

**UNIVERSITY OF DUBLIN**

**TRINITY COLLEGE**



# Laboratory Animal Science

Sophister Module  
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## HISTORY OF THE USE OF ANIMALS FOR RESEARCH

The first record of the use of animals in mans quest

### The Plague or Black Death ,

The great epidemic of bubonic plague that ravaged Europe in the 14th century, killing between one-third and half of the population. The cause of the plague was the bacterium *Yersinia pestis*, transmitted by fleas borne by migrating Asian black rats. The name Black Death was first used in England in the early 19th century.

for knowledge was about the year 500 BC when Alcmaeon of Crotona severed the optic nerve of a pig to determine the function of that structure. He can be called the first medical scientist.

The Auguries and Oracles of Roman times regularly inspected the entrails of chickens in an effort to predict the future.

In the 17th century Asseli discovered the function of the lymph system by observing the absorption of fat in the open intestine of a dog, while in the 18th century Boyle showed the existence of a vacuum by asphyxiating a kitten in an evacuated glass bowl. In the 19th century, Pasteur and Lister demonstrated the role of bacteria in disease and produced the first vaccines.

The development of biological science continued apace from then to the present day with the discovery of the action of insulin and the development of heart-lung by-pass machines, to name but two landmarks.

The moral climate regarding the use of animals has varied throughout the centuries.

Experimenters such as Galen (130-201 AD), physician to the Roman Emperor, Marcus Aurelius, were more concerned with ensuring that they would not be accused of human experimentation than with concern for the welfare of the animals. There were some expressions of concern by the early workers with the agonies suffered by their victims; however, these were suppressed as a weakness which they could not afford if they were to continue in the pursuit of knowledge.

The Plague or Black Death , that ravaged Europe in the 14th century, was responsible for the awakening of thinking people to the fact that religion and current medicine had little effect on the course of this disease. Contemporary medicine was powerless to slow down or halt the spread of the disease. Thinkers like Roger Bacon questioned the God centred view of the world and began to look for proofs. Thus began the renaissance and the seeds of the scientific method.

The presence or absence of a mortal soul still dominated the debate in the 16th, 17th and 18th centuries. It was believed that animals, while showing expressions of pain, were not capable of appreciating it as humans did, because of the absence of a 'soul'.

This theory suited the Church of the time and was enshrined in Rene Descartes writings depicting the '*Beast Machine*' which stated that while animals appeared to express pain when hurt , that this was but a poor imitation of mans ability to feel and appreciate pain. He contended that because of the absence of intellect that animals were not able to

understand pain and therefore not able to feel it. However he accepted that they had all the pathways to feel it if only they had a soul to appreciate it. Any antivivisection sentiments expressed at this time, were more often to do with the stance taken by the active researchers who were questioning the conventional treatment of human diseases. This was no more acceptable to a conservative society then, than it is now.

It was not until late in the 19th century and the early years of the 20th century that concern for the animals were expressed. Guidelines on humane treatment and appropriate control of animal experimentation were laid down. In fact, a lot of the thinking and resulting decisions of that period can be used as moral and ethical benchmarks against which to judge todays actions.

In 1831, a physiological experimenter, *Marshal Hall*, set down the following five principles which should guide the decision on whether or not to use animals for a specific experiment.

- (i) '*Is the experiment necessary?*' Could observation alone attain the required results? Could we make more use of naturally occurring phenomena such as disease outbreaks and other epidemiological information?
- (ii) '*Has the experiment a possibility of achieving the desired result?*' Is the hypothesis a valid one and has the project been planned correctly with all the instrumentation etc in place?
- (iii) '*Can the protocol be modified to reduce the discomfort of the animal?*' Can anaesthesia or analgesia be used to control pain or discomfort?
- (iv) '*Has the experiment been done before?*' An obvious point but one often overlooked by experimenters who may be tempted to rush into animal experimentation on a 'lets-see-what-happens' basis rather than to spend sometime in the library.
- (v) '*Will the protocol produce valid results?*' i.e. Is the design of the experiment such that the results obtained will be valid? Are enough animals being used to achieve statistical significance? or is the appropriate species being used?

### Bacon Roger 1214–1292

English philosopher, scientist, and a teacher at Oxford University. He was interested in alchemy, the biological and physical sciences, and magic. Many discoveries have been credited to him, including the magnifying lens. He foresaw the extensive use of gunpowder and mechanical cars, boats, and planes. In 1266, at the invitation of his friend Pope Clement IV, he began his *Opus Majus* /Great Work, a compendium of all branches of knowledge. In 1268 he sent this with his *Opus Minus*/Lesser Work and other writings to the pope. In 1277 Bacon was condemned and imprisoned by the church for 'certain novelties' (heresy) and not released until 1292. He followed the maxim 'Cease to be ruled by dogmas and authorities; look at the world!'

in all member states has and will control the use of animals in the future.



Figure 1 Galen on a Yemen stamp Signifying his contribution to Islamic knowledge

To these principles of Marshall Hall could be added the three aspirations of Russel and Burch (1959) of '**Reduction, Refinement and Replacement**'.

**Russel and Burch** were commissioned in 1959 to study the situation regarding the use of animals in research. The results of their study was published in a seminal work called the *Humane Experimental Technique*. In it they assessed the necessity of using animal's in research and the condition under which they were kept. They coined the use of the 3 R's which guide all responsible decisions regarding the use of animals in research today.

They are:

**Reduction** of the number of animals.

**Refinement** of the techniques used

**Replacement** of living animals by in vitro techniques where ever possible.

The moral guidelines of *Marshall Hall* are enshrined in the 'Cruelty to Animals Act, 1876' which controlled the performance of experiments involving animals. This Act has served well to the present day and has only recently been replaced in the UK and Europe in response to the modern 'Animal Rights Movement' which has made society re-appraise its values in regard to the role of animals.

The EC Directive which currently determines the treatment of all animals used for a scientific purpose

**Aquinas St Thomas c. 1226–1274**

Neapolitan philosopher and theologian, the greatest figure of the school of scholasticism. He was a Dominican monk, known as the 'Angelic Doctor'. In 1879 his works were recognised as the basis of Catholic theology. His *Summa contra Gentiles/Against the Errors of the Infidels* 1259-64 argues that reason and faith are compatible. He assimilated the philosophy of Aristotle into Christian doctrine. His unfinished *Summa Theologica*, begun 1265, deals with the nature of God, morality, and the work of Jesus. His works embodied the world view taught in universities until the mid-17th century, and include scientific ideas derived from Aristotle.

**HISTORICAL HIGHLIGHT SHOWING THE DEVELOPMENT OF THE USE OF ANIMALS TO ADVANCE KNOWLEDGE**

500 BC - 1800 AD

- 500 BC Alcmaeon of Croton: optic nerve function
- 350 BC Hippocrates: function of oesophagus
- 150 AD Galen: The four Humours Sanguine, Phlegmatic, Choleric Melancholic

Middle ages little scientific advances

- 1500 AD Aselli -lymphatic vessels; Pecquet - thoracic duct; Harvey - blood circulation
- 1600 Lower - blood transfusion
- 1700 Boyle - vacuum
- 1800 Pasteur - vaccines
- 1920 Banting & Best Insulin for diabetes
- 1930 Diphtheria vaccine
- 1940 Helen Tussig; Hole in the heart corrective surgery
- 1950 Salk and Sabin; dead and then living attenuated polio vaccines.
- 1960 First heart transplants; Man to Man: Christiaan Bernard; Animal to Man: Norman Shumway
- 1970 Drugs to treat ulcers: (Ceser Milstein) - Monoclonal antibodies produced
- 1980 Cyclosporin;immuno-suppressant drug discovered. Monoclonal diagnostic kits for pregnancy detection were in use.
- 1990 DNA probes and Transgenic animals.

The use of animals for research has always been an emotive issue. Galen (150 AD) Physician to the Roman Emperor Marcus Aurelius, is reported to have commented on the pain felt by the *Barbery Apes* which he dissected. Encouraging researchers not to be deterred in their search for knowledge by the distress of the animals, but to firmly proceed if they were to discover the truths of living beings , aid man in his quest for knowledge and to defeat the sickness and disease which beset him.

## Laboratory Animal Science

Development of moral control on the scientific use of animals	
c150	Galen (130-201)
1200	Thomas Aquinas (1225-74)
1679	Wepfer 'Tu Quoque'
1600	Rene Decartes (1596-1650) 'Beast Machine'
1700	Alexander Pope (1688-1744) Samuel Johnson (1709-1784) 'Against vivisection'
1831	Marshall Hall **
1849&1854	First Cruelty to Animals Acts
1873	Francis Cobb-Power - <i>Society for the Protection of Animals in Florence</i>
1875	<i>Victorian Street Society -For The Protection Of Animals Libel To Vivisection</i>
1898	<i>British Union For The Abolition Of Vivisection (BUVA)</i>

Legal control only came about in 1876 when the first UK act of parliament the 'Cruelty To Animals Act' was passed.

This act controlled the use of animals in research and the principles which it set up have been included in modern legislation.

In Ireland the use of animals in research is controlled by the same 1876 Act. This act has been used to implement EC directive (86/609) of 1986 (SI17/94). This directive is the European Unions' instrument to ensure that all member states have similar laws for the protection of animal used in research.

The instrument (SI17/94) has various sections to it.

DEVELOPMENT OF LEGAL CONTROL ON THE SCIENTIFIC USE OF ANIMALS	
< 1876	NO CONTROL
1876	UK. Cruelty to Animals Act
1911	Animal Welfare Act
1965	Protection of Animals Act
1986	Control of Dogs Act
1986	Council of Europe Convention
1986	UK. Scientific Procedures Act
1989	EC Directive Implemented Ireland
1994	SI 17/94

Defining the conditions to be satisfied before an animal is used, establishing training levels for persons involved in animal work and laying down the housing and welfare standard to be provided in animal accommodation.

The main features of modern law are as follows

- **All work for a scientific procedure**
  - must have prior approval.
  - must have scientific value.
  - must cause the minimum distress.
  - must use the minimum no. Of animals.
  - must use the lowest type of animal.
  - must have established a humane end point.
- **All workers**
  - must have considered in vitro alternatives.

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have a duty to care for the animal they use.  
must be adequately trained -

- To achieve a successful outcome
- To be competent at handling animals and performing the necessary procedures.

- **All animals**
  - must be purpose bred.
  - must *not* be reused.
  - must be appropriately housed, fed and cared .
  - must be observed daily.
  - must be humanely killed if the approved severity is exceeded.
- **The premises must be**
  - of appropriate design.
  - registered and approved for the species used.
  - be managed by 'competent people'.
  - have an animal welfare program with a veterinary supervisor.

### STRUCTURE AND FUNCTIONING OF AN ANIMAL HOUSE

Modern animal units are mostly run by dedicated staff who have specific training in the care and

Marshall Hall's Principles of experimental research with animals **
(i) 'Is the experiment necessary?'
(ii) 'Has the experiment a possibility of achieving the desired result?'
(iii) Can the protocol be modified to reduce the discomfort of the animal?'
(iv) Has the experiment been done before?'
(v) Will the protocol produce valid results?'

breeding of animals used for scientific procedures. The training required is laid down by the CoE (Council of Europe) convention on the use of experimental animals as adopted by the EU (EC Directive 86/609). The day to day care people are designated category A and must have recognised qualifications.

Typically, a unit is a central service which is independent of the specific department which they serve in the institute.

The unit is required to be registered and recognised by the Competent Authority, which in Ireland is the Department of Health, and the people working in the unit must be trained to defined standards.

The structure and appointment of the rooms within the unit will conform to the requirement laid down in appendix A of the EC Directive as implemented by SI 17/94.

The unit will be designated as a 'User Establishment' by the Competent Authority; it may also be designated as a 'Breeding and Supplying Establishment', recognised as a supplier of purpose bred animals.

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There are requirements to keep records and to ensure that all work is appropriately licensed.

The purpose of an Animal Unit is to provide accommodation and appropriate environment for animals used for scientific purposes.

To this end it must provide for:

- Animals
- Research workers
- Animal Care staff
- Administrative staff

### ANIMAL REQUIREMENTS

These can be divided into two basic divisions. The **macro-environment** or the room in which the animals is kept or the **micro-environment** which is the cage in which the animal actually lives.

As a rule of thumb we aim to control the macro-environment which will, we hope, give appropriate conditions in the animals cage.

There are modern systems which simply control the environment in the animals cages. These are called ventilated racks (fig 1) which provide conditioned air to each individual cage. However these are expensive and are only warranted if the design of the experiment requires such elaborate specifications.

### **MACRO ENVIRONMENT**

#### **SIZE: EC Recommendations/Species**

The size of the room depends on the type of animal held in it. For larger animals such as pigs, horses dogs etc, it will normally be determined by the floor space available. The appropriate space requirement are to be found in the guidelines attached to the EC directive.

For the more common laboratory animals, the space will depend on the type of racking used, the size of cages and the capacity of the heating and ventilation system.

How often the cages are cleaned will depend on the number of animals in a cage and the bedding material used. When providing bedding for animals cages it is important to ensure that it is bacteriologically and chemically clean and is not a source of infection or contamination to the animals. It is also important to remember that animals use their sense of smell to identify familiar cage mates and surroundings. The use of strongly scented disinfectants should be avoided as they place additional stress on animals. A balance between hygiene and maintaining the existing markings

should be preserved. It is usual to change animals bedded on saw dust twice weekly whereas animals

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bedded on beet pulp may be changed weekly or less often depending on the stocking density.

The common bedding materials are Sawdust, Peat moss, Beet pulp. Corn cob is used a lot in the US.

### **LIGHT: 100 - 600 LUX**

The level of light required in a room depends on the species held in the room. The common species such as rats and mice are often albino, lacking in pigmentation. These animals are sensitive to light and the high intensity of light in a room fully

#### CATEGORY A Persons must be competent at

1. cleaning, feeding and watering of experimental, breeding and other animals
2. competence in basic handling of the common laboratory animal species
3. daily observation and inspection of animals for general condition (including noting food/water intake)
4. restricted methods of euthanasia following well defined procedures
5. general maintenance of animal rooms
6. recording animal room environment and room procedures.

#### TYPES OF SPACE NEEDED FOR :

##### ANIMALS

Breeding, holding, quarantine, experimental, infected/toxic, germ free, immunodeficient stress free etc.

##### RESEARCH WORKER

Equipment rooms, theatre & recovery, procedure rooms, laboratories ,special facilities e.g. isolators, post mortem room.

##### STAFF

Canteen, administrative offices, records, toilets, wash rooms and lockers.

##### UTILITIES

Cleaning equipment, storage, cleaning rooms, washing etc., autoclave, dirty and clean cage storage, food and bedding store, storage & disposal of waste.

illuminated to 600 lux will cause retinal damage

especially to those in the top layer of cages nearest the light source.

These animals are dusk and dawn feeders (crepuscular). They are most active in reduced light. The levels at which they are most comfortable are 60 to 100 lux which is regarded as dim for humans. Modern rooms now incorporate a dual lighting system which has a high level (600 lux) to enable technicians to work in the room and a timed source which will be of a lower intensity (60-100 lux).

To standardise the environment in a constant spring, it is customary to have the lighting on a timed cycle usually 12 hours light and 12 hours dark. This aids breeding and also aids in the standardisation of the animals used.

## VENTILATION 20 CHANGES/HOUR

### Ventilation Systems

There are 2 main types:

1. Positive pressure
2. Negative pressure

They have applications in different circumstances.

The purpose of ventilation is to extract stale air and to provide fresh conditioned air to the room. The level which is required will depend on the species kept in the room and the density of the stocking. It is typically in the region of about 20 air-changes /hour. This means that the air in the room is changed every 3 minutes.

Ensuring that this happens in an evenly distributed way and that the air changes in the room reflect similar changes in the cages is a problem encountered when designing animals rooms.

The structure and positioning of inlet and extract point is the subject of on going debate. Some favouring floor extract and others ceiling extract. An additional factor which has now to be incorporated into the design of air flow in animals rooms is the requirement to reduce the amount of animals dust and dander to which workers are exposed. It is a requirement of the Health and safety at work regulations which are aiming to lower the incidence of animal related allergies.

Currently the air flow configuration favoured, is one in which the fresh air is directed on to the human first. The flow is from the centre of the room to the cages and then extracted through the ceiling.

### POSITIVE OR NEGATIVE PRESSURE

The direction of the air flow will depend on the use made of the room. In a standard animals room the requirement is to keep the animals in that room distinct from animals in other rooms. Stopping smells and organisms flowing from one room to another. Positive pressure ventilation enables this position be maintained. In a positive pressure ventilated room the in coming air is conditioned and forced into the room. The extract air is exhausted at a reduced pressure, maintaining a positive pressure in the room. This ensures that leakage is always out of the room and not into it.

In certain rooms, such as areas where infective organisms or toxic substance are being used it is necessary that all exhaust air is captured and extracted under controlled conditions, possible being filtered on the way. These rooms will be maintained under negative pressure ensuring that

there is no leakage of material to other areas. The personnel using these rooms should be aware of the substances being used in the rooms and warning notices should be prominently displayed.

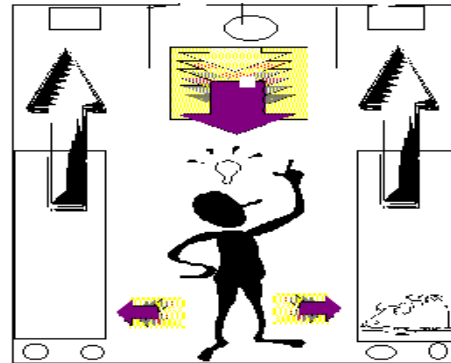


Figure 2 Air Flow Direction, showing the fresh air delivered to the centre of the room to reduce allergen exposure.

### **TEMPERATURE 20-22°C**

The temperature of most laboratory animals is in the range of 18 to 21 degrees Celsius.

Again the guidelines lay down the appropriate ranges for the species. It is important to remember that animals without hair will need slightly higher temperatures.

Animals can, if given nesting material, adapt fairly well to lower or higher temperatures. However large variation must be seen as a stressor which alter the animal physiology. This may affect the animals response in an experimental situation.

### **HUMIDITY 45-65 %**

This is the amount of water vapour which is in the air expressed as a percentage. The humidity can have adverse affects on the well being of animal. The rat in particular is prone to a condition called 'ringtail.' under prolonged low humidity conditions ie <40%. The rat regulates it heat control by increasing the blood flow in it tail. In dry conditions there is excessive evaporation and drying of the skin resulting in cracking of the skin at the base of the tail. High humidity may increase the incidences of respiratory diseases.

### **NOISE < 60 dB**

High levels of noise can be distressing to animals. Especially if they are sudden. Fire drills and building work can have very dramatic effects on litter viability. Under stress conditions many animal eat their young.

It is fairly easy to know when there is too much noise in an animal room as long as it within our auditory range. This however cuts off at about

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20 khz . Most rodent on the other hand have ranges which are far higher then this and much of their communication is conducted at frequencies out side our range. (Rodents 20khz > 60khz). This is important because most engineering solutions which involve noise quenching is concentrated

### HUMAN REQUIREMENTS

Confinement, Separation, Health, Hygiene, Cost, Adaptability, Ease of Use.

### ANIMAL REQUIREMENTS

Activity, Food, Sleeping, Nesting, Defecation/Urination, Breeding.

within the human range and don't take into account these higher ranges. Items like fluorescent tubing , computers , fans and vacuum cleaners etc. give off high levels of ultrasound which may interfere with the communication between animals or simply may be so loud as to be stressful . Putting a rat beside a computer which is generating 90 Db of sound at 30 khz may be the equivalent of standing beside a jumbo jet taking off. Is it any wonder that experiments involving behaviour measurement sometimes appear erratic in the presence of measuring or tracking equipment.

## MICROENVIRONMENT or CAGE

Size: The animal requirements must determine the size of the cage. Minimum sizes are shown in the appendix to the EC directive.

Food: The appropriate type should be available either ad lib or as a predetermined ration. Water must be available *ad lib* at all times. Whether food

### CAGE SIZES AND CAPACITIES

MOUSE BOX 1x 30x 13 cm  
holds 3x 30g mice or a breeding pair and their litter to 21 days

SMALL RAT BOX 41 x 24 x 13 cm  
( Low Lid 13 cm) holds 10 x 30g mice  
( High Lid 20 cm) 4 x 300g Rats or 1x 600 g Guinea pig

LARGE RAT BOX 30 x 55 x 18 cm  
(Flat lid 18 cm) 7 x 300 g Rats or 20 x 30g

MICE  
(High Lid 25 cm) 2x 600g Guinea pigs

is available *ad lib* will depend on the dietary practice in the unit. Most animals will be fed *ad lib*, however, rabbits can present specific problems if they are allowed unrestricted access to food. They become bored and over fat. If rabbits stop eating for some time then they often develop mucoid enteritis and/or ketosis and may die.

Socialisation etc. The animal will divide the cage space into sleeping, defecation and socialisation

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areas, if there is adequate space to do so. It is becoming popular now to consider the concept of environmental enrichment. This involves the inclusion of additional things in the cage such as tubes and small boxes to provide a more stimulation environment for the animals. This is particularly popular when primates are being housed. It is becoming more popular in rabbit housing where 'free range' rabbits are becoming more common.

The cage where the animals lives, perform two functions. It must provide for both Human and Animals requirements.

The quality of the cage must be durable. It is subjected to wear an tear from both washing handling and from the efforts of animals to explore and define their environment. They are normal made of either opaque or transparent polycarbonate. Whether the cage should be clear or opaque is an area of discussion. Many carers in the UK feel that animals prefer non transparent cages as they will feel more secure in them. While their European counterparts prefer the transparent cages. From a maintenance point of view it is much easier to inspect animals in transparent cages on a daily basis. If an animals is sick it is easier to observe without interfering with it when it is housed in clear cages. It is possible that the feeling about solid cages is based on an anthropomorphic point of view. It presumes that a rat or mouse can distinguish fine detail and can perceive clear images through the wall of the cage. Based on the absence of a clear optic horizon in the retina of these animals it is unlikely that they can focus and see clear fine images as humans can . Most of their sensory perception is based on touch and smell. Work which we have done on preference testing indicates that the animals shows no preference for either a clear or solid cage.

What seem to be important to the animals is that there is some area which is darker then the rest of the cage. Here animals may build their nest and subordinate animals may escape from more dominant cage mates. Experiments have been done using cardboard rolls and plastic insets to determine animals preferences. The problem about adding a lot of extra clutter to a cage is that it increases the cleaning and maintenance time of cages. It is also more difficult to maintain a hygienic environment. These are problems which still have to be overcome for the smaller of the laboratory animals species. It is important that animals have nesting material both to help temperature control and to provide areas of seclusion for nesting and breeding animals.

## SUPPLY OF ANIMALS

Animals enter into animal units from two main sources.

1. Imported from commercial breeders or other institutes.
2. Bred in house.

If animals are being imported into the unit their disease status will have to be taken into consideration before allowing them to be mixed with animals already in the unit.

It is advisable to have a quarantine facility which allows assessment of animals before they are integrated into the unit.

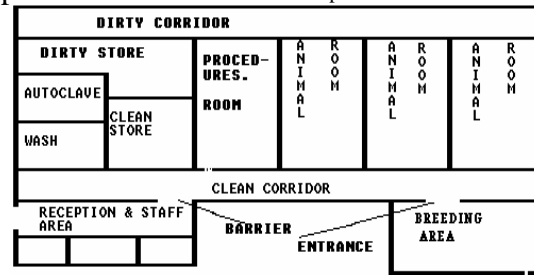
Animals coming from commercial sources will typically have come from a colony which has been subjected to a health monitoring scheme. In Europe the most commonly used scheme has been developed by The Federation of European Laboratory Animal Associations (FELASA). The results of the monitoring will be available for inspection.

Animals coming from other sources may have a more unreliable health status. It is advisable to regard these animals as potentially infective and they should not be integrated into the main unit until steps have been taken to determine their health status.

If a unit is breeding its own animals the breeding areas should be regarded as special. They should be viewed as the most important part of the Unit. If breeding colonies acquire some disease, the integrity of the continuing supply of animals is compromised. It is common for breeding colonies to be housed separately to the main animal areas. There should be some form of health monitoring in place.

### FACILITIES AND EQUIPMENT

The construction of an animals unit must be of material which can withstand regular cleaning and washing. All corners and edges should be curved to aid cleaning. There should be rodent barriers on each animal room door, including all outside doors. There will need to be a hygienic management system which allows for a dirty clean circulation between rooms. This ensures that any items coming from outside can be disinfected and quarantined as necessary. It allows for a system whereby dirty caging and bedding material are removed from the rooms without coming in contact with the fresh cages, food and bedding material. One way to achieve this is to have a double corridor system



In this system the air flow is arranged so that the leakage of air is from the clean corridor to the dirty corridor. The rooms have two doors with entry of clean material from the clean corridor and exit through the dirty one. This sets up a circulation which maintains the integrity of the cleaning system.

In addition it is sensible to restrict entry to the animal holding areas and provide some barrier this may be as simple as the donning of a lab coat to showering with full cloths change. This will depend on the health status of the animals contained in the rooms.

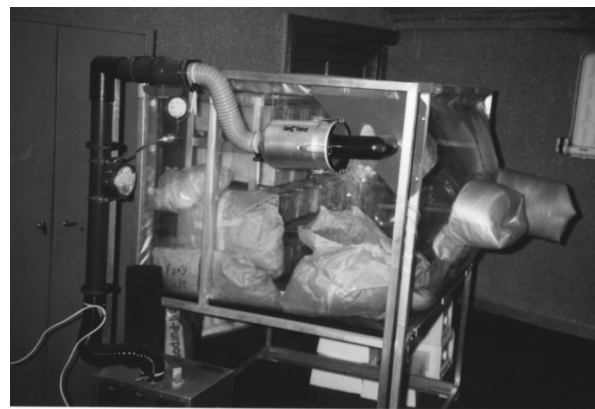
It may be necessary to provide specialised isolation areas such as isolators for either very sensitive animals or very 'dirty' animals.

There are various ways of isolating animals, from simple filter top through filter cabinets to full blown isolators.

### SERVICES

#### Provision of water and drainage

Fresh water of a potable quality should be supplied. If automatic watering is provided a facility for flushing the tubing and header tanks should be present. Food may need to be sterilised. Water may be kept from harbouring micro-organisms by acidifying it.



Isolator

The provision of drains in the centre of the floor is an area which has its devotees and its opponents. They facilitate wet cleaning by hosing the room. However, the presence of the drain and its gully trap provide an area which is impossible to keep fully

clean. My personal preference is for a room without drains which can be wet cleaned and vacuum sucked dry. However if drains are to be provided it is desirable to be able to seal them when they are not in use A rodent trap should be provided in every room to keep those rodents who should be out, out and to keep any escapees in.

### STAFF AND WORKERS

People who are working in a unit must be provided with appropriate facilities , offices, canteen, toilets etc. research staff may need to be provided with special room within the unit to house their equipment etc. It is worth while, if possible, to keep all animals within the confines of the Animals unit. This helps preserve the integrity of the health of the colony. It also ensures the standardisation of the conditions in which the animals are maintained. This requires the provision of laboratory equipment for use by researchers or at least the provision of space in which to house it. This is often a problem as space is normally at a premium. However it is a goal to be aimed for where possible.

### UTILITIES

A lot of space may need to be allocated to this area. Equipment such as plant for controlling the air handling , cage washers , autoclaves, etc. will have to be provided . As will special rooms such as post-mortem rooms, surgical theatres, special containment facilities to class 1, 2 or 3 standards.

Upwards of 30% of the space allocated to an animals unit may be used in non animals holding areas. These may be areas which are not immediately apparent to the worker but are vital in the efficient running of a animals unit

Waste disposal deserves a special mention in that it is becoming and increasingly difficult area. The legislation in this area has become very restrictive and is controlled by the 'Waste Management Act 1996'.

This means that all waste must be rendered safe before being sent for final disposal. Categories of waste which are regarded as Hazardous are Infected, Toxic, Radiological. The responsibility for the rendering of the waste safe is in the hands of the producer. This must be taken into consideration when designing an experimental protocol.

All appropriate precautions need to be taken when handling waste.

## **Alternatives To the use of animals in scientific research**

Article 23 (1 ) of the EC directive 86/609 requires that ' *The commission and Member states should encourage research into the development and validation of alternative techniques which could provide the same level of information as that obtained in experiments using animals , but which involve fewer animals or which entail less painful procedures and shall take such steps as they consider appropriate to encourage research in this field. The commission shall monitor trends in experimental methods.* ';

This has been translated within the EC into ECVAM (European Centre for Validation of Alternate Methods) This is situated in Ispra Italy and its aim is to, as its name implies, to research the replacement of existing in-vivo methods with invitro methods.

I suppose a position statement on behalf of scientists is appropriate here. If I say that the position of the scientist with regard to the use of animals is largely neutral. A scientist will use what ever is the best method available to him/her to give valid reproducible results. Obviously within the constraints of moral and legal controls.

I would hope that if this was accepted as the position then the reasons for using animals will always be justifiable with regard to methodology. Obviously no person is fully and truly objective. They will reach a position of action using all their reasoning power and also all their prejudices and experiences.

It is not in the scientists interest to automatically decide to use animals if they are not the best choice available. So they will be constantly trying to find new methods to solve problems. If the research community do not provide alternatives to using animals then animals will be used. Scientist do not use animals because they have some specific hang up about using and hurting animals . They use them because they are the best available to them. If there are alternatives then it is up to the concerned scientific community to develop them and to fund them. This is happening . Not by the action of militant extremists but by the daily slog of research scientists who take this as their job. Such people are working for FRAME and ECVAM The John Hopkins Centre for alternatives etc.

Why this hang up on finding replacements for animals.

The answers are two fold.

#### 1. Scientific.

Animals experiments are by their nature less reliable then more calibrated systems.

## 2. Ethical stand point

The use of animals is being questioned more and more by society and the continuing use of them without justification is becoming harder and harder to justify with consequent increased difficulty in getting permission to perform scientific procedures in animals..

1959 Russell and Burch in the Book 'The principles of Humane Experiment; technique' set out 'the removal of inhumanity: the three 'R's' Replacement Reduction and Refinement. (p64 Ed).

These basic guidelines have directed the search for alternatives.

While these three words are useful benchmarks they should be seen as such as in fact there are no real solid frontiers between the concepts. Many reductions in the use of animals will come from refinements in some areas such as the production of monoclonal antibodies in cell lines while or replacements from the development of techniques such as horseshoe crab blood coagulation to replace pyrogen testing in rabbits.

The other aspect that must be considered is that new techniques are often unable to replace the traditional test fully at present and additional techniques will be needed to address the areas which are not solved by the new tests. For example the horseshoe coagulation test deals well with gram negative bacterial derived pyrogens but is not so sensitive to gram positive bacterial pyrogens.

**Reduction.** The use of animals world wide is about 75 to 100 million per annum In the Netherlands and the UK this usage has dropped by 60% in the last 10 years.

In Ireland the figure in 1984 was 36,000 and by 1992 this had fallen to 22,000 animals . However in 1994 the figure had risen again to 42,000.

There is now a general trend of increased use of transgenic animals which are mostly mice . This is because of the upsurge in research in the area of genetics and genetically modified animals.

The progress in experimental toxicology is slower as the scientific basis of toxicology is less precise than that of pharmacology and also the questions asked of toxicology are inevitably more general. This has led over the years to a set of recommended test required by regulatory agencies and they are comfortable with them. Moving them from this complacency is difficult and has to be made on a much larger world scale rather than at the individual scientists bench. However there an organisation which is EU sponsored the European Centre for the Validation of Alternate Methods (ECVAM,) which is validating standard tests for use in toxicological and safety tests

A major success here has been the replacement of the notoriously unreliable LD50 test by the fixed dose toxicity test and the increasing replacement of the LAL test to replace the standard pyrogen tests. The EU and the UK have recently effectively banned the use of animals to test cosmetics.

**Refinement :** The use of animals has been refined in many areas often in the numbers used for specific experiments the use of humane end points the better use of statistical models which require fewer numbers of animals of more refined or predictable strains. The use of modern analgesics and surgical methods. The use of cell lines derived from animals and the use of tissue slices and isolated organised techniques. These techniques do not replace animals but use animals in different and more productive ways.

**Replacement.**

This area is the Mecca of all Alternative researchers. It involves developing techniques which replace the use of animals in experimental situations.

It is divided into two, areas of research.

Basic scientific research and toxicity and safety testing.

*Basic research.*

Because research in this area is normally working in on the frontiers of knowledge progress in this area has been slow and it is unlikely that there will be many major advances. The use of invitro cell lines to grow monoclonal antibodies is an area in which mice have been replaced. is one example

*Toxicity and safety testing*

This area is one in which the potential for replacement is much more likely. As the tests used are largely repetitive under well defined conditions the possibility of replacement is high for specific conditions. The advances in this area reflect this potential.

20 years ago the testing of a compound for carcinogenicity potential was largely done by life time studies in rats and mice. Since the relationship between mutagenicity and carcinogenicity has become fairly established the short term mutagenicity tests such as the Ames test have become routine, no compound which fails a battery of mutagenicity test will ever be tested in animals. The investment would be too large for a suspect mutagen. An Ames test or DNA repair test can be done for a few hundred pounds whereas a 2 year carcinogenicity test in rats or mice would cost in the region of £200,000 top perform.

The areas of irritancy testing has also been investigated mainly by the cosmetic firms and isolated organs such as rabbit eyeball or isolated bovine or porcine cornea are used to predict irritancy in the eye good correlation has been found for the decrease of light transmission when exposed to irritants. These work on some forms of irritant. However some products instead of causing increased irritancy have caused increased sloughing of layers and so increasing rather than reducing light transmission. There are engineered membranes such as the EYTEX system which have been found to have useful correlation to 'Draize' irritancy in some compounds for instance alcohol based compounds.

In the end of the day these tests only show the irritancy of the compound The Draize test which is to be replaced also measures inflammation.

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Additional tests need to be developed to determine this aspect of the effect. There are models which expose isolated bovine cornea to a compound and measuring the chemotactic factors released by exposure to neutrophils.

Invitro cytotoxicity test have been developed where a dye marker is used to determine the toxicity of compounds to invitro cell lines. One way it is used is to preload a cell line with neutral red marker and to expose the line to a compound. Measurement of the release of dye is related to death of cells in the line.

LAL test (Limulus amebocyte lysate) or horseshoe crab coagulation test as a replacement for pyrogen testing in rabbits. Has replaced rabbit pyrogen testing in rabbits for gram negative pyrogens but has the problem of not picking out gram positive pyrogens very successfully.

### *Validation.*

The biggest problem with the profusion of alternative tests is their validation. Unfortunately many of the test work under specific conditions and for specific compounds but fail under other conditions. One of the problems of irritancy tests is that if they cause their irritant effect in a way other than coagulation of protein then the effect is not demonstrable in these systems. For instance the ability of anionic surfactants to coagulate proteins does not correlate well with its irritancy as demonstrated by in-vivo tests.

### *Computers*

One major tool which has been used to replace the use of animals is the computer. Obviously it can be used for mathematical. Modelling of compounds and prediction of toxicity from databases depending on the structure of the compound. However it is probably in its information gathering capacity in the use of libraries and databases world wide in which it has replaced the use of animals in many laboratories. Simply discovering that somebody else has already done the work before and providing that information to the scientist may enable them to skip a step in the daily slog of assembling information to prove or disprove a specific theory.

### *Teaching*

This is an area where animals have been often replaced by videos and computer programmes in fact the granting of licences for this area of animals use is much more restricted then it was 20 years ago. There is a data base of teaching material called NORINA which lists all the teaching aid which are available it can be picked up on the internet at <http://netvet.wustl.edu/norina.htm#records>

Will animals ever be fully replaced by alternative techniques?

The two problems which have to be overcome are Specificity of the tests and their validation.

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I would suggest that it is likely in the short term alternatives will play a bigger and bigger role in the scientist's armoury but are not going to replace the use of animals completely. Within the regulatory area steady progress will be made. For instance it is likely that the EC will ban the use of animals testing in the cosmetic industry in the next few years both because irritancy alternatives are available and also that a body of information on the irritancy of most compounds used in this industry has been gathered and is available for reference.

### **Appendix.**

NORINA: (<http://oslovet.veths.no/>) Norwegian Inventory of Audiovisuals

The Laboratory Animal Unit, Norwegian College of Veterinary Medicine, Oslo, has compiled an English-language database of audiovisuals for use in the biological sciences. The compilers are Karina and Adrian Smith, in collaboration with Richard Fosse, Laboratory Animal Services, University of Bergen. The primary purpose of the database is to offer an overview of possible alternatives or supplements to the use of animals in student teaching, at all levels from schools to university. The database consists at present of around 2200 entries, including computer programs, interactive video, films and more traditional teaching aids such as slide series, 3-D models and classroom charts. There is also a section for Contact Persons who are developing and/or using audiovisuals at their institution, and for suppliers of audiovisuals. We invite users, developers and suppliers of audiovisuals to send in details for future upgrades of the database.

#### Database Record Detail

Each record in the NORINA database contains the following fields:

Record number (a unique number enabling searches by record number)

Type of Record - a classification of the audio-visual aids into one of the following categories (or combination of categories):

Audio Tape, Book, CD-ROM, Classroom Chart, Computer Program, Film, Film Loop, Filmstrip, Miscellaneous, Model, Overhead Transparencies, Reference Articles, Review Sheets & Transparencies, Slides, Super 8 Cassette, Video Disc, Video Film.

In addition approximately 100 Contact Persons (supplying or working on audio-visual aids) are cited in the database.

#### Sample Database Record

Record number: 1

Type of record: Computer Program

Type of record: Computer Program

Category: Pharmacology (animal)

Program name: Ileum

Computer type: Macintosh, IBM and BBC

Version: 2.0, 1990

Price: 149 Pounds Sterling (multi-user, department licence for unlimited copies)

Program source:

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Biosoft, P.O. Box 10938, Ferguson, MO 63135, USA; Biosoft, 49 Bateman Street, Cambridge, CB2 1LR, England

Telephone: USA: Int-1-314-524-8029; England: Int-44-223-68622

### Details:

This program simulates the effects/actions of various drugs on the isolated (in vitro) guinea pig ileum. Twelve common agonists and a variety of blockers can be 'administered' to the preparation, which will contract the muscle and the actions of many different drugs can be demonstrated on tissue, and simulated on the program. By randomly selecting any one of 20 unknown drugs it is possible to test the user's skill at identification by experiment. Random elements have been incorporated into the program to simulate inherent biological variability in the response to the same dose of agonist. A chart trace can be printed as a permanent record. Drugs available: atropine, hexamethonium, physostigmine, mepyramine, lignocaine, promethazine, acetylcholine, methacholine, carbachol, furmethide, vasopressin, angiotensin, barium, potassium, hexyltrimethylammonium, vasopressin, angiotensin, barium, potassium, hexyltrimethylammonium, tetramethylammonium, dimethylphenylpiperazinium, unknowns.

### Comments and references:

Suitable for graduate and professional audience. For Apple II, IBM or BBC (40 or 80 track). Manual included. Biosoft have their own catalogue of biomedical software. Abstracted in the booklet 'Animal-Related Computer Simulation Programs for Use in Education and Research', Animal Welfare Information Centre (AWIC), National Agricultural Library (see record 1373). Mentioned in the catalogue edited by Prof. W. Threlfall (see record 45), Memorial University of Newfoundland (1989), entitled 'Audiovisual Materials concerning the Care, Use, Behaviour and General Biology of Animals' (copyright Atlantic Provinces Council on the Sciences). See record 699. Key words: Ileum, experiment, in vitro, guinea pig, drug, simulation, muscle, blocker, agonist, antagonist. Mentioned in a catalogue from Universities Federation For Animal Welfare (UFAW) and British Univ Key words: Ileum, experiment, in vitro, guinea pig, drug, simulation, muscle, blocker, agonist, antagonist. Mentioned in a catalogue from Universities Federation For Animal Welfare (UFAW) and British Universities Film & Video Council (BUFVC), called 'Animals in Science Teaching, A Directory of Audio Visual Alternatives.' (1988).

### Program author:

Dr. Ian E. Hughes, Dept. of Pharmacology, Worsley Medical and Dental Building, University of Leeds, Leeds LS2 9JT, Yorkshire, England  
Updated: 17-01-1994

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### Sample List of Titles

Computer Programs  
Exercise Physiology  
Exercise Physiology,  
.Exercises in Muscle Contraction  
.Exercises in Muscle Contraction  
.Experiments in Biology Lab  
.Experiments in Human Physiology  
.Experiments in Metabolism  
.Female Reproductive Cycle/Fertilization and Early Pregnancy  
.FICKSYST (Fick Principle) , Flexicom

## Techniques in laboratory animals use

There are many and varied techniques which researchers have derived for working with animals. These can be best divided up into the following headings

1. Handling
2. Common Manipulations Removal of body fluids and administration of substances
3. Anaesthetics
4. Surgery

### Handling

Each species has its own particular characteristics. Good handling techniques will reduce the risk of injury from bites and scratches and will increase the confidence of both the handler and the animal. This reduces the stress on both parties. It is important to remember that all the animals used in laboratories are used to being handled and are unlikely to bite if handled correctly. If a person gets bitten or scratched it is most likely because they have done something to provoke it.

It is not possible to learn animal handling from a book. It can only be learnt by doing it while being supervised by an experienced handler. A few pointers is all that can be usefully given in print.

### Mice and Rats

It is normal to remove these species from the cage by grasping them by the base of the tail. They are then put on to a soft non slip surface. It is then possible to take a firm hold of the loose skin at the back of their neck. It is important not to hold the tip of the tail as it may come off if subjected to any stress. This is traumatic for the animals and may necessitate its destruction.

It used to be common to see workers lifting mice out of cage using a forceps. This is extremely bad practice.



**Gerbils and Hamsters** are handled by cupping them in your hands. It is bad practice to use the tails of these animals. Firstly because the hamster has no tail to speak of and secondly the skin of the gerbils

<u>MOUSE</u>	
WEIGHT	= 30 GRMS.
CIRCULATING BLOOD	= 7% OF 30G = 2ml
MAX. BLOOD SAMPLE	= 0.2ml
MAX. TERMINAL BLEED	= 1ml
USUAL SITE	CARDIAC /TAIL VEIN?
<u>RAT</u>	
WEIGHT	= 300 GRMS.
CIRCULATING BLOOD	= 7% OF 300G = 20ml
MAX. BLOOD SAMPLE	= 2.0ml
MAX. TERMINAL BLEED	= 10ml
USUAL SITE	CARDIAC/TAIL VEIN?
<u>RABBIT</u>	
WEIGHT	= 2.5 KG.
CIRCULATING BLOOD	= 7% OF 2.5KG = 175ml
MAX. BLOOD SAMPLE	= 17ml
MAX. TERMINAL BLEED	= 88ml
USUAL SITE	EAR VEIN/ARTERY & CARDIAC

tails is very libel o come away in your hand. This type of accident is called a gloving injury. It is important to wake up a hamster before staring to handle it. They may often be asleep and do not take kindly to being woken by probing fingers . Use a pencil to disturb the nest and then when it is awake cup it in your hand.

**Rabbits** need to be handled carefully for two reasons. Firstly they have extremely powerful hind legs and can give a severs scratch if they make contact with bare skin. So it s advisable to were a coat covering your arms . Secondly it s possible for rabbits to break their backs if handled incorrectly . This is normally fatal for the rabbit. It is not correct to hold rabbits by their ears *a la* magician. They should be taken from their cage supported under the pelvis and placed on a soft surface . They should then be held next to the chest of the handler. They can be carried around like this and if held securely they are comfortable and wont struggle.

**Guinea pigs** must also be handled carefully . They are unlikely to do you any damage but it is possible to cause internal bleeding if they are handled roughly. They are normally held by grasping around the thorax and supporting their rear end.

### Common Manipulations

#### *Blood Sampling*

Blood is the most common body fluid removed. Blood is an organ and its removal may have three potentially detrimental effects.

a. Handling and restraint of the animal is stressful. It is important that the correct procedure is used and that the animal and handler are comfortable with each other. It am be useful to use sedation or anaesthesia for some methods of blood sampling.

- b. Venepuncture cause pain and distress . Again sedation general or local anaesthesia may be indicated
- c. The removal of blood causes physiological responses. The amount and frequency of blood removal must be carefully calculated.

### Sites of Blood Removal

The site for the removal of blood will depend on the species. Cardiac puncture is a common method . It requires full general anaesthetic and requires considerable practice to do safely. Superficial veins are available in some animals such as the tail vein of the rat and mouse. The radial of the dog and cat and the ear artery and vein of the rabbit.

### Quality of samples

To achieve meaningful results and avoid needless repetition, any samples taken must be of good quality, and be preserved correctly. If the sampling technique is poor blood may clot or haemolyse and render the results invalid. Blood may be collected using various techniques. It is mist common to use syringes and needles. An alternative to conventional needles are butterfly needles or indwelling cannulae. These often help preserve the vessel if an animals moves.

<u>BLOOD SAMPLING ANIMALS</u>	
<u>BLOOD VOLUMES</u>	
APPROXIMATELY 7% OF BODY WEIGHT EXPRESSED IN MLS. IS CIRCULATING BLOOD.	
<u>SAMPLE SIZE</u>	
1.	TO ALLOW RECOVERY 10% OF CIRCULATING BLOOD CAN BE TAKEN.
2.	AT A TERMINAL BLEED 50% OF CIRCULATING BLOOD CAN BE RECOVERED.

Needles should be as large as possible allowing the blood to move fast and prevent it clotting.

### Injection of Materials

Substance may be administered by mouth, intravenously, intramuscularly, intraperitonealy , intradermaly or subcutaneously etc.

The rout will depend on the characteristics of the substance.

The comon sites used in the various animals are as follows;

### INJECTION SITES

**INTRAMUSCULAR**

<u>SPECIES</u>	<u>SITE</u>	<u>VOLUME</u>	<u>NEEDLE</u>
MOUSE	THIGH	0.05ml	23G
RAT	THIGH	0.3ml	21G
RABBIT	THIGH	0.5-1ml	20G
DOG	THIGH	2.5ml	20G

**SUBCUTANEOUS**

<u>SPECIES</u>	<u>SITE</u>	<u>VOLUME</u>	<u>NEEDLE</u>
MOUSE	NECK/SCRUFF	2-3ml	20G
RAT	NECK/SCRUFF	5-10ml	20G
RABBIT	NECK/SCRUFF	30-50ml	20G
DOG	NECK/SCRUFF	100-200ml	20G

**INTRAPERITONEAL**

<u>SPECIES</u>	<u>SITE</u>	<u>VOLUME</u>	<u>NEEDLE</u>
MOUSE	PERITONEAL	2-3ml	20G
RAT	CAVITY	5-10ml	20G
RABBIT	do	30-50ml	20G
DOG	do	100-200ml	20G

**INTRAVENOUS**

<u>SPECIES</u>	<u>SITE</u>	<u>VOLUME</u>	<u>NEEDLE</u>
MOUSE	TAIL VEIN	0.2ml	25G
RAT	TAIL VEIN	0.5	23G
	SUB LINGUAL VEIN		
RABBIT	EAR VEIN	1.0-5.0ml	21G
DOG	RADIAL VEIN	10.0-1.5ml	1G

**ORAL**

Ref. Handbook of laboratory animals management and welfare: Wolfensohn, LLoyd.

**SAFETY PROCEDURES IN AN ANIMAL UNIT**

Adapted From TCD'S Biorresources Unit

This statement is additive to a general Safety procedures. It only addresses animals related issues.

**USE OF HAZARDOUS CHEMICALS OR DRUGS IN ANIMALS**

If an animal is to be exposed to a toxic compound for an acute or chronic procedure, precautions must be taken as to the method of exposure and disposal of the excreta and carcass.

If Carcinogenic or Mutagenic substances are to be used, special precautions must be taken in this respect and an appropriate protocol must be agreed with the Safety Committee on Carcinogens. When using these substances, account must be taken of all situations in which humans may come in contact with the substance from the delivery through to the processing of tissue and final destruction of the carcass. It must be considered that it may not be possible to work with some types of compounds under present facilities.

Narcotics and anaesthetics etc are kept in a locked cupboard and if you want them, please request access to them from a Technician who should ensure that you have need for them and are familiar with their use. DO NOT help yourself; these drugs can be very dangerous to humans.

**Halothane**

The usual volatile anaesthetic used in Halothane. The use of this substance requires special precautions. Halothane is potentially teratogenic and may affect the foetus in the first trimester of pregnancy. It should be used in a well ventilated room and the scavaging equipment provided should be used. If you are pregnant DO NOT enter any room where this drug is used without assuring yourself that it is safe.

**Freunds Adjuvant**

This substance is often used in procedures designed to raise antibodies. Special care should be taken not to inject oneself or somebody else accidentally. It is necessary to wear protective glasses or goggles while mixing as a splash in the eye can cause a severe reaction. If an eye splash occurs wash the area with water or eye wash solution and seek medical advice.

**COMPRESSED GASES**

The only compressed gases carried in the unit are CO<sub>2</sub>, O<sub>2</sub> and NO<sub>2</sub> beside the escape stair well. All these cylinders are on rental from B.O.C Gases and are regularly inspected when being refilled.

**ACCIDENTS**

Any bite, cut or scratch received in the Animal House, however trivial, must receive immediate attention and be recorded in the accident book.

Syringes and needles may inflict injuries, they are kept in a locked drawer and access to these is by a

## Laboratory Animal Science

key obtainable from the Technicians. Syringes and needles should be handled with care. It is easy to inject yourself as well as somebody else. DO NOT walk around with an un-capped needle. All loaded syringes should be carried in trays to prevent accidental injection in the event of a fall. They should be disposed of as 'sharps'.

### *INFECTIONS FROM ANIMALS*

Animals harbour infectious agents such as bacteria, fungi and viruses and these may be harmful to man. Certain regions of the body provide a direct route by which infectious agents may enter, such as respiratory, alimentary, urinary tract and eyes. Broken skin provides a means of direct entry for micro-organisms.

It is wise for persons who are handling animals to have an up-to-date tetanus vaccination; these can be got at the Student Health Centre.

### *SPECIAL PROCEDURES IN BIO HAZARD AREAS*

Any animals which are to be injected with any living organism must be housed in a specific facility for this type of work. These animals or their products should NEVER be brought into the main holding unit.

Work with micro-organisms requires a hazard assessment protocol. This must be signed by the Bio Hazard Officer. Organisms currently listed as category A, pathogens by the Dangerous Pathogens Advisory Group (DPAG), (1) can only be used with the expressed permission of the Bio Hazard Officer for such work.

### *PARASITES*

Parasites infecting an animal may pass to man or transmit disease to man. Normal hygienic procedures such as hand washing etc should be practised.

### *ALLERGIC CONDITIONS IN MAN*

Allergic conditions in man may result from contact with animal fur or hair and sometimes from feathers, exudates, bedding and fodder. The allergy may manifest itself immediately or may be acquired over a succession of exposures to the allergen. Any person with a history of an allergic condition (asthma etc) should advise their supervisor or the Director of the Unit as special provisions (masks etc) may need to be provided.

### *ANIMAL ACCOMMODATION*

#### Access

Only authorised personnel are permitted in animal accommodation. It is important that animal accommodation is secured against the entry of unauthorised persons.

Arrangements can be made for late working (after 5pm or week-ends). A key permit must be obtained from the unit and presented to the security personnel at the security Gate with a college

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identity card. The late working book must be filled in. If an emergency arises security.

### Animal Welfare

If there are problems of animal welfare, ring security, and ask them to page the person on duty. Wait by the phone for a return call.

### Clothing

The appropriate protective clothing must be worn in animal accommodation and should not be worn outside the limits of that accommodation.

### Warning Notices

Where animals under experiment may be an infected, or present a radioactive or toxic hazard they must be kept in the containment area. Written instructions must be prepared for staff or other workers that might encounter the risk and approved warning notices prominently displayed at the entrances to the contaminated area. The notice must specify the precautions to be taken before entering the area.

### Hygiene

There must be no eating, drinking or smoking in animal accommodation. Protective clothing must be removed before going for refreshment breaks and the hands well washed.

### *HANDLING ANIMALS*

#### Small Animals

No persons may handle an animal unless they have assured the Director of the Unit that they are capable of doing so in the proper way. People who are inexperienced must ask the help of an experienced handler and they should receive full instruction in animals handling from a member of the staff at the earliest opportunity. If an animal gets out of its cage and is caught and returned, the escape must be reported to a member of staff.

#### Large Animals

Because of their size, farm animals can be dangerous. It is essential that a person experienced enough and strong enough to deal with the animal always accompanies the inexperienced or less strong person. An individual, however experienced, must ensure that one other person is aware of his/her involvement with large animals. It is advisable that help is always within call and where necessary be present.

### *INOCULATION OF ANIMALS*

Special cabinets, face shields and other protection may be considered necessary when inoculation hazardous materials into animals. 'Luer-lok' hypodermic needles are recommended because the needle cannot fly off when inoculation. Great care must be taken to ensure that the animal holder is never at risk during inoculations; the hands must be positioned so that if the syringe slips the needle cannot accidentally pierce their skin. It is essential that help be available when an accidental inoculation of a substance may be hazardous to

## Laboratory Animal Science

humans. If an antidote is required ensure that provision for its availability has been made. The needles must be discarded into yellow Sharp boxes for subsequent incineration.

All the infected materials from inoculation sessions should be autoclaved before disposal.

Freunds Adjuvant: this substance is often used in procedures designed to raise antibodies. Special care should be taken not to inject oneself or somebody else accidentally. It is necessary to wear protective glasses or goggles while mixing as a splash in the eye can cause a severe reaction. If an eye splash happens wash the area with water or eye wash solution and seek medical advice.

### *SAMPLING FROM ANIMALS*

Material taken from animals for laboratory examination must be transported in approved leak-proof containers. Post mortem examinations of animals must be done in a room that can be decontaminated. Carcasses and tissues for incineration must be put into "sulu bins" then the freezer.

As with handling of animals only persons who are competent in the handling and taking of samples from animals should do so. In 'once off' situations members of the units staff will normally take the samples otherwise researchers are required to have training in the technique of taking the sample they want.

### *PREVENTATIVE INOCULATIONS AND SCREENING OF WORKERS.*

#### Tetanus

All persons who handle animals or work on farms or in the field are strongly advised to be vaccinated against tetanus.

#### Tuberculosis

All staff or researchers who handle animals which may have tuberculosis must be Mantoux test positive and should be prepared to accept annual chest X-rays tests. Persons who are mantoux negative will be offered BCG vaccination against tuberculosis until they are Mantoux positive (ie protected).

### *THE POST MORTEM ROOM*

#### The Hazard Of Contact Infection

The hazard of contact infection in a post-mortem room is obvious, if splashes of blood and faeces are clearly visible. The hazard of aerosol infection is not as obvious but the post-mortem room is probably the area where most aerosols are created. Apart from infective hazards, accidents can also be caused by cutting instruments, slippery floors, fixatives, disinfectants and electricity.

#### Good Practice

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Workers must abide by any local rules which govern the operation of a post-mortem room. They should not put colleagues at risk by practising faulty techniques, being careless with tools and equipment or deviating from the accepted system of entry. The latter could result in the spread of contamination to clean areas of the laboratory. It is essential that gloves be used when performing a post-mortem. Great care must be taken not to contaminate surfaces such as door handles etc with dirty gloves. Gloves should be disposed of before opening doors and presses etc.

### *DISPOSAL OF ANIMAL CARCASSES*

Carcasses must be placed in the specially marked bins provided which is collected at the end of each day. Great care should be taken to ensure that scalpel blades, hypodermic needles and other sharp instruments are not placed in the plastic bags with the animal carcass. These must be disposed of in the special containers that are provided in the post-mortem and minor procedures area. All animal carcasses must be disposed of by commercial rendering or incinerated. It is normally necessary for them to be stored in a deep freezer prior to disposal.

### *DISPOSAL OF CONTAMINATED WASTE*

All waste material must be disposed of in appropriate boxes. There are appropriate boxes for all material:

1. Yellow 'sharp boxes' for needle and syringes or blades etc
2. Specially marked bins for non-contaminated glass
3. Specially marked bins and storage in the freezer for all normal biological material (radioactive and biohazard material needs special arrangements).

#### Toxic Chemical Hazards

Solid material presenting a chemical hazard must be disposed of in an appropriate manner (consult the chemical safety officer and relevant literature etc). The college employ a contract firm to dispose of this toxic waste. No chemicals may be disposed of in the normal refuse system without prior consultation with the Chemical Safety officer.

Any package containing a toxic chemical must be labelled appropriately and stored in a safe place prior to disposal.

### **IT IS POLICY THAT THE PERSON WHO GENERATES THE TOXIC WASTE IS RESPONSIBLE FOR ITS DISPOSAL.**

The unit will not be responsible for the storage or disposal of this type of waste.

#### Radio Nucliotides

No radioactive compounds may be brought into or used in the Unit without the approval of the 'Radiological Safety officer.' The safe use and

## Laboratory Animal Science

disposal of all materials which may be contaminated throughout the course of the experiment will be discussed and determined at the development of the protocol. The same principle of 'Producer Responsibility' as applies to toxic waste applies to this type of waste.

### Biological Hazards

Solid material containing pathogenic micro-organisms must be sterilised by an appropriate method before being placed in the waste sack. Proteinaceous material (i.e. dried micro-organisms and other tissues) should be placed in a separate package and then sealed prior to being put into the waste sack. This to prevent dusting and potential allergic sensitisation of those workers engaged in the handling and subsequent sorting of the wastes.

### 'Sharps' Hazards

Syringe, needles, scalpel blades, broken glassware, initially intact glassware and other sharp debris (ie Pasteur pipettes) must never be placed directly into a plastic sack. They should be put in an approved sharps box.

Syringes and needles exposed to radio nuclides should not be put in the normal sharp box, a special one should be designated for radio nuclides and it should be marked accordingly. separate arrangements should be made for the disposal of these type of sharps.

Initially intact glassware must also be separately packaged in a special grey box before being consigned to the refuse disposal system. This instruction applies to glass test tubes just as much as it applies to larger items such as bottles, beakers, flasks etc.

### *FIRE ALERT: AN EVACUATION PROCEDURE IN AN ANIMAL UNIT*

1. If the fire alarm rings you must evacuate the building at once; close doors behind you and DO NOT use the lift.
2. If you are handling an animal at the time, return the animal to its cage and secure cage.
3. Ever reasonable effort must be made to return an animal to its cage, however, in an extreme situation where it is not possible to do this and there is any possibility that the animal may awaken while unattended, it must be killed.

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Ensure death and leave at once. Inform the Unit safety officer of the situation.

4. If you are alone in the unit at the time of the alarm you must return the animal to its cage or Kill it as necessary and evacuate the building at once.

### **BIBLIOGRAPHY**

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