Experimental Animals

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The use of animals as experimental subjects has contributed to many important advances in scientific and medical knowledge.

Although scientists have also developed non-animal models for research, teaching, and testing, these models often cannot completely mimic the complex human or animal body.
All animals except man

Certain immature forms:

(i) mammals, birds and reptiles: throughout gestation or incubation periods.

(ii) fish, amphibians and octopus vulgaris: from the time at which they become capable of independent feeding.

An animal is regarded as “living” until the cessation of circulation or destruction of its brain.
Ethical principles for regulation of experiments
An experiment should never be performed if the necessary information could be obtained by observations.

No experiment should be performed without a clearly defined and obtainable objective.

Scientists should be well-informed about the previous work in order to avoid unnecessary repetition of an experiment.

Justifiable experiments should be carried out with the least possible suffering (often through the use of lower, less sentient animals);
REGULATION AND PRINCIPLES
Use of appropriate species, quality, and number of animals.

Avoidance or minimization of discomfort, distress, and pain in concert with sound science.

Use of appropriate sedation, analgesia, or anesthesia.

Establishment of experimental end points.

Provision of appropriate animal husbandry directed and performed by qualified persons.

Conduct of experimentation on living animals only by or under the close supervision of qualified and experienced persons.
Commonly used laboratory animals

Species: Rattus norvegicus

Strains:
- Sprague Dawley rat
- Wister rat
- Long-Evans rat
- Zucker rat
- Hairless rat (Rowett nude)
Commonly used laboratory animals

The Laboratory Mouse
Commonly used laboratory animals

The Guinea Pig
The Rabbit
The Hamster
The Gerbil
Animal Environment, Housing, and Management
A good management program provides the environment, housing, and care that permit animals to grow, mature, reproduce, and maintain good health; provides for their well-being; and minimizes variations that can affect research results.
Housing

- Allow normal physiologic and behavioral needs.
- Allow social interaction.
- Allow the animals access to food and water and permit easy filling, refilling, changing, servicing, and cleaning of food and water utensils.
- Secure environment, free of sharp edges or projections.
- Allow observation with minimal disturbance.
Animal Accommodation

Cages must be made of harmless material, easily cleaned and sterilized. There must be adequate room to allow a normal range of movement. It allows 2 cubic feet per rat. Add hammocks and other toys.

Large Ventilated Cages in NG (17.5 x 12 inch, 210 sq inch)

<table>
<thead>
<tr>
<th>Body Weight</th>
<th>Maximum Number Rats per Cage</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-200 grams post weaning</td>
<td>9</td>
</tr>
<tr>
<td>200-300 grams</td>
<td>7</td>
</tr>
<tr>
<td>300-400 grams</td>
<td>5</td>
</tr>
<tr>
<td>&gt;400 grams</td>
<td>3</td>
</tr>
</tbody>
</table>
Mouse needs housing space. Mice love to climb and do NOT need open (bare) floor space.

- Large Ventilated MicroIsolator (10x18 inch, 180 sq. inch)

<table>
<thead>
<tr>
<th>Body Weight:</th>
<th>Maximum Number Mice per Cage:</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>30</td>
</tr>
<tr>
<td>10-15</td>
<td>22</td>
</tr>
<tr>
<td>15-25</td>
<td>15</td>
</tr>
<tr>
<td>&gt;25</td>
<td>12</td>
</tr>
</tbody>
</table>

Breeding:
Rats are bred in male: female ratios of 1:1 or 1:2. The male, female and litter may remain together until the pups are weaned.
Date of birth for the litter must be recorded on the cage card by the investigator.
Feeding

Animals should be given free access to food and water.

Amount: 15 to 20 g/rat/day

### FOOD COMPOSITION

<table>
<thead>
<tr>
<th>Nutritional component</th>
<th>Metabolisable energy content (kJ/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACRO nutrients (energy producing substances):</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>37</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>17</td>
</tr>
<tr>
<td>Protein</td>
<td>17</td>
</tr>
<tr>
<td>MICRO nutrients:</td>
<td></td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
</tr>
<tr>
<td>Trace minerals</td>
<td></td>
</tr>
</tbody>
</table>

**AD LIBITUM FOOD INTAKE**
Temperature
Carefully controlled, maintain the temperature within a ±2°C range, and continuously monitored.

Relative Humidity
Should be maintained at 55% ± 10%.

Ventilation
Provide sufficient air of an appropriate quality
Lighting

Most laboratory mammals are nocturnal. For the majority of laboratory animals a daily cycle of 12:12 hours is suitable.

Noise

Sound level should be kept below about 50dB.
Rats life cycle

- Rats reach sexual maturity at 5 weeks.
- Females of breeding age come into heat every 4 to 5 days.
- Gestation period is normally 22ds (17 to 22ds).
- Female approaches menopause at about 18 months of age.
- Rats have average is 10 to 12 litters.
- Babies can be weaned at 4-5 weeks.
Determining the sex of rats

Male rats can get rather stinky and dirty while out exploring.

Females rats have 12 nipples on their chest.

Male rats have very larger testicles.
ANIMAL HANDLING AND RESTRAINT

Safe for both the handler and the animal.
Rats

- The rat should be picked up by grasping it around the shoulders, and then lifted clear of its cage.
- The handler's forefinger should be positioned under the mandible to prevent the animal from biting.
- If the rat struggles, it can be calmed by applying minimal restraint and allowing the animal to rest on the handler's sleeve.
- If the rat appears aggressive, it can be lifted by the base of the tail.
Mice

- Grasped by the tail, preferably the proximal third, and lifted clear of its cage.
Guinea Pigs

- Approach the animal rapidly and smoothly, and grasp it around the shoulders with one hand.
- The guinea pig can then be lifted clear of its cage.
MINOR (NON-SURGICAL) PROCEDURES
Intraperitoneal injection

The assistant should grasp one hindlimb and direct the needle along the line of the limb into the posterior quadrant of the abdomen.
Intramuscular injection

An assistant should immobilize one hind leg by grasping the quadriceps between his forefinger and thumb, and the injection made into the middle of the muscle mass.
Subcutaneous injection

The rat should be restrained by grasping by the skin of the scruff, and the injection made into the area of skin "tented" by this procedure.
Vaginal smear

Insert a moistened cotton bud swab into vagina, gently removing the cells from the vaginal lumen and walls and transferring the cells to a glass slide.

The tip of the swab stick is inserted carefully into the rat’s vagina to a depth of approximately 1.0 cm, with a rotating action at an angle of about 45° to the animal’s body.
HUMANE KILLING

Kill animals must be to bring death in a rapid and painless manner.
Chemical Methods

- Intravenous or intraperitoneal injection of pentobarbital (100-150mg/kg).
- Exposure of animals to raising concentrations of CO2.
- Inhalation of diethylether.
Physical Methods

- Decapitation and dislocation of the cervical vertebrae (mice, rats, hamsters, gerbils and small birds).
- Larger animals such as pigs can be rendered unconscious by one of the recognised methods of slaughter.
- Animals should not be killed in the presence or sight of other animals.
Anesthesia
Chemical Methods

• Intravenous or intraperitoneal injection of pentobarbitone (35 mg/kg).

• 10% chloral hydrate (300 mg/kg, intraperitoneal).

• Inhalation of diethylether.
Collection of blood sample
1- Retro-orbital:
A microhematocrit blood tube into the corner of the eye socket, directing the tip at a 45-degree angle toward the middle of the eye socket. Rotate the pipette between your fingers during forward Passage.

2- Cardiac Puncture

3- Tail vein
RAT DISSECTION
• Using scissors, make the incisions in the rat.

Liver
Small Intestine
Stomach
Spleen
Large Intestine
Caecum
Fixation should be carried out as soon as possible after removal of the tissues.
Intra-cardiac perfusion

Injection of fixative in left ventricle.
Open right atrium.

Intracardiac perfusion with 500 ml of an ice cold, freshly prepared solution of 4% formaldehyde in phosphate-buffered saline.
Fixation is a complex series of chemical events that differ for the different groups of substance found in tissues.

The aim of fixation:

1- To prevent autolysis and bacterial attack.
2- To fix the tissues volume and shape during processing.
3- To prepare tissue and leave it in a condition which allow clear staining of sections.
4- To leave tissue as close as their living state.

Fixation is coming by reaction between the fixative and protein which form a gel, so keeping every thing as their in vivo relation to each other.
Factors affect fixation

- PH.
- Temperature.
- Penetration of fixative.
- Volume of tissue.

According to previous factors we can determine the concentration of fixative and fixation time.
Types of fixative:

There are five major groups of fixatives:

- Aldehydes
- Mercurials
- Alcohols
- Oxidizing agents
- Picrates
Aldehydes include formaldehyde (formalin) and glutaraldehyde.

Formalin penetrates tissue well, but is relatively slow.

The standard solution is 10% neutral buffered formalin. A buffer prevents acidity that would promote autolysis and cause precipitation of formol-heme pigment in the tissues.

**Volume of solution:** tissue 20:1

**Duration:** 24-48 1hr per 1mm
10% neutral buffered formalin

Total volume 1 litre

40% formadehyde 100 millilitre (ml)
Sodium phosphate, monobasic, monohydrate 4 g
Sodium phosphate, dibasic, anhydrous 6.5 g
ddH2O to 1 litre (l)
Picrates
Fixatives with picric acid. Foremost among these is Bouin's solution.

**Bouin's solution**

Picric acid, saturated aqueous solution, 750 ml
37-40% formalin, 250 ml
Glacial acetic acid, 50 ml
Mercurials

Contain mercuric chloride and include such well-known fixatives as mercuric chloride formalin and Zenker's.

Their best application is for fixation of hematopoietic and reticuloendothelial tissues.
Alcohols, including methyl alcohol and ethyl alcohol, they cause too much brittleness and hardness. However, they are very good for cytologic smears because they act quickly and give good nuclear detail.

Oxidizing agents include permanganate fixatives (potassium permanganate), dichromate fixatives (potassium dichromate), and osmium tetroxide. They are used very infrequently.
Recognition of Pain and Distress

- Isolation from other members in a group.
- Hunched posture.
- Not eating and/or drinking. Diarrhoea/absence of faeces.
- Reduced grooming, coat staining (in ano-genital region).
- Discharge from eyes, nose or other orifices.
- Reluctance to move, favoring one or more limbs, staggering, circling.
- Unexpected aggression or vocalising in response to handling.
- Excessive weight loss.