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A Novel Method for the Rapid Bleeding of Rats from the Tail Vein [1]

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A method for bleeding rats from the tail vein was developed. Sufficient vasodilation of the tail was accomplished by the use of a specially designed warming device. With the help of a physical restrainer, up to 5 ml of blood can be collected from the tail vein and no anaesthesia is required. This bleeding procedure is fast, simple to perform, and imposes practically no risk to the animal.

Key words: *bleeding — blood — vasodilation — rat — restrainer — tail vein*

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Introduction

Blood can be obtained from rats by at least 2 techniques: bleeding from the retro-orbital plexus (Pettit, 1913; Halpern and Pacaud, 1951) and cardiac puncture (Chase, 1967; Campbell et al., 1970; Herbert, 1978). Both methods require anaesthetization of the animal before the operation can be performed. Retro-orbital bleeding is impractical on occasions when a relatively large volume of blood is required and this approach often renders the animal prone to infection. Cardiac puncture carries the risk of a high mortality rate, especially when performed by inexperienced individuals. In view of these difficulties, we have developed a simple technique in bleeding rats from the tail vein using a specially designed, but simple, tail-warming device. This technique proves to be fast, easy to perform, and capable of providing up to 5 ml of blood in a regular bleeding.

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Materials and Methods

Animals

Male hooded rats weighing about 300 g were purchased from the Charles River Breeding Laboratories, Montreal, Quebec. Animals were kept individually in stainless steel cages and were allowed free access to laboratory rat chow and drinking water.

Restrainer

The rat restrainer was constructed of clear plexiglass (6 mm thick) and its design is shown in Fig. 1. It is basically a cylindrical chamber fitted with 2 perforated circular end plates. The circular plate at the tail end of the chamber is removable. Each of the 2 end plates can be adjusted lengthwise to keep the animal properly restrained. A restraining chamber with an internal diameter of 5.5 cm is found to be appropriate for animals weighing in the range of 300–375 g.

Tail-warming device

The tail warmer was made of thermal wires obtained from an electric thermal blanket (Sunbeam, Brantford, Ont.) coiled closely together to form an open cylinder

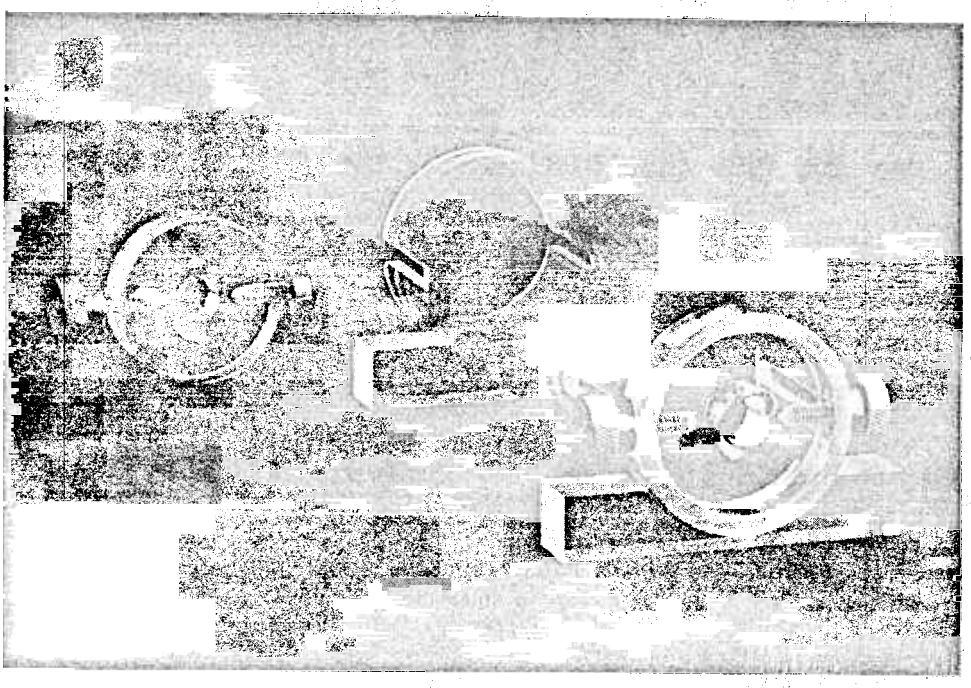
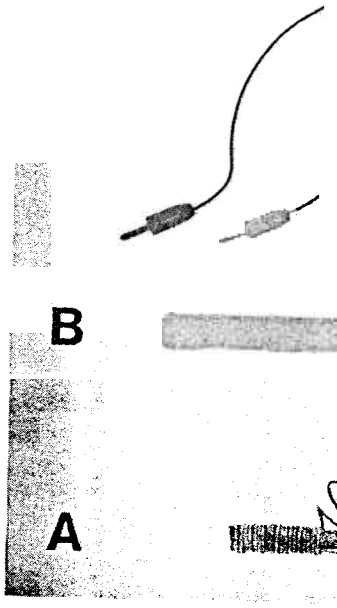


Fig. 1. Design of the rat restrainer. Plexiglass cylindrical chamber: thickness, 6 mm; length, 23 cm (maximum), 13 cm (minimum); internal diameter, 5.5 cm; side slots for ventilation, 5 cm×6 mm. Each circular end plate has a central opening (6 mm in diameter) and is fitted with 2 locking-screws.



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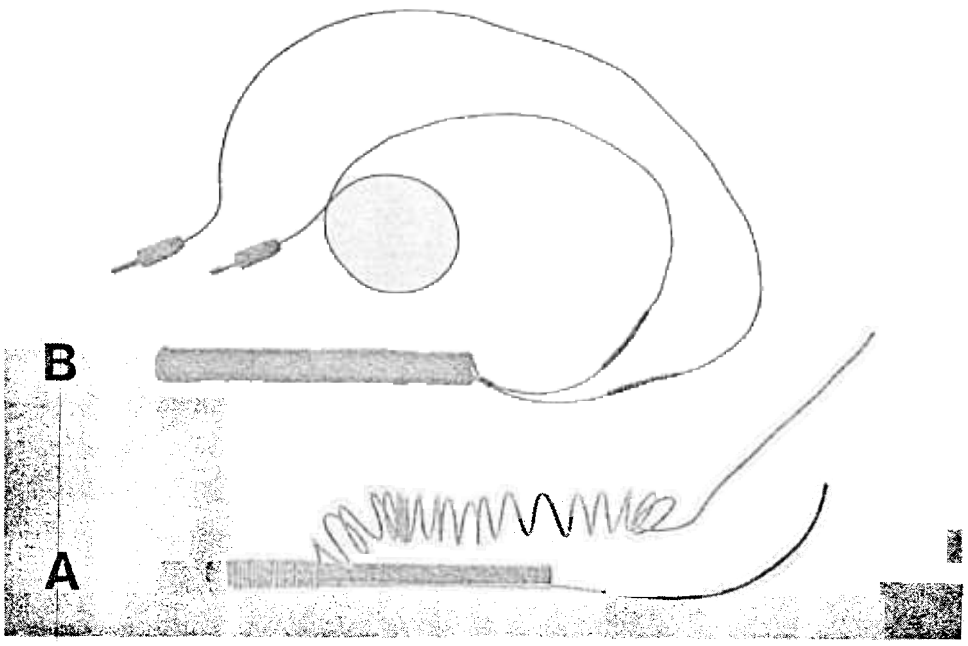


Fig. 2. Construction of the tail-warming device. (A) A glass rod (1 cm in diameter) was wrapped with adhesive tape, the adhesive side facing out, and the thermal wire was coiled tightly around it. (B) The 2 free ends of the completed thermal coil were connected to a second pair of wires fitted with banana plugs.

as shown in Fig. 2. The thermal coil was assembled simply by looping the thermal wire around a glass rod (diameter, 1 cm) and holding the coils together with adhesive tapes (Dominion Tape, Cornwall, Ont.). The glass rod was then removed and each of the 2 free ends of the thermal wire was connected to a hook-up wire (Electrosonic, Willowdale, Ont.) fitted with a banana plug at one end. It was found that 3 m of thermal wire were required for making a tail warmer of approximately 16 cm in length and 1 cm in internal diameter. The tail warmer can be connected to a portable DC power supply (Model 6204A, Harrison Lab., N.J.) adjustable to provide an electrical potential in the range of 6.5–8.5 V, generating temperatures between 41°–48°C within the core of the tail warmer.

Bleeding procedure

The rat to be bled was allowed to enter the plexiglass restrainer with the tail of the animal exposed through the opening (12 mm) of the circular end plate which was then locked into place to keep the animal properly restrained. The tail of the rat was cleaned with a cotton swab moistened with 70% ethanol. The tail warmer, previously set at 46°C, was used to enclose the whole length of the tail (Fig. 3). Warming the tail for a few minutes was usually found to be adequate for vasodilation to occur. After the warmer was removed, the dilated tail veins became visible and a sterile

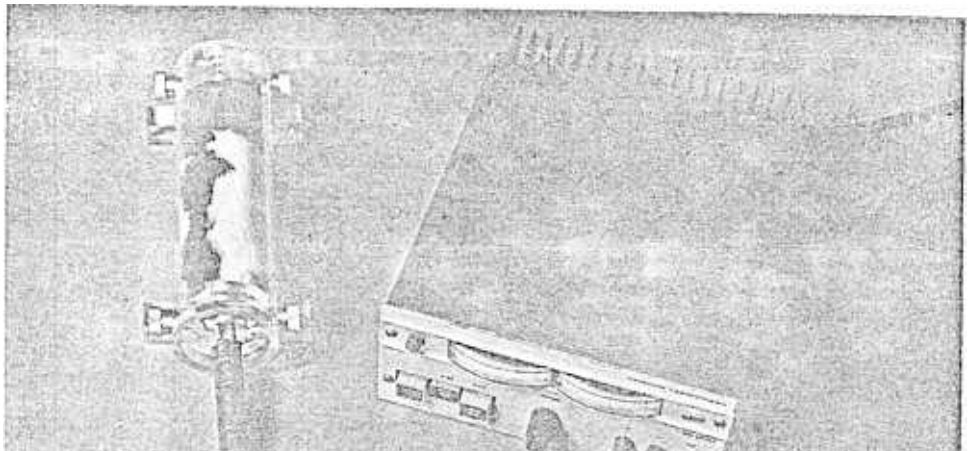
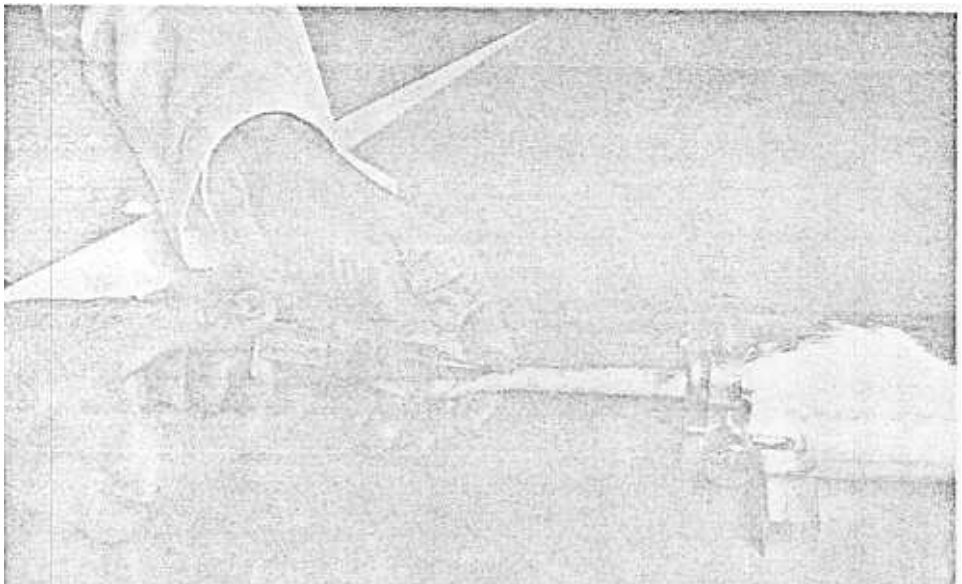


Fig. 3. Application of the tail warmer for dilation of the tail veins. By appropriate adjustment of the DC power supply, the tail-warmer was set to a predetermined temperature, for example, 46°C. The warmer was then inserted to enclose the entire length of the animal's tail. After a few minutes, the warmer was removed and the tail inspected for visible vasodilation.



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Results and Discussion

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22-gauge hypodermic needle, connected to a 5 ml disposable syringe, was inserted into the lumen of one of the distended veins for the steady withdrawal of blood (Fig. 4). Best insertion was obtained with the bevel of the needle facing upward, and the needle inserted at an angle of approximately 3° along the direction of the vein. The point of insertion was chosen to be in between 2 skin scales. The dorsal as well as the 2 lateral tail veins were found to be appropriate for this bleeding procedure.

Results and Discussion

Rats may be bled up to a volume of 5 ml at a time with no apparent ill effect on the animal (Campbell et al., 1970). The method described in this report can be used to obtain this quantity of blood without having to anaesthetize the animal, clearly an advantage in experiments where anaesthesia would be undesirable. This bleeding method, unlike that of cardiac puncture (Herbert, 1978), also avoids the risk of traumatizing the animal's heart and hence is particularly suitable for use in experiments where serial blood sampling is required. The rat restrainer used in this study was designed to have 2 continuously adjustable end plates. This design provides greater restraining efficiency than other devices with lock-step end plates. It must be emphasized that it is very important to keep the animal maximally restrained for proper bleedings.

The route via the tail vein of a rat has been used primarily for the purpose of injections (Herbert, 1978). The use of the intravenous route for bleeding is hampered by the lack of an efficient vasodilating device for the tail. In order to promote sufficient venous return to the tail for bleeding purposes, the tail veins need to be adequately dilated. The thick scaly skin of the rat tail renders it impractical and ineffective to apply a chemical vasodilating agent, like xylene which is very efficient, for example, in dilating the marginal veins (Campbell et al., 1970) and the central artery (Gordon, 1981) of the rabbit ear for bleeding. With the use of the tail-warming device, sufficient dilation of the tail veins can be achieved within minutes. The use of a 100 W desk lamp is helpful for the visualization of the veins as well as for the insertion of the needle. It was found appropriate to use a 22-gauge needle for proper entry into the lumen of the tail vein. After the needle has been inserted into the vein, the plunger of the syringe should be pulled back slowly and steadily, and the pulling action should be stopped momentarily after the withdrawal of approximately every 0.1 ml of blood. This approach allows the venous return to refill the partially collapsed vein before additional blood is withdrawn. It must be emphasized that following this guideline is essential for successful and trouble-free bleedings.

Fig. 4. Withdrawal of blood from the tail vein. The dorsal as well as the 2 lateral tail veins can be used for the withdrawal of blood. After the needle was inserted into the lumen of the distended vein, the luer-lok hub of the syringe was allowed to rest on the thumb of the hand holding the tail. Blood was withdrawn by pulling the plunger of the syringe intermittently, thus allowing the venous return to refill the collapsed vein. For trouble-free bleeding, it is imperative that the animal be properly restrained by prior adjustment of the 2 circular end plates of the restrainer.

This bleeding method has been used repeatedly (over 100 bleedings) to obtain blood from rats. Depending on the need of the experiment, 1–5 ml of blood can be collected using syringes with or without heparin. Heparinized blood was used for the preparation of peripheral blood lymphocytes and non-heparinized blood, for the collection of serum. To prevent unnecessary haemolysis, the needle of the syringe must be removed before transferring the collected blood to an appropriate container. The use of sterile, disposable plastic syringes (5 ml) was found to be far more superior than glass syringes. The tight-fitting design of the plastic syringe circumvents the problem of the back-flow of blood over the plunger of most glass syringes.

This bleeding technique should be useful to investigators whose experimental design may require a relatively large volume of rat blood for the collection of normal or immune sera. By taking the appropriate aseptic precautions, this bleeding procedure is also useful in obtaining heparinized blood for cell culture studies.

Acknowledgements

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