# SECTION-A

## GENERAL DISCUSSION ABOUT LABORATORY MICE AND RATS

This part of the book envisages fundamental knowledge on handling, housing, breeding, nutrition etc. of laboratory mice and rats. Handling involves two most important responsibilities on the part of experimenters. These responsibilities are primarily a most scrupulous care to the effect that the animals suffer no pain and secondly a sincere regard for the health and well-being of the animal colony. As for the well-being of the colony, the experimenters should assume much of the responsibility. This is not pure caretaker work. They should take pride in visual appearance of the animal colony, in having sleek, well-nourished animals in clean sanitary lodgings. Familarity with their habits, demeanours and care for their well-being will enrich dividends, in a minor way in a reduced incidence of animal bites and in a major way in an increased incidence of successful results in pharmacology research.

This section also includes some important biological and physiological data of laboratory mice and rats in tabular form.

# The Laboratory Mouse▲

CLASS : Mammalia ORDER : Rodentia

FAMILY: Murinae

SCIENTIFIC NAME: *Mus musculus*

Amongst all animals used in the laboratory experiments the mouse is considered as the most widely used one which is, available for use all over the world. To sort out number of research problems, mouse stands as the animal of choice, because of its tiny size, early sexual maturity (puberty), high fecundity (fertility), short gestation period (length of time from conception to birth) as well as relatively higher position in the evolutionary scale. In the field of laboratory tests, about millions of mice are used all over the world in a year. In the field of cancer research, reproductive biology, diagnosis, study of drug, screening, immunology, toxicology and genetics, numerous variety of inbred strains of mice are available for being profusely used.

The Swiss albino mouse has been selected here as the representative of the species for resume of discussions herein, this animal being most popular amongst the numerous inbred strains available.

## Exterior Features of Mouse

The body of the mouse is slender with smooth hair-coat, long pointed snout, prominent round ears and long flat front sharp teeth. The average weight of an adult mouse is about 30 gms. and the length is about 7.5 cm. from snout to the base of tail. The tail is hairless. The female mouse has five pairs of nipples, three pairs on the thoracic region and two pairs on the abdominal region.

## Anatomical Peculiarities of Mouse

Like other rodents, in mice a single pair of upper incisors are present. The incisors are chisel-shaped in wear owing to the absence of enamel on their posterior surfaces and having persistently open roots. They grow throughout life and are always of great size. Canines are never present, and there is wide diastema between the incisors and cheek-teeth into

which the cheeks can be tucked so as to separate the front part of the mouth from the hind during the process of gnawing. An adaptation of the jaws enables the animal for gnawing. The grinding-teeth vary in number and frequently have persistently open roofs. To provide the necessary power for gnawing. The jaw-muscles are very greatly enlarged. The brain of mouse is little, convoluted and the cerebellum is not covered by the cerebral lobes. The sternum of the mice has a long and narrow body. The skull is elongated narrow in front, broader and depressed behind. The nasal cavities are very large, with complex air sinuses in their upper part. The orbit and temporal fossa are continuous. A remarkable feature of the skull is the presence of many large opening corresponding to the infraorbital foramen. The bones of the arm and forearm show considerable variation. In the alimentary canal there is large caecum. The placenta is duciduate. The mice do not vomit because they lack vomiting centre [absence of CTZ].

## Housing of Mouse

Normally in animal house, mice are housed in the cages of shoebox type, made of plastic, galvanised iron, anodized, aluminium or stainless steel and the floor of such cages may be either screen bottomed or solid. The screen bottom cages are always suspended and the solid bottom cages may either be suspended or standing on a rack. In case of screen bottom (wire mesh) cages, the diameter of the wire and the mesh size should be appropriate to afford maximum comfort to the living animal housed on it. A floor mesh (grid) size of 3 × 3 openings per inch being made of 18 guage (0.0475 inch) wire is ideal for housing mice. A tray is placed at the bottom of the cages when screen bottom cages are in use, for the purpose of collecting urine and faeces droppings, Usually, some materials are provided as bedding in solid bottom cages as such bedding would absorb urine and moisture from faeces. It also aids comfort to the animal. For such bedding, rice husk are used widely. The size of the cage of animals, should commensurate with the age and number of animals to be housed in the cage and the minimum floor space should be 97 sq. cm. per adult mouse. The minimum height of the cage should be 12.7 cm, (5.0 inch). Housing in a cage in groups for weanings or adult females is possible; but housing of adult males in groups will create bitter fighting pell-melI in the cage. Maximum housing number of mice in a cage must not exceed 20. A cage with dimensions of 25 × 20 × 15 $cm^3$ will comfortably bear either a pair (a male and a female) or one male with two females with litters (the young brought forth at a birth) or six adult mice.

## Requirement of External Environment for Mouse

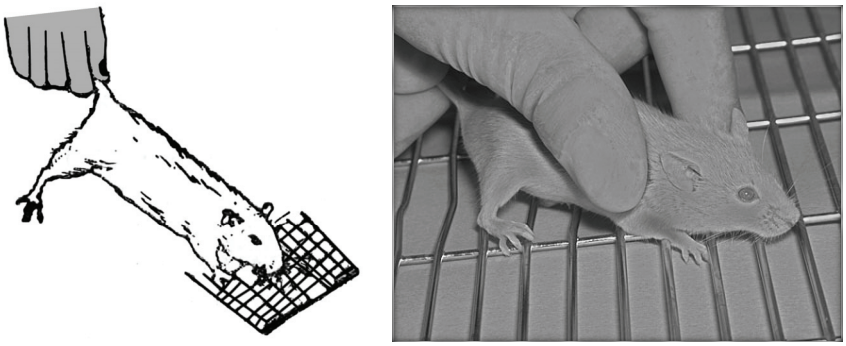
The temperature of the mouse-housing room may be maintained any where in the range of 20-25°C. An optimal temperature for the mouse is 22 ±1°C. The relative humidity (RH) should be held between 45% and 55%. Twelve to fifteen changes of air per hour in the animal room, are recommended. Lighting and darkness be divided in equal number of hours viz 12 hours of lighting and 12 hours of darkness, which should be provided in the room for optimal growth and reproduction. Intensity of light inside the animal room should be between 350- 400 lux one meter above the level of floor. Mice are very sensitive to noise particularly in the midst of higher- frequencies of human auditory range and beyond it ; hence noisy atmosphere has to be eliminated to the maximum for healthy living of mice.

## Nutritional Requirement of Mouse

Laboratory mice are fed either with commercially available pellet diet or diet formulated in the animal facilities. By use of a variety of cereals, pulses, seeds etc. the diet can be formulated for use. In order to ensure proper growth and reproduction, mice should be fed on stock diet containing 16 – 25% protein, 4 – 6% fat, and 45 – 55% carbohydrate. In addition, normal diet should contain all the major vitamins in definite proportions and essential minerals in specific quantities. The diet should be tasty to the animal and one which can be easily formulated and stored conveniently. An adult mouse normally consumes 4 to 5 gms. of diet per day. The consumption of daily diet of animal varies depending on the physiological status of the animal as well as environmental temperature. Food consumption, increases considerably in the pregnancy or lactating stage of animal. Clear water should be provided to the animal for drinking *ad libitum.* An adult mouse drinks about 6 ml. of water per day.

## Handling of Mouse

Mice are by nature very timid, social, nocturnal and escape prone rodents. Mouse can be picked up by holding at the base of tail. The animal however, should not be caught by the tip of the tail, since it would cause severe pains to the animal. For the purpose of inspection on manipulations, the mouse is lifted by tail, placed on a rough "toe-gripping" surface, such as the top of a case, grasped on the scruff of the neck by thumb and forefinger. The animal is then inverted and the tail is held between the little finger and palm.



(a) (b)

**Fig. 1** Holding of Mouse, a, b.

(c)

**Fig. 1** Holding of Mouse, c.

## Breeding of Mouse

The mouse is a prolific (fertile) breeder. Puberty occurs at the age of four to six weeks. However, mating is done when the animals are around 45-60 days old. Mice bred too early (less than six weeks of age) or too late (after ten weeks of age) causes reduction of fertility. 60 days old male mouse weighs around 35 gms. and the female around 30 gms.

| Age at sexual maturity (days) | Breeding season | Estrus | | Gestation Period (days) | Young No. / Litter |
|---|---|---|---|---|---|
| | | Type | Cycle (days) | | |
| 30–45 | all year | P | 4 | 19–21 | 6-12 |

## Inbred Strains of Mouse

A strain is considered as inbred when it has been mated full brothers and sisters (bxs) for 20 or more consecutive generations. Even then it is not completely inbred in the true sense because it gives an inbreeding co-efficient of only 98.6% which means that on the average 1.4% of the originally heterogygous genes are still heterogygous. The total list of inbred starins and clearly defined substrain now exceeds 300. Wide use of inbred strains has been found in almost every field of biomedical research. The chief drawbacks of inbred strain are that the animal become generally less healthy, more susceptible to disease and reproductive capacity is reduced (inbreeding depression).

## Estrus Cycle of Mouse

The mouse is habituated to a recurring estrus cycle of four days' duration. At the interval of 4 days, the females being housed in close association with males, cycle regularly; and this is caused by a pheromone released by the males. Single female housed away from the males do not cycle regularly, or the interval between cycles tends to be longer in them. In case of large groups of females being housed together, they tend to go into anestrus and do not cycle, as because the females become pseudo, regnant and this is known as the "Lee-Bool-effect". Such group of females when accommodated with a male, begin to cycle, and come into estrus or heat, after about 72 hours exposure to the male. This phase is known as the "Whitten effect".

According to the cell types found in the vaginal smear, it is estimated that estrus cycle of the mouse is divided into five stages, viz pro-estrus, estrus (early), estrus (late), metestrus and diestrus. Being influenced by 'heat' or estrus, the female is most receptive to a male and mating will take place if a male is present with her in the same cage. But estrus cycle does not take place when the mouse is pregnant or lactating. However, immediately after delivery, the female leans to estrus and this stage of estrus is known as the post-partum estrus. Irregularity of estrus cycle occurs in the case of pseudopregnancy.

## Mating Technique of Mouse

The monogamous and polygamous methods are resorted to usually. In the former system a male and a female are paired together and the youngs are segregated from the mother prior to the next parturition (act of bringing forth). By dint of this system the advantage of post-partum estrus is

utilised to produce maximum number of litters within shortest time. For the purpose of evaluation of the individual female production, accurate breeding records have to be maintained. This system, however, creates disadvantages also. A good number of males have to be maintained in cages, which would entail increase of labour, enlargement of space and larger provision of equipments.

One male mouse is caged with 2 to 6 females while applying polygamous or harem method. Females with pregnancy are housed in a separate cage being removed from the group. After the weaning of young, the female is arranged to associate with a male for subsequent mating. By this system more youngs are weaned per litter and they are healthier than those raised by post partum matings, The total number of litters produced per breeder is fat less. Successful mating in mice can be determined by the detection of sperms in the vaginal smear or by the presence of a copulatory plug (solidified semen) in the vagina. The mouse introduced for mating should be examined everyday morning for the presence of copulatory plug. Pregnancy can be detected 'by palpating the animal around 8-10 days of gestation. Daily weighing will also reveal an increased rate of weight gain by about 13 days of gestation.

On an average, length of gestation extends to 19-21 days, but this may, however, prolong to 24 to 30 days in case of post-partum matings, according to the size of the suckling litter.

## Timed Pregnancies of Mouse

Numerous ways of timed pregnancies in mice are noticed. Taking advantages of the rebound cycles that occur in the whitten effect is one way. And the other is to perform vaginal smears and mate only those mice in heat, which is however, time consuming. For the presence of vaginal plugs, mice has to be examined daily,

The size of litter differs considerably according to strain and age. The mouse gives birth to 6-12 pups per litter, the first litter being generally smaller. Till the age of about one year mice breed well and during this period 8 to 10 litters emerge per breeding. The productivity diminishes after the first 6 litters. The litters are likely to be comparatively less frequent and smaller in size and pre-weaning mortality is greater.

A new born (1 day old) pup weighs about one gm. Just at the point of birth the young are hairless and their eyes and ears remain completely closed. Hair on the body begins to grow after about 3 days of birth and

within about 10 days hair coat fully covered; the eyes and ears open on attaining the age of 12-13 days. Pups attaining the age of 14-16 days start to eat solid food and they are weaned (separated from the mother) when they are 21 days old. At this age, weight around 10-14 gms. is noticed in each young.

## Sexing of Mouse

One day-old mice even can be sexed by measuring the anogenital distance. This distance in male is greater than the same in female.

## Life Span of Mouse

The longivity of laboratory mouse ranges from $1\frac{1}{2}$ years to $2\frac{1}{2}$ years.

## Common Diseases of Mouse

Any element of extra stress on the animals metabolism may create to predispose them to disease. Poor or low nutrition, over-crossing, lack of hygiene, insufficient ventilation, rough handling and exposure (directly or by indirect contact) to mice suffering from infectious disease will all lead to an out break of disease in a colony.

A healthy mouse will be active with smooth and glossy coat. An ill mouse will be inactive and will have a rough haircoat. It will have no eagerness for food and will lose weight. Symptoms like conjunctivities, diarrhoea, accelerated respiration, wheezing or chattering, swelling on the tail, enlargement of feet, etc. are indicative of existence of some disease in mice.

Mice are sensitive to both streptomycin and chloroform. Mice often die if streptomycin is injected even in low concentrations. Many inbred strains of mice, particularly the males, are sensitive to chloroform vapour and may die following accidental exposure,

## Collection of Blood from Mouse

Blood samples can be obtained from mice by cardiac puncture or tail vein puncture by needle (25 to 27 gauge and $\frac{1}{2}$" to 1" long fitted with 1 or 2 ml syringe).

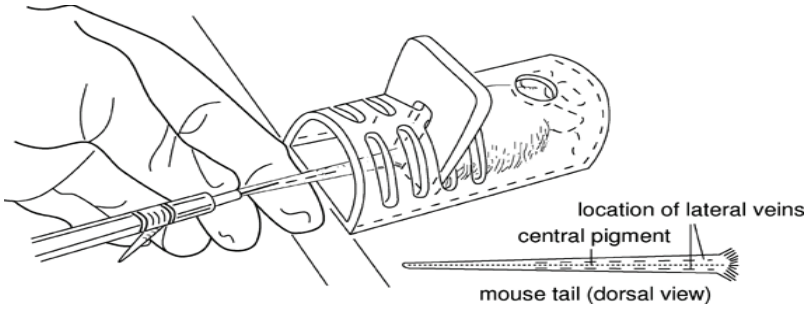



**Fig. 2** Intravenous injection (Mice).

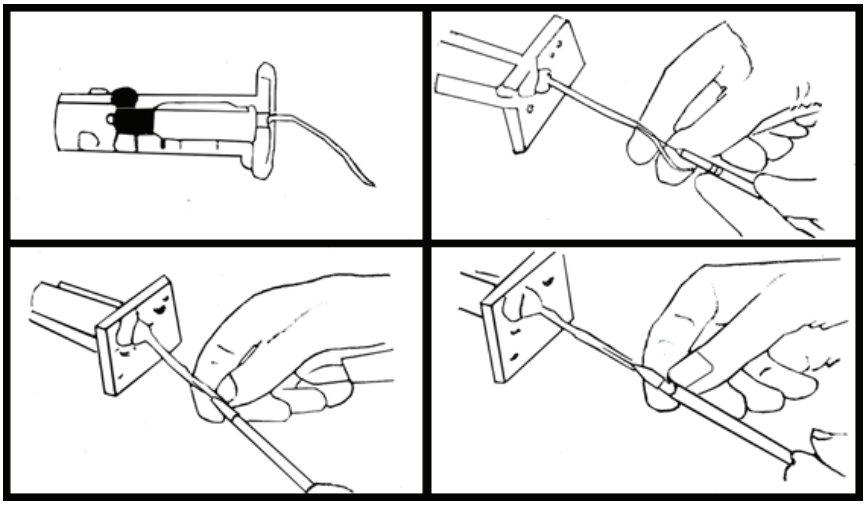


**Fig. 3** Blood sample collection from mouse tail vein.

The most efficient method of collecting blood causing least strains to the animals is from orbital sinus with the help of a capillary tube. The animal is restrained in such a way that the loose skin of the head is tightened while holding the head with the left hand with the help of index finger when the eye is made to protrude by traction on the skin adjacent to the eye. The thumb is pressed just behind the angle of the jaw resulting in the engorgement of the retro orbital plexus. The tip of a blood pipette is then inserted at the medial canthus into retro orbital plexus with gentle rotation by the end of the other hand. As the vessels are ruptured, blood wells up in the periorbital space. The tip of the pipette is then slightly withdrawn so that the blood flows into the pipette by capillary action.

Bleeding stops once the normal ocular pressure is allowed to impinge on the venous plexus by releasing the animal.

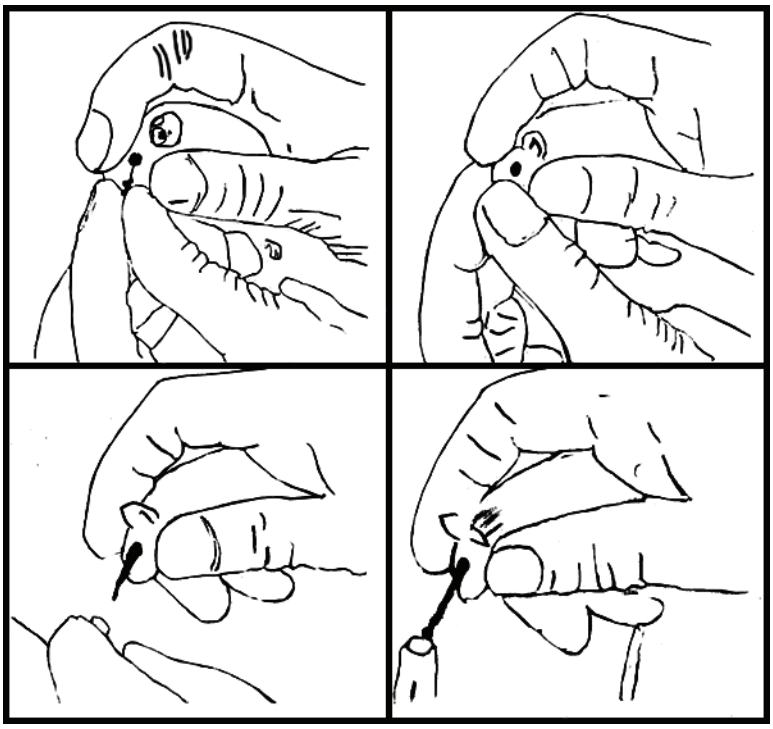


**Fig. 4** Blood sample collection from mouse orbital sinus.

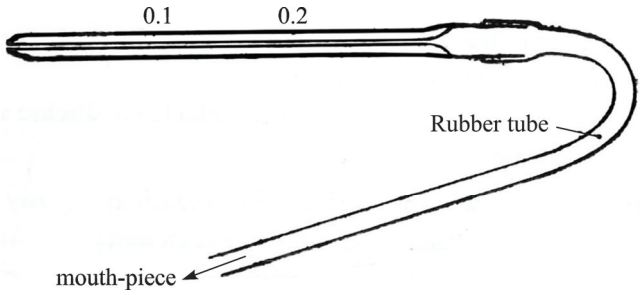



**Fig. 5** A blood pipette.

The total volume of blood of a mouse is about 6- 8% of its body weight. A maximum of 7.7 ml. can be collected safely in one spell of bleeding.

## Administration of Drug to Mouse

Administration of medications can be made by oral (p. o), subcutaneous (s. c), intramuscular (i. m), intraperitoneal (i. p) and intervenous (i. v) routes. Appropriate volume for injection is 1 ml / 100 gms body weight. Subcutanous injections can be made into the scruff of the neck. Intramuscular injection is given into the hind leg, whereas intravenous injection is usually

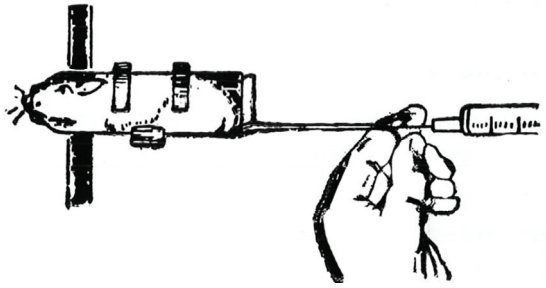


**Fig. 6** I.V. injection to a mouse.

**Fig. 7** P.O. injection to a mouse.

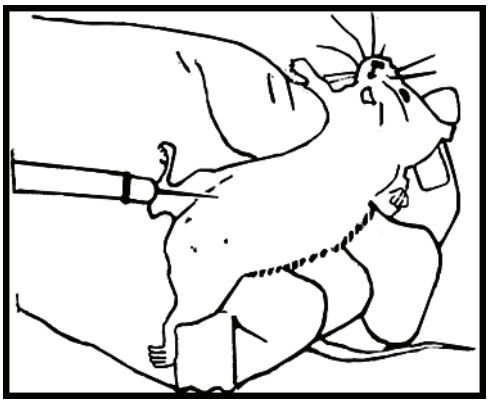


**Fig. 8** P.O. injection to a mouse.

**Fig. 9** Intramuscular injection (Mice)

given into the tail veins. The tail can be heated by dipping the same in warm water or rubbing it with xylene will make the tail vein to dilate becoming clearly visible, Intraperitoneal injections should be administered into lower, half of the abdomen on either side of the midline with the needle at about 1-20 degree angle to the skin.

## Euthanasia of Mouse

Ether or carbondioxide in a jar can be used to kill mouse painlessly. Alternatively, the animal can be killed by cervical dislocation.

▲ Based on LAIS centre news.