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Simple and Reliable Method for Serial Sampling of Blood from Rats

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Abstract □ A technique for serial sampling of whole blood, plasma, or serum from unanesthetized, unrestrained rats is described. This technique is sufficiently rapid, reliable, and independent of the need for elaborate preparation, specialized equipment, or practiced skills to appeal to a wide range of experimental and teaching situations requiring multiple sampling from many animals. A simple surgical procedure implants a one-piece jugular cannula cut from a commercial coil of silicone polymer tubing. Multiple blood samples are almost immediately available for 5 weeks or more. Plasma or serum samples are readily obtainable from each blood sample without transfer from the syringe in which it is collected. Intravenous injection through the cannula does not prejudice later sampling protocol.

Keyphrases □ Blood sampling, serial—simple and reliable method, technique and equipment described, rats □ Serial sampling, blood—simple and reliable method, technique and equipment described, rats

The size of the rat makes it a convenient laboratory animal for instructional classes and limited facility situations or where statistical treatment of data necessitates a number of subjects. For pharmacokinetic studies in particular, a simple technique for serial sampling of blood from these animals would be most useful.

The methods available, however, are unreliable, traumatic for the subject, difficult for the operator, or require elaborate preparation or specialized equipment. The following modification of the Popovic and Popovic (1) and Weeks and Davis (2) methods involves a simple, rapid surgical procedure, requiring neither elaborate preparation nor equipment more specialized than medical-grade tubing. Cardiac blood samples from the unanesthetized, unrestrained rat can then be taken repetitively and almost instantaneously. Plasma or serum samples are available for very little extra outlay of time or effort.

The modifications described thus eliminate problems counter to the demands of those very situations that favor choice of the rat as a laboratory subject. The technique is well suited to a wide range of experimental and teaching situations requiring multiple samples from large numbers of animals.

EXPERIMENTAL

Surgical Procedure—The rat is lightly anesthetized with ether, and the hair is removed from about a 2-cm square of the skin on the back of the neck. The animal is then held on its back by taping the forelegs to a board with adhesive tape. Light anesthesia is maintained for the rest of the surgical operation by applying a small wad of tissue paper over both the rat's nose and mouth and moistening the wad with ether occasionally.

Hair is clipped from above the right jugular vein, and a 2–3-cm skin incision is made in an anterior direction from above the midpoint of the right collar bone. By blunt dissection, about 1 cm of the external jugular vein is exposed forward from its emergence from below the collar bone. A 14-gauge hypodermic needle, from which the hub has been removed, is inserted under the skin at a point in line with, and about 1 cm anterior to, the exposed section of the jugular vein. It is passed subcutaneously but directly (*i.e.*, over the right shoulder but under the ear) to pierce the skin in the center of the clipped area on the back of the neck and just above the shoulder blades.

A 150-mm length of silicone polymer tubing is threaded through the needle, and the latter is removed through the back of the neck to leave the tubing lying subcutaneously. A syringe charged with heparinized saline is attached to the dorsal end of the tubing, and the tubing is rinsed with a little of the solution.

The exposed section of the vein is ligated anteriorly, and the ends of the tie are left uncut. By taping these ends to the operating board beside the subject's head, slight traction can be applied to the vein. A short oblique cut is made in the vein with a pair of fine scissors just below the ligature. By lifting the resultant flap with a pair of fine forceps and rotating the tubing, the ventral end of the tubing can easily be inserted into the vein. Thirty millimeters of tubing is passed toward the heart.

The tubing is secured by tying firmly around the vein just above the collar bone and again closer to the point of insertion of the can-

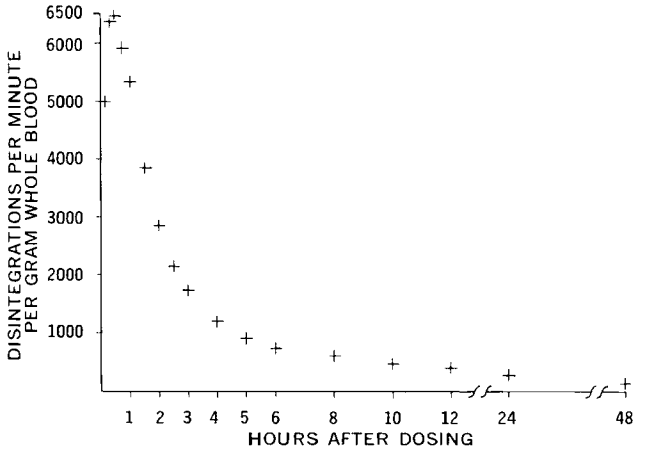

Figure 1—*Pharmacokinetic study. A 282-g rat was dosed intraperitoneally with 7.5 × 10⁶ dpm ¹⁴C-antifolate Compound 1, 12 hr after cannula implantation.*

nula. The ends of the anterior ligature are firmly tied around the exposed tube; another tie is made below the ligature but above the point of insertion, thus securing the exposed cannula to the depleted section of the vein. Possible occlusion of the cannula can be monitored after each tie by movement of the plunger of the syringe still attached.

Parted subcutaneous tissue and musculature are reunited by discontinuous sutures. Where possible, the sutures are passed around the tubing. Three or four usually suffice. The ventral skin incision can then be closed, also with discontinuous sutures (six to eight). A firm tie is made around the tubing and skin just before the tubing emerges at the back of the neck; the ends of the tie are left long and are used to form a small loop above the skin. After injection of 0.2 ml heparinized saline, the cannula can be cut with a bevel to a length of 10–20 mm and stoppered with a light plug fashioned from 24-gauge stainless steel wire.

A translucent, seamless silicone tubing[1] (0.51 mm i.d., 0.94 mm o.d.) was used. Sterilization was found to be unnecessary. The tubing was wiped free of visible lint prior to insertion into the vein. Vein wall obstruction of the cannula was never a problem if the tubing was beveled by trimming to an arrowhead and the tip of the arrowhead was removed by a transverse cut. Correct location of the cannula in the vein was assisted by marking the tubing, *e.g.*, 40 mm from the bevel. The heparinized saline used was normal saline containing heparin, 10 units/ml.

While not expensive, the cannula could be cleaned without difficulty and reused indefinitely.

Cannulated subjects were individually housed. All animals survived surgery, regained consciousness within minutes after the operations described, and moved apparently unencumbered after about 0.5 hr. Presence of the cannula caused no obvious discomfort. Where possible after effects of light ether anesthesia presented no objections, animals could be used for sampling procedures almost immediately.

Sampling Procedure—The following technique was employed for obtaining blood samples for pharmacokinetic studies from cannulated male Wistar rats, 250–300 g, approximately 4 months old. Each was housed individually in a urine–feces separating apparatus, having a cage of 25-cm diameter horizontal cross section. (Any similarly sized, shallow roofless cage would be suitable.) Handling of the animal was found to be unnecessary. Rather, movement of the animal (rare) during the withdrawal procedure was followed by a complementary movement of the hands manipulating the cannula or attached equipment.

A small clip was positioned at the back of the neck, both to clamp the suture loop and occlude the exposed tubing. The plug was removed from the cannula. A syringe fitted with a 23-gauge needle and containing 0.2 ml heparinized saline was inserted into the cannula. The cannula was released from the clip, which was allowed to hang from the suture loop. Then 0.2 ml of blood was withdrawn into the syringe, held vertically to minimize mixing of blood and saline.

[1] Silastic 602-135, Dow Corning International Ltd.

Table I—Short Sampling Interval

Sampling Time, min after Dosing[a,b]	Weight of Blood Samples, g Whole Blood	Content, dpm ¹⁴C/g Blood
60.0	0.2068	5851
61.0	0.1988	5864
62.0	0.2006	5728
63.0	0.1962	5461
64.0	0.1930	5231
64.5	0.1953	4957
65.0	0.2017	4754
65.5	0.2043	4571
66.0	0.2100	4527
66.5	0.2058	4323

[a] A 282-g rat was dosed intraperitoneally with 7.5 × 10⁶ dpm ¹⁴C-antifolate Compound 1, 10 days after cannula implantation. [b] Standard procedure (withdrawal of 0.2 ml of blood prior to each sample and return with 0.2 ml of heparinized saline after each sample) was omitted after 64.0 min for 30-sec sampling.

The syringe was removed from the needle and stored vertically in a needle scabbard taped to a wall, and a fresh syringe was fixed to the needle still attached to the cannula. The sample of blood was withdrawn and the syringe was removed. The first syringe was then returned to the needle, and the blood followed by the upper layer of heparinized saline was injected into the animal. The cannula was then reguided between the jaws of the clip, and the needle was removed.

Use of the clip enabled rapid access to samples without excessive blood loss or introduction of air. Entry of air could be further minimized by additional application of the clip between syringe manipulations. Use of the loop of suture material prevented drag on the cannula. Subjects wore the clip arrangement for up to 12 hr when sampling was frequent. When sampling intervals were longer, *e.g.*, overnight, the lighter plug replaced the clip and the loop was not necessary. Hold-up volume of the cannula and attached needle plus dead space between the needle and syringe was 20–25 μl. Removal of the needle bevel aided preservation of the tubing material.

During one pharmacokinetic study, each rat provided 15 samples of 0.2 ml blood at 10, 20, 30, 45, and 60 min and thereafter every 30 min up to and including 6 hr after dosing. In another such study, 17 samples were removed from each subject with the same regimen to 1 hr but with the remainder of the samples spaced to 48 hr[2] (Fig. 1). In all cases, samples could be taken within seconds of the nominated times.

Sampling could be comfortably achieved every minute. For more frequent sampling, the advantages of rinsing the cannula disappeared. By omission of rinsing and blood volume replacement, samples could be withdrawn every 30 sec without rush (Table I). These samples contained the contents of the dead space, giving for each 0.2 ml sample about 10% contamination from the previous sample. This contamination could be reduced by skillful use of the air introduced when syringes were changed without application of the clip.

Two rats were retained for 5 weeks, after which time blood samples could still be freely removed. Both subjects continued to show no patent ill effects. For these longer term animals, a loose rather than firm tie was made at the back of the neck with a fairly coarse sewing silk and the loop was constructed as before. A small piece of adhesive tape was folded over the cannula and loop such that both were fixed between the two adhesive surfaces. The resulting protective sheath was trimmed.

Where the cannula was used as frequently as daily, no special rinse-out precautions were necessary. For the first use of the cannula after more than 24 hr, initial withdrawal of a small blood sample was advisable. Cannulas not undergoing use were rinsed weekly, at which time clots may have formed in the line. These clots

[2] Whole blood samples were digested with Soluene (Packard Instrument Co.) and isopropanol in preweighed vials, bleached with hydrogen peroxide, and counted in acidified Instagel (Packard Instrument Co.). Correction for counting efficiency was made by external standard.

could often be withdrawn before injection of 0.2 ml heparinized saline. Occasionally they had to be reinjected into the animal with the saline.

Preparation of Plasma or Serum Samples—Whole blood samples collected in the described manner were nonhemolyzed. Plasma or serum could be obtained from each blood sample without transfer from the syringe in which it was collected. The syringe itself was centrifuged in an inverted position after provision was made to prevent depression of the plunger by centrifugal force. This was very simply achieved by slipping over the plunger a short piece of beverage tubing whose lumen diameter did not allow passage of the body of the syringe. It was even more simply achieved with syringes of the plastic disposable type by shortening the plunger with a large pair of scissors.

After centrifugation, hematocrit values could be conveniently obtained from the syringe scale before removal of each plasma sample. Plasma or serum was removed either by aspiration from the top or by depression of the plunger (reinsertion of the severed end of the plunger).

Intravenous Injection—The cannula described also was used successfully as a site for intravenous injection, where the injection was followed by 0.2 ml of heparinized saline. This procedure did not influence blood samples later obtained from the same site. Injection of liquids as viscous as dimethyl sulfoxide presented no difficulty, and even pure glycerin could be injected (0.5 ml within 1 min).

DISCUSSION

Cannula implantation techniques appear to have been largely avoided for pharmacokinetic studies in the rat, probably because of elaborate preparation, requisite surgical skill, or unreliability of repeated blood sampling associated with this approach. Because alternative techniques do not offer great control or facility for blood sampling, use of the rat as a pharmacokinetic subject has been more limited than its use elsewhere.

The techniques described here are designed for pharmacokinetic studies and are based on the properties (3, 4) of a silicone polymer cannula rather than polyethylene, which has been used in almost all reported instances of implantation of blood-collecting cannulas in rats. The installation of the silicone cannula is simple and rapid, and its use is convenient and reliable.

The advantages of a one-piece cannula are achieved by utilizing the greater softness and flexibility of silicone polymer tubing to fulfill both internal and external requirements. Laborious cannula manufacture is avoided, since the tubing is used virtually as supplied commercially.

Exteriorization of the cannula by previously described circuitous and difficult routes and the provision of elaborate anchors for the cannula are bypassed since the cannula is sufficiently secured by the firmly tied sutures, which partially embed into the outer wall of the soft tubing. Furthermore, without the rigid curves present in previously described cannulas it is possible to rotate the tubing after it is placed subcutaneously, thereby facilitating its insertion into the vein. Since exposition of the jugular vein and suture after insertion of the cannula are not complex tasks, the simplifications described in both inserting and exteriorizing the tubing render the surgical procedure sufficiently undemanding for untrained personnel to perform.

Use of a clip to regulate flow during frequent sampling is far more convenient and effective than use of plugs and is without the expense, unavailability, and dead volume problems of taps. These advantages, significant for pharmacokinetic work, could not have been realized with polyethylene tubing, which does not recover clamping.

Failure of previous cannulas to allow repeated sampling has arisen from three main areas:

1. Tendency for removal by the subject or operator. Attempted removal of the silicone tubing by the subject has rarely been observed. Less discomfort was a probable consequence of its greater softness and flexibility and also of the direct route of exteriorization. The elasticity of the tubing also allowed a certain degree of tension to be applied to the cannula without detrimental effects.

2. Obstruction of the orifice by intravascular structures (*viz.*, vein wall) under negative pressure. This has not been a problem since use of the bevel design described. With the ability to rotate the tubing, even this more complex bevel could be inserted into the vein without difficulty.

3. Blockage within the cannula. The use of a one-piece cannula has eliminated connecting sites with their tendency to promote blockage and resist flow of viscous fluids. Furthermore, permanent surfaces in contact with blood were solely of silicone polymer, which has been reported to be free of the tendency to effect thrombus formation. Blockage has not been a problem with frequently used cannulas nor with properly maintained, longer term cannulas.

The sampling techniques described have been combined with urine collection procedures (5) and the technique of Light *et al.* (6) for bile collection in the unanesthetized rat. These combinations have enabled the establishment of statistically well-defined pharmacokinetic models for certain antifolate drugs, including a close investigation into the contribution made by enterohepatic cycling.

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