Micro-Method for Intravenous Injection and Blood Sampling

By H. SALEM, M. H. GROSSMAN†, and D. L. J. BILBEY‡

The described methods have been found to be valuable in investigations into the body distribution and fate of pharmacological substances and are herein reported for the benefit of other workers in the field of pharmacology and toxicology.

IT HAS BEEN FOUND both expedient and practicable to combine two techniques in which substances are introduced directly into the bloodstream of small animals and recovered for determination in serial samples of blood.

The injection of prepared amounts of a substance in either solution or suspension (chemicals, drugs, bacteria, particulate matter ± radioactivity) are injected into the bloodstream of a mouse or other small experimental animal by the dorsal vein of the penis. For this purpose it has been found convenient to use a 1-ml. "tuberculin" syringe graduated into 100 divisions and fitted with a fine hypodermic needle (gauge 26–30). The animal is secured by an assistant who holds it by the loose skin behind the neck in the right hand and by either the tail or hind feet in the left in order to stretch the animal and hyperextend the vertebral column (Fig. 1). The operator then places gentle pressure over the lower abdomen and secures the tip of the penis between the thumb and forefinger of the left hand as shown in both Figs. 1 and 2. The dorsal vein of the penis is easily entered by a fine hypodermic needle and the contents of the syringe can be injected with ease. It should be noticed that in this maneuver the hands of the operator and his assistant are kept in close contact to prevent uncoordinated movements.

The advantage of this method is in its applicability to animals having no easily accessible site for intravenous injection. Although we have used the mouse to illustrate this technique, we have successfully employed it using guinea pigs and hamsters.

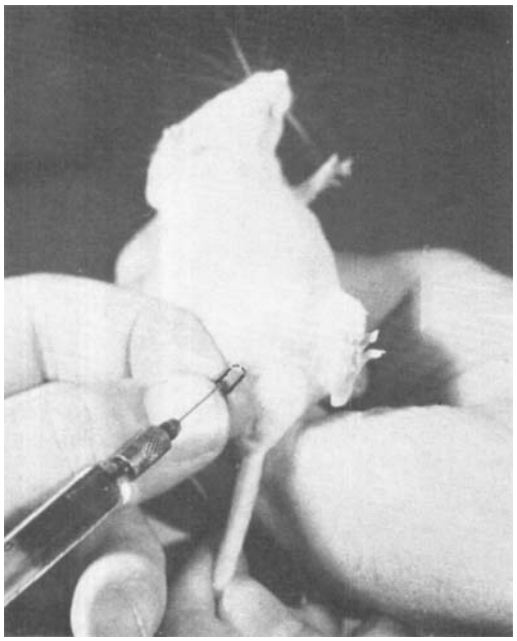

Fig. 1.—The method of holding the mouse, the retraction of the penis, and the insertion of the needle for injection into the dorsal vein.

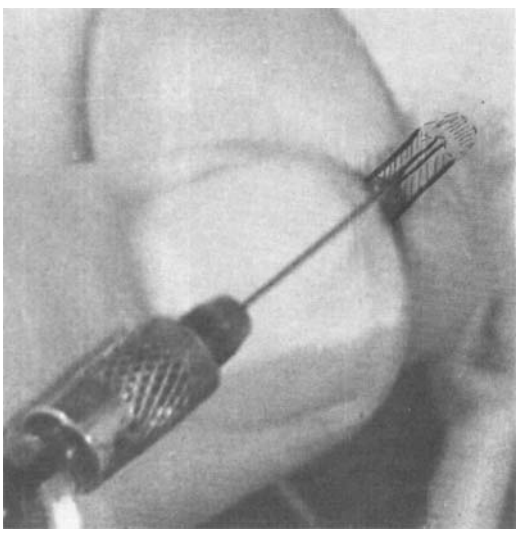

Fig. 2.—The method of insertion of the hypodermic needle into the dorsal vein of the penis. Note that the hands of the operator and assistant are in close contact to ensure stability during the injection.

Received February 20, 1963, from Smith Kline and French Laboratories, Philadelphia, Pa.
Accepted for publication March 26, 1963.
† Present address: University of Toronto, Toronto, Ontario, Canada.
‡ Present address: Faculty of Medical Science, University of London, King's College, London, England.

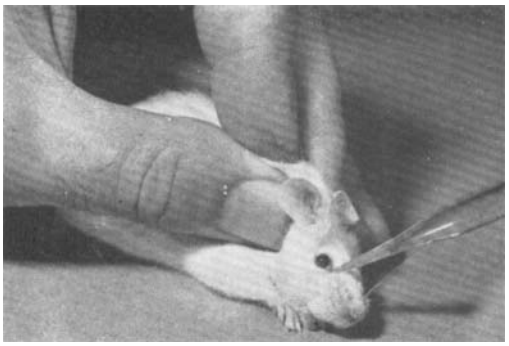

Fig. 3.—The angle at which the fine pipet should be placed at the medial canthus prior to the withdrawal of blood.

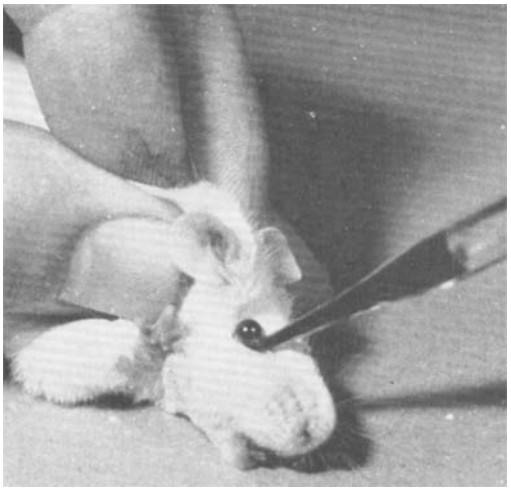

Fig. 4.—The removal of blood from the retro-orbital plexus and the positioning of the operator's thumb.

Small samples of blood are removed by the veins of the retro-orbital plexus (Fig. 5). This method has been described by Halpern and Pacaud (1) and later by Hans Nöller (2) and consists of the removal of a desired amount of the blood (usually 0.01 to 0.50 ml.) by a fine glass pipet which is inserted into the medial canthus of the orbit and which fills both by capillary action and venous pressure following the rupture of one or more of the fine branches of the orbital venous plexus. The manner of holding the animal and the angle at which the pipet is inserted is shown in Figs. 3 and 4. The middle and forefingers of

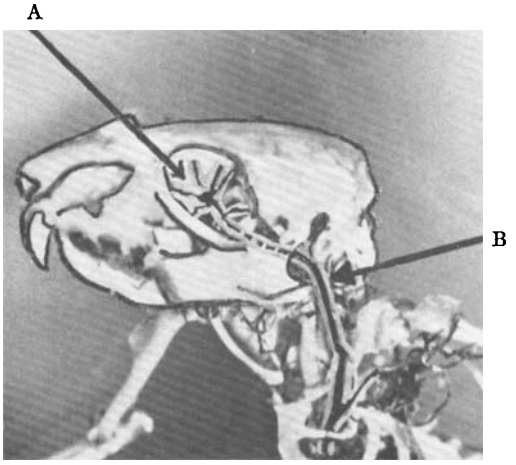

Fig. 5.—A rat skull prepared to show the veins of the head and neck in order to indicate the position of the drainage from the retro-orbital plexus of veins. *A*, indicates the orbit in which may be seen the main vessels of the plexus; *B*, shows the point at which the internal jugular vein leaves the skull and where pressure by the thumb serves to engorge the retro-orbital venous system with blood.

the left hand maintain the head of the animal in position. It is most important to press gently but firmly with the thumb just behind the angle of the jaw as shown in these illustrations. This last procedure causes a restriction of venous return by the internal jugular vein (Fig. 5) and consequent engorgement of the retro-orbital plexus. The longer the pressure is maintained, the greater volume of blood is withrawn. In order to prevent coagulation of the blood inside the pipet, the end of it is placed in a solution containing an anticoagulant before the taking of each sample. No damage to the actual eye is incurred in this procedure, and it is possible to remove up to as many as 30 serial samples from one animal. The blood that is withdrawn is available for various investigations and may be chemically, photometrically, or radioactively analyzed—cultured and plated for bacterial growth—or made into a film for observations under the microscope.

REFERENCES

(1) Halpern, B. N., and Pacaud, A., *Compt. Rend. Soc. Biol.*, **145**, 1465(1951).
(2) Nöller, H. G., *Klin. Wochschr.*, **33**, 770(1955).