understanding and hopefully control of that disease.

Training in Specialised Therapeutic Procedures

This project will train surgeons in new, minimally invasive (keyhole surgery), surgical procedures.

Minimally invasive surgical procedures are significantly better for patients as they are associated with less time in hospital, faster recovery, less pain, easier post-operative care and much faster return to active life. Consequently many new minimally invasive procedures are being developed to replace larger, open procedures - particularly in response to the Governments new screening programmes for bowel cancer and aortic aneurysm among others.

These screening programmes are identifying 30-40% more patients requiring surgical intervention for their conditions and the number of surgeons qualified in the new procedures is very limited. Untrained use of these new procedures results in unacceptable mortality and morbidity. We will teach surgeons these new procedures, in terminally anaesthetised pigs, to ensure rapid competency and safety. Simulators will be used as part of the teaching but, as yet, there are no simulators that truly represent the full physiological state necessary to teach these procedures.

We will endeavour to develop better simulators as these courses progress. By carrying out a number of procedures in one animal we can reduce the number needed and, as all animals will be deeply and terminally anaesthetised, there will be no suffering or adverse effects. The pig has been chosen for these courses as we need to represent the same size and physiology as humans, in particular with regard to blood system, lymph system, tissue response and general anatomy.

These courses will ensure an adequate supply of appropriately trained surgeons who will be able to fulfil the needs of our increasing numbers of patients using new minimally invasive procedures safely and effectively.

Investigation of the Role of Nox and Reactive Oxygen Species in the Pathogenesis of Metabolic Disorders and Cardiovascular Diseases

The overall aim of this project is to discover the roles of the Nox enzyme and its product, reactive oxygen species (ROS) in the development of metabolic disorders and cardiovascular diseases for the purpose of discovering new targets to treat or to prevent these diseases.

Cardiovascular disease is a major cause of death and illness in subjects with obesity, insulin resistance and type II diabetes and represents a major healthcare problem. Studies from us and others have discovered that excessive production of reactive oxygen species by an enzyme called NADPH oxidase (Nox) causes oxidative damage to the lining of blood vessels, the endothelium, causing endothelial dysfunction which is a feature in the early stage of the development of cardiovascular diseases. Compelling evidence has shown that factors such as angiotensin II, high blood glucose and high fat (or cholesterol) diet may activate Nox and cause oxidative stress throughout the body contributing to the development of endothelial dysfunction, insulin-resistance, obesity, type-II diabetes, hypertension and atherosclerosis. However, the role and the mechanism(s) of Nox activation in these diseased conditions are largely unknown.

Cardiovascular diseases and metabolic disorders involve malfunctions of multiple organs/tissues and cells in a living animal or human. We have to use animal models of these diseases to understand the role and the mechanisms of Nox and ROS in the development of hypertension, atherosclerosis, obesity, insulin-resistance, type 2 diabetes and other cardiovascular complications. There is no alterative way to achieve this. Every alternative way (such as in vitro cell culture and ex vivo organ functional assessment) has been considered and applied in our project whenever it is possible.

During our study, genetically modified mice with Nox deficiency will be bred. These mice will then either be used to provide tissues for our work or they will be used in studies for AngII-induced hypertension or high-fat diet induced obesity, insulin resistance and type 2 diabetes. The numbers and sizes of the groups of mice will be determined by statistical analysis, but will be kept to the minimum required for significant results. We will try to use the same animal for several sets of experiments such as for cardiac stem cell isolation (heart), for vessel contraction (aorta), for bone marrow cell isolation (legs) to reduce the number of animals.

Our research investigates the reduction of oxidative stress which is expected to protect animals from endothelial dysfunction and reduce disease symptoms. This project will provide an insight into the role and the mechanisms of Nox and its product, reactive oxygen species, in the development of metabolic and cardiovascular diseases. The crucial information from this project will be used to discover the new targets for the development of novel therapies for patients.

The Physiology of Left Ventricular Hypertrophy

The project aims to measure the changes to the electrophysiological properties of ventricular myocardium after the induction of left ventricular hypertrophy and upon regression of the condition. In particular the role of intracellular Ca^{2+}-dependent phosphatases in generating the electrophysiological changes will characterised.

Left ventricular hypertrophy is an adaptive response of the heart to increased stroke work after an increase of afterload. However, cardiac growth is associated with an excess of arrhythmias in patients, a risk that persists even after the afterload has been normalised and the heart has regressed to its normal size. The reason for this excess of arrhythmias is unknown but represents a significant increase in mortality and morbidity to patients.

We have developed an animal model of this condition by artificially constricting the thoracic aorta in guinea-pigs, a model that allows removal of the constriction after hypertrophy has developed to follow regression. Data from this model closely matches those from human hypertrophied myocardium, because the cell physiology of guinea-pig ventricle is very similar to that of human myocardium. The model thus represents an excellent way to understand the changes that take play to myocardium that predispose the heart to arrhythmias.

In this project, left ventricular hypertrophy will be generated by constricting the thoracic aorta: in one group the constriction will be removed to allow regression to occur. In a second group the constriction will remain. A final group will undergo sham-operations, with no constriction imposed, to act as age-matched controls. In the initial phase of the study (first two years) 54 animals will be required and data will seek the fundamental causes of persistent electrophysiological changes. In subsequent phases identified pathways that are shown to be altered in hypertrophy and its regression will be further characterised, requiring about 30 animals per year.

An animal model is necessary as human tissue is increasingly unavailable, as surgical procedures to correct the causes of hypertrophy have progressed. Moreover human biopsies come from hearts with other potential co-morbidities that may confound interpretation of the data. An equivalent model using cultured cells or in silico approaches is not feasible as the model requires that ventricular hypertrophy must be generated in the heart before the experimental study can be initiated.

Because of my experience with this model the number of animals that are required have been greatly reduced; operative and post-operative mortality is greatly reduced. Animal suffering is kept to a minimum by using good operative procedures and the prevention of co-morbidities such as heart failure. Moreover, control data obtained from previous studies means that they may be used as base-line measurements against which data from interventional animals may be compared, thus reducing the total number of animals required.

It is anticipated that it will be possible to identify changes to specific cellular pathways that underlie arrhythmogenesis during hypertrophy and its regression. This will expose novel drug targets that may be used to minimise the risk of arrhythmias during cardiac growth and upon its regression.

Reduction of the Sequelae of Ischaemia

Any tissue which suffers a restriction in blood-flow (ischaemia) will show either temporary or permanent changes some which will be detrimental and can lead, if untreated or treated inappropriately, to severe damage. Some restrictions in blood-flow happen without any interventional stimulus as in stroke, heart attack, diabetes and obesity. Some restrictions happen during, and sometimes after, many forms of surgery and some happen due to accidental trauma. If blood is re-introduced inappropriately after a period of restricted blood flow then a condition known as “ischaemia reperfusion damage” (IR) can occur.

This research will advance our knowledge of how ischaemia/IR occurs and progresses and where are the most appropriate places along this progression that we can intervene to reduce or prevent the effects. This knowledge will be important in developing new treatments or prevention strategies for ischaemic episodes as in stroke, heart attack, seizures and diabetes.

Likewise, this research will identify new procedures and other regimes which are able to prevent or reduce ischaemia/IR in a variety of surgical areas such as transplantation, implantation, novel techniques and new instrumentation.

In the short term this could lead to better tissue management for preventing immediate ischaemia/IR and in the long term may lead to prolonged tissue storage and banking
The development of new devices to limit haemorrhage would benefit all surgical cases where there is a potential for bleeding that can result in increased ischaemia/IR and associated tissue damage.

All of these advances will reduce patient morbidity and pain, increase quality-of-life, reduce dependency on the NHS and may save lives in some cases. These treatments will have uses in both human and veterinary medicine.

For all proof-of-concept studies and size translation studies the numbers will be limited with between 4 and 6 animals only being used to establish whether the treatment should progress. The least sentient animal consistent with necessary results will be used. Ensuring that only those procedures, materials or devices that have proven potential by way of *in-vitro* and *ex-vivo* assessment progress to live animal studies will keep animal use to a minimum
For all transplantation and implantation studies, where possible tissues/organs will be retrieved from animals undergoing termination from other projects.

Where specific donors are needed the maximum amount of tissue/organs will be harvested to minimise donor numbers. For studies to be used for regulatory submissions we will be bound by the relevant regulations for appropriate statistical analysis. We will also use our statistician to make sure we use the least numbers of animals possible.

Where studies allow, rodents will be utilised first and if the procedure is successful then it will be transferred to larger animals such as pigs and sheep for compatibility with human requirements. Creation of small areas of reduced blood flow for assessment of potential treatments of ischaemia/IR under general anaesthesia will not result in lasting harm to the animals. Those who are recovered from anaesthetic for long term assessment may show transient effects of the ischaemia/IR but this will be closely monitored and if pain is suspected then this will be treated under veterinary supervision or, in the case of prolonged symptoms the animals will be killed by a schedule 1 procedure.

Some tissues that have undergone tissue banking by cold storage methods will be transplanted to test their viability, these animals that exhibit any untoward symptoms will be managed with pain relief and anti-inflammatory drugs and if too persistent the animals will be killed by a

schedule 1 procedure.

For all of the studies the severity limit will be moderate and close monitoring of all animals used will keep pain, suffering and distress to the lowest possible levels whilst keeping the animal's welfare to the highest standard possible. This is achieved by the use of analgesia, appropriate anaesthetics for species, antibiotics where relevant, attention to fluid balance and appropriate animal nursing and husbandry.
All procedures will be carried out under aseptic conditions using aseptic technique and we do not expect to see any post-operative infection.

All treatments applied under this license will have to show substantial potential for the prevention or reduction of ischaemia/IR *in-vitro* and *ex-vivo* before we will take them into studies using animals. We will continue to investigate cellular culture and other *in-vitro* analyses alongside these studies which may reduce animal use by replacement or refinement during the course of this license.

There are currently no non-animal alternatives for these studies as a fully functional physiological system is required to create ischaemia/IR and test the treatments.
We have recently been able to successfully argue against the use of controls in some of these studies and will continue to do this in order to reduce the numbers of animals used.

Animals will only be used after in-vitro and ex-vivo models have shown substantive evidence that a regimen will significantly reduce or prevent ischaemia/IR.

There are no synthetic models which can be utilised to evaluate the progress or treatment of ischaemia/IR as a fully functional physiological system is required – thus there is no substitute for the whole animal.

Early "proof-of-concept" studies and size translation studies will use limited numbers of least sentient animals consistent with appropriate results. Only regimens with substantial evidence of success potential will be progressed through to GLP studies. Where studies are conducted according to regulatory body requirements, the minimum number of animals will be used and arguments against "controls" will be pursued where possible.

For transplantation and implantation we will, where possible, use donor tissues/organs retrieved from animals under termination from other projects.

Where specific donors are needed the maximum amount of tissue/organs will be harvested to minimise donor numbers.
Those tissues directly harvested from Protocols 1 and 2 are usually at the proof of concept stage and the organs are analysed to prove efficacy of treatment.
In some studies we can argue that function assessment of the targeted tissue can be observational rather than statistically driven which means fewer numbers will be needed.
Where statistical analysis is required we will take advice from the statistician as to the least number of animals we can use to provide the necessary data.

All the models we will use under this license have been developed, established and refined over the last 20 years. We have managed to successfully establish some procedures in mice which were traditionally carried out in larger species such that we can provide early information in the "proof-of-concept" phases of ischaemia/IR research.

Using an increased bank of assessments in which we can now include non-invasive imaging has further refined our techniques and we will endeavour to develop additional refinement as these studies progress.

Where possible the lowest vertebrate group is used for each procedure. Size of an animal is an issue that has to be considered when utilising surgical techniques. Mice are used when the surgery is possible (and does not lead to greater surgical loss due to size). The species chosen for each procedure also depends on the tractability of the animal species, statistical requirement and clinical relevance. Where a procedure has generated good results in, for example, rodents, this may then be applied to a ‘higher’ species, for example, pigs, to evaluate the results that may be generated in man.

Some animals are chosen because their size makes them easier to use for operative procedures and because of their physiological and physical similarity to humans, for example, pigs are utilised for skin and bowel procedures and pigs, sheep and goats are used because of better surgical access, size of organs and longer l