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Diploma in Pharmacy 2nd Year
Pharmacology
Experiment

To study the techniques of blood collection from animals.

Aim:

To study the techniques of blood collection from animals.

Reference :

‘ Dr. P.Mudagal Manjunatha , “Practical Manual of Pharmacology” Published by Nirali Prakashan, Page no 27 – 34

Theory :

There are various efficient methods for taking blood from small laboratory animals, which is necessary for many different types of scientific research. Numerous regulatory organisations and recommendations have regulated the use of animals in research and the procedures used to take blood from laboratory animals

General Principles of Blood Collection in Animals

→ The procedure authorised by the Institute animal ethics committee should specify the procedure for blood collection.
→ It must be least painful and stressful. Blood samples may be collected both with and without anaesthesia.
→ Blood collection from any animal using any method requires adequate training.
→ Blood samples are frequently collected from the venous, arterial blood vessels or heart chambers.

→ Blood collection frequency is important. It is best to treat non rodents every two weeks. Lagomorphs (such as hares and rabbits) can be employed if the study requires numerous blood samples.

→ In healthy, normal adult animals, the maximum amount of nonterminal blood that can be taken at one time without replacing lost fluids is 10% of the total amount of circulating blood and this can be done once every 3-4 weeks. A maximum of 0.6 ml/kg/day or 1% of an animal's total blood volume can be taken every 24 hours, if repeated blood samples are needed at frequent intervals.

→ A temporary cannula can be used to collect blood samples if the study involves repeated blood sample collection which can minimise stress and pain experienced by the animals.

→ Blood volume in adult animals range from 55 to 70 ml/kg of body weight. Aged or obese animals need to be treated with care. Fluid replacement may be necessary if the volume of blood collected is greater than 10% of the total blood volume. The National Institutes of Health (NIH) advise Lactated Ringer's Solution (LRS) as the best fluid replacement. Ample precautions should be made to prevent hypovolemia in the animal if the volume of blood collected exceeds 30% of the total volume of circulating blood.

General Methods for Blood Collection

1) Blood Collection Not Requiring Anesthesia :

- Saphenous vein (rat, mice, guinea pig)
- Dorsal pedal vein (rat, mice)

2) Blood Collection Requiring Anesthesia (Local/General Anesthesia) :

- Tail vein (rat, mice)
- Tail snip (mice)
- Orbital sinus (rat, mice)
- Jugular vein (rat, mice)

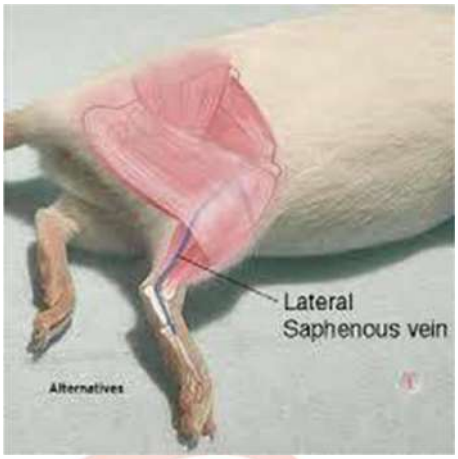
- Temporary cannula (rat, mice)
- Blood vessel cannulation (rat, guinea pig, ferret)
- Tarsal vein (guinea pig) viii) Marginal ear vein/artery (rabbit)

3) Terminal Procedure :

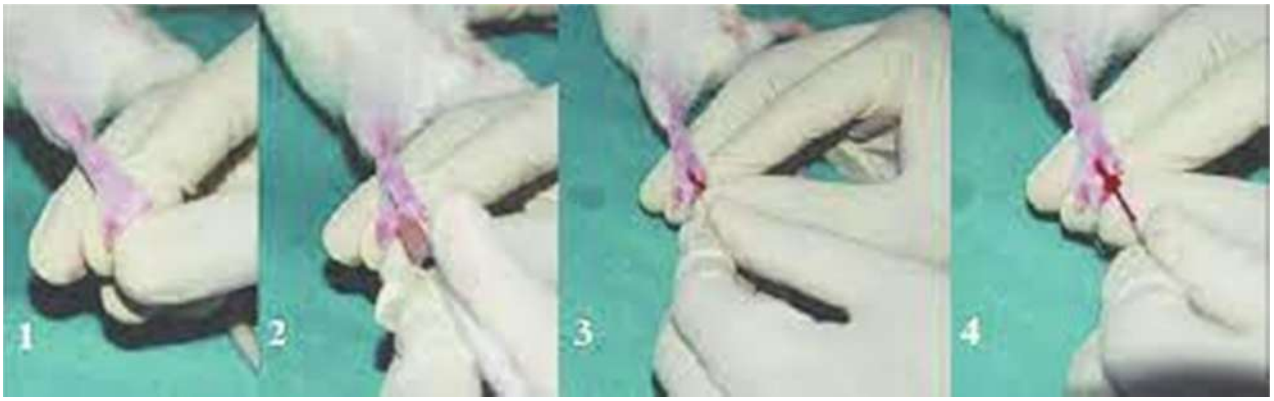
- Cardiac puncture (rat, mice, guinea pig, rabbit, ferret)
- Orbital sinus (rat, mice)
- Posterior vena cava (rat, mice)

Procedure

1) **Procedure for Saphenous Vein Blood Sample Collection:** While handling animals and rodents, gloves, towel, cotton, sample collecting tubes, and 20G needle are required materials
   i. Sampling should be done while taking aseptic measures using the lateral saphenous vein
   ii. Electric trimmer should be used to shave the back of the hind leg until saphenous vein is visible. Hair removal cream may also be used.
   iii. The animal should be restrained manually or using a suitable animal restrainer
   iv. The hind leg is immobilised, and the knee joint may be gently pressed with slight pressure.
   v. 20G needle should be used to puncture the vein and enough volume of blood should be collected with a capillary tube or a syringe with a needle To stop the bleeding, the punctured site should be compressed.
   vi. **Precautions :** While collecting blood.
      a) The local anaesthetic cream should be applied on the collection site.
      b) More than three attempts should not be made.
      c) Continuous sampling should be avoided.
      d) More than four samples should not be collected in a day.



2) **Procedure for Dorsal Pedal Vein Blood Sample Collection:** Animals, rodents handling gloves, cotton, capillary tube, 23G/27G needle, sample collection tubes, and an animal warming chamber are required materials.
   i. The animal should be kept in restrainer
   ii. The hind foot around ankle should be held and medical pedal vessel is located on the top of the foot.
   iii. Absolute alcohol should be used to clean the foot and dorsal pedal vein should be punctured with 23G/27G needle
   iv. Blood drops that would normally appear Figure 16: Blood Collection from the Dorsal Pedal Vein on the surface of skin should be collected in capillary tube and small pressure should be applied to stop the bleeding

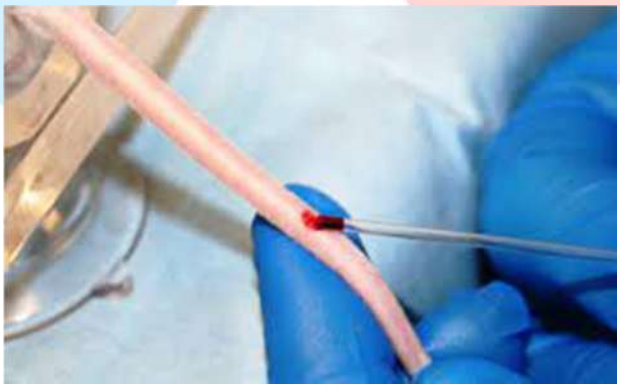


3) Procedure for Tail Vein Blood Sample Collection: Animals, rodents handling gloves, a towel, cotton, sample collection tubes, and an animal warming chamber are required materials.

i. This procedure should be recommended for large volume of blood collection.
ii. The animal should be made comfortable in the restrainer between 24°C to 27°C.
iii. The tail should not be rubbed from the base to the tip as this can produce leukocytosis. If the vein is not visible, the tail should be dipped in about 40°C warm water.
iv. Local aesthetic cream should be applied on the surface of the tail 30 minutes before the experiment.
v. A 23G needle should be inserted into the blood vessel and a capillary tube or a syringe with a needle should be used for collecting blood In case of problems, 0.5 to 1 cm of surface of the skin should be cut open and the vein should be pricked with bleeding lancet or needle as well Figore 17: Blood Collection from Tail Vein as blood should be collected with a capillary tube or a syringe with a needle
vi. Pressure/silvernitrate ointment/solution should be applied to stop bleeding after collecting blood.
vii. Temporary surgical cannula should be used if multiple samples are required.
viii. Restrainer should be washed regularly for preventing pheromonally induced stress or cross infection.

4) **Procedure for Tall Snip Blood Sample Collection:** Animal, anaesthetic agent, cotton, a surgical blade, and tubes for collecting blood are required materials.

i. This procedure for blood collection should only be done in mice.
ii. This procedure should be avoided as much as possible because it may result in long-term harm to the animal's tail. If necessary, only terminal anaesthetic should be used
iii. Local anaesthesia should be applied on the tail before collecting the blood and a cut should be made 1 mm from the tip of the tail using scalpel blade
iv. The tail tip should be dabbed to stop the flow of blood.



5) Procedure for Orbital Sinus Blood Sample Collection : Animal, anaesthetic agent, cotton, capillary tube, and tubes for collecting blood are required materials.

i. This procedure is also known as orbital venous plexus bleeding and periorbital, posterior-orbital, and periorbital bleeding. is used in experimental settings with recovery.
ii. General anesthesia should be used for collecting blood sample.
iii. Topical ophthalmic anaesthetic agent should be applied to the eye before bleeding
iv. The animal should be scruffed with thumb and forefinger of the non- dominant hand and the skin around the eye should be pulled taut.

v. A capillary should be inserted into the medial canthus of the eye (30 degree angle to the nose)

vi. Only a small amount of thumb pressure is required to puncture the tissue and enter the plexus or sinus.

vii. Blood will flow via the capillary tube once the plexus or simas is punctured.

viii. The capillary tube should be removed gently and wiped with sterile cotton when the required volume of blood is collected from plexus. Gentle finger pressure should be applied to stop bleeding.

ix. Animal should be examined for post-operative and periorbital lesions in 30 minutes after blood collection

x. Caution :

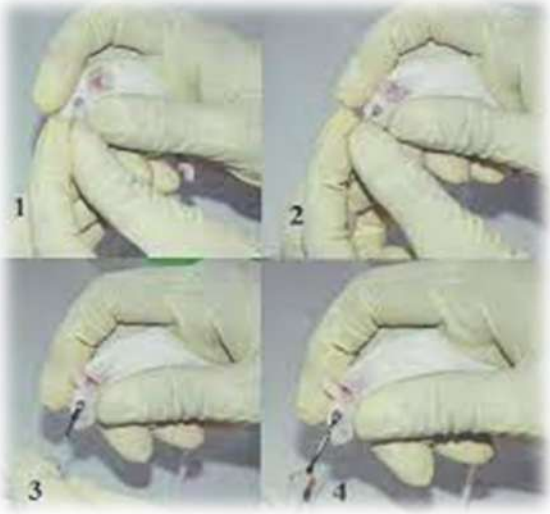
a) Blood sampling should not be repeated

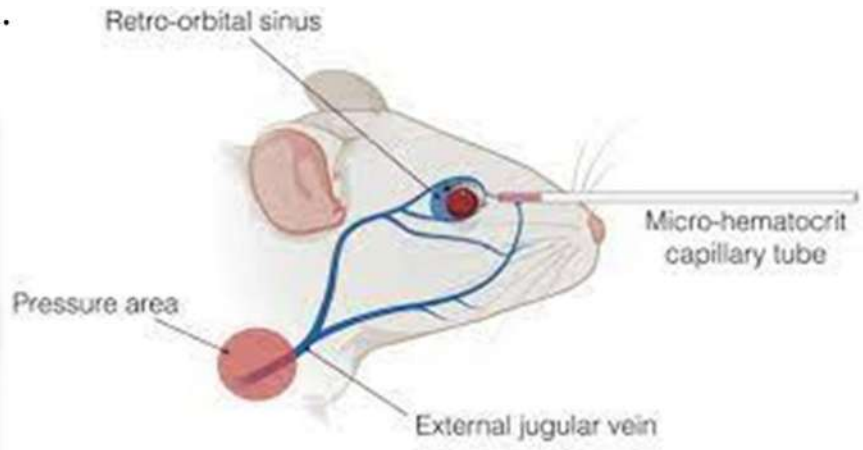
b) Skill should be required for collecting blood.

c) Eye injury can result from even a small error

d) There should be a two-week interval between two bleedings.

xi. Negative side effects from this surgery have been reported in 1 to 2% of patients. and they include bleeding, corneal ulceration, keratitis, pannus formation, and rupture of the globe, damage to the optic nerve and other intra-orbital structures, and necrotic dacryoadenitis of the harderian gland.





6) Procedure for Jugular Vein Blood Sample Collection: Animal, anaesthetic agent, cotton, 25G needle, and tubes for collecting blood are required materials

- In this procedure, blood sample should be collected in quantities ranging from very small to one millilitre instead of warming the animals.
- Two people are required to collect the blood sample for this procedure. which should be performed under general or inhalation anaesthetic.
- The animal should be restrained and monitored. The blood sample should be then collected from the animal.
- The neck region of the animal should be shaved and kept in hyperextended position. The jugular veins are located 2 to 4 mm lateral to the sternoclavicular joint and are blue in colous. A 25G needle should be inserted in the caudocephalic direction (back to front) to collect blood slowly to prevent the collapse of these small blood vessels. Animals should be handled gently, and only a 3 to 4 mm needle should be used to puncture a blood vessel.
- The needle should be slowly removed and the site should be monitored for bleeding if the attempt to collect blood is unsuccessful. Another effort should be made if there is no bleeding. In the case of bleeding, further attempts should be avoided since they could cause vein collapse.
- Finger pressure should be applied to stop bleeding.
- **Precautions:**
    - More than three attempts should not be done.
    - Local anaesthetic cream should be applied 30 minutes before sampling.

7) Procedure for Blood Sample Collection With Temporary Cannula: Animal, anaesthetic agent, cotton, 25G needle, animal warming chamber and tubes for collecting blood are required materials.

i. A temporary cannulation should generally be made in the tail vein and used for a few hours.
ii. The animal should be restrained and local anaesthetic cream should be applied to 1-2 cm above the tail tip
iii. The tail should either be cannulated or 25G needle should be used.
iv. The animal should be warmed (37-39°C for 5-15 minutes) in tail bleeding procedure in order to dilate blood vessels.
v. Animals should be kept separately in large cages after being cannulated.

8) Procedure for Blood Vessel Cannulation: Animal, anaesthetic agent, cotton, 25G needle, 1.V. cannula, surgical blade, heparin (or any anticoagulant) and tubes for collecting blood are required materials

i. Close and continuous monitoring of the animal should be done in this procedure.
ii. Blood vessel cannulation should generally be done in the femoral artery, femoral vein, carotid artery, jugular vein, vena cava and dorsal aorta.
iii. In this procedure, surgery is required and suitable anaesthesia and analgesia should be used for reducing pain.
iv. The animal should be kept alone in a large and spacious cage after undergoing surgical cannulation.
v. Blood samples with a volume of 0.1-0.2 ml each should be collected over 24 hour.
vi. The cannula should be flushed with an anticoagulant after collecting blood and the collected volume should be replaced with LRS and cannula should be tightly closed.

vii. Aseptic techniques should be used to conduct the experiment. The area surrounding the cammulation site for indications of infection haemorrhage, cannula blockage, and oedema should be examined.

viii. The maximum blood volume to be obtained and the needle size are listed in Table

Table 3: Needle size used for blood vessel cannulation in different species

Species	Needle to be used	Maximum collection volume
Mice	23-25G	1 ml
Rat	19-21G	10-15 md
Rabbit	19-21G	60-200 ml
Guinea pig	20-21G	1-25 ml

9) **Procedure for Tarsal Vein Blood Sample Collection :** Animal, anaesthetic agent, cotton, 25G needle, IV cannula, surgical blade, heparin (or any anticoagulant), hair remover and tubes for collecting blood are required materials

i. This vein should be identified on hind legs (appearing in blue colour) of large animals

ii. Anaesthetic cream for hair removal should be applied.

iii. Blood should be collected slowly after 20-30 minutes.

iv. Maximum sample of 0.1 to 0.3 ml per leg should be taken.

v. Pressure should gently be applied to stop bleeding.

vi. **Caution:** More than 6 samples from each of the animal's legs should not be taken

10) Procedure for Marginal Ear Vein Blood Sample Collection:

O-xylene. 95% alcohol, 26G needle are the required material.

i. Animals should be placed in restrainer.

ii. Alcohol should be used for clearing ear and local anaesthetic should be applied before 10 minutes.

iii. O-xylene should be used as a topical vasodilator.

iv. Surgical blade should be used to cut the vein.

v. Sterile cotton should be placed on the collection site after collecting blood

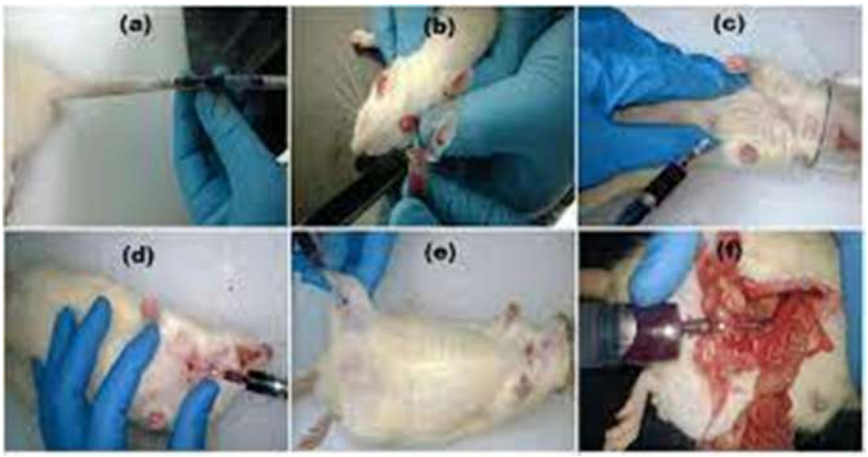
11) Protocol for Cardiac Puncture: Animal, anaesthetic agents, towel, cotton, 19 to 25G needle with 1 to 5 ml syringe, surgical blade, tube (internal diameter of 0.1 to 0.3 mm) for thoracotomy, plastic disposable bag, and tubes for collecting blood are required materials.

i. Generally, cardiac puncture should be done for the terminal stage of the study in order to obtain a single, large volume of high-quality blood from the experimental animals.

ii. Animal should be under terminal anaesthesia during collecting the blood sample

iii. Proper needle should be used to draw blood samples, whether a thoracotomy is performed or not. Blood sample from heart should be collected ideally slowly from the ventricle in order to prevent the heart from collapsing

iv. Precautions: Sampling may not succeed if the animal has dextrocardia.



12) Protocol for Blood Sample Collection through Posterior Vena Cava: Animal, anaesthetic agents, cotton, 21 to 25G needle with 1 to 5 ml syringe. surgical blade, small glass rods, surgical scissor, and tubes for collecting blood are required materials.

i. Generally, posterior vena cava blood sample should be done for the terminal stage of the study.
ii. The animal should be given anaesthesia before having a "Y" or "y" shaped cut made in its belly and the intestines should be gently removed.
iii. The liver should be pushed forward and the posterior vena cava (between the kidneys) should be identified.
iv. Blood should be collected from the posterior vena cava using 21 to 25G needle.
v. This procedure should be repeated 3 to 4 times in order to collect more volume of blood sample.

Result :

The techniques of blood collection from animals were studied.

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