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LITERATURE SURVEY

Methods for Vascular Access and Collection of Body Fluids from the Laboratory Rat

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Keyphrases □ Vascular access—in the rat, literature survey □ Body fluids—collection in the rat, literature survey □ Rat—vascular access, collection of body fluids, literature survey

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> When I first tried animal experimentation for the purpose of discovering the motion and functions of the heart by actual inspection and not by other people's books, I found it so truly difficult that I almost believed with Fracastorius, that the motion of the heart was to be understood by God alone.
>
> —William Harvey, M.D., 1628

INTRODUCTION

The rat has been a useful laboratory animal in virtually every area of biological and medical research since its introduction to the laboratory in adrenalectomy studies in 1856 (1). The history of the development of the rat as a laboratory animal is a fascinating story that has been well presented elsewhere (2, 3). Briggs and Oehme (4) recently provided a very useful summary of the types of pharmacokinetic and toxicological studies commonly conducted to evaluate the safety and efficacy of xenobiotics in the rat.

Pharmaceutical, pharmacological, and toxicological studies of xenobiotics in the rat often require collection of urine, feces, blood, bile, and various other body fluids. A search of the literature was conducted to locate methods for collection of these various fluids and excreta from the rat. The goal of this literature survey is to provide infor-

[1] Employed at the Wellcome Research Laboratories, Research Triangle Park, North Carolina, while part of the background literature search was completed.

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Table I—Summary of Normal Parameter Values in the Adult Laboratory Rat [a]

Parameter	Normal Range or Mean
Life span	2.5–3 years
Surface area	0.03–0.06 cm^2
Water consumption	80–110 ml/kg/day
Food consumption	100 g/kg/day
Body temperature	37.5°
Gestation	21–23 days
Litter size	8–14 pups
Birth weight	5–6 g
Heart rate	330–480 beats/min
Blood pressure	
Systolic	88–184 mm Hg
Diastolic	58–145 mm Hg
Stroke volume	1.3–2.0 ml/beat
Cardiac output	10–80 ml/min (mean = 50 ml/min)
Plasma volume	36.3–45.3 ml/kg (mean = 40.4 ml/kg)
Blood volume	57.5–69.9 ml/kg (mean = 64.1 ml/kg)
RBC volume [b]	3.63 ± 0.10 ml/100g (mean ± *SEM*)
Respiration rate	66–114 per min (mean = 85.5 per min)
Tidal wave	0.60–1.25 ml (mean = 0.86 ml)
Plasma pH	7.4
Urine pH	7.3–8.5
Albumin Pools [c]	
Extravascular	365 mg/100 g body weight
Intravascular	127 mg/100 g body weight

[a] Values abstracted from Baker *et al.* (20) unless otherwise indicated. [b] Bruckner-Kardoss and Wostmann (53). [c] Sellers *et al.* (56b).

mation that will inform the experimental pharmaceutical scientist regarding available methodologies and aid the experimentalist in choosing the best method for the intended application. In addition, it is hoped that this publication will prove to be a valuable tool for introducing research trainees to the wealth of biological samples and data that can be gathered from the live animal using existing experimental methods.

Periodically, various authors have provided a review of methods in use for laboratory investigation of the rat (5–21). Such updates must be done, since scientists working with rats are constantly developing new apparatus and better techniques with special application to laboratory problems. This ongoing methodology research supports efforts to minimize animal (and scientist) stress and thereby ensure a humane research environment.

Kraus (21) has written the most recently published review of laboratory research methods for the rat. This review (including 672 references) provides a comprehensive introduction to all types of methodology including animal handling, body fluid collection, xenobiotic administration, anesthesia, and euthanasia methods. Excellent reviews of more basic information such as handling methods, laboratory personnel safety, injection procedures (volume, pH, and methods), blood collection techniques, methods for general anesthesia, and techniques for common surgical procedures have been provided by Waynforth (12) and Singh (22). A very useful introduction to basic surgical skills, from proper procedures for donning sterile gloves and gown to stages of anesthesia, has been assembled by Singh (23). The importance of aseptic techniques in rat surgery has been emphasized by Popp and Brennan (24). The reviews of previous authors were used as a gateway to the extensive literature in this area.

This review attempts to gather, for summary and comparative purposes, the literature on methods of vascular access and collection of body fluids for the rat. Papers were selected from the published literature through January 1982. Although extensive literature is summarized, some citations inadvertently may have been omitted. Nonetheless, most available methods are summarized. Since many scientists from diverse fields of study have developed methodologies for the rat, it often is difficult to attribute the origin of a particular method to a specific scientist or laboratory. Therefore, originators of most methods are not identified.

It is widely assumed that the rat is devoid of superficial blood vessels accessible to percutaneous puncture. Therefore, the novice may assume that technically complex surgical methods must be mastered to collect the blood and other biological fluids essential to whole-animal studies. Unfortunately, these assumptions have fostered, by default, a trend toward studies on isolated cells and subcellular fractions when whole-animal studies would be more appropriate. In reality, there are a multitude of surgical and percutaneous methods for acute and chronic vascular access, as well as methods for collection of other biological fluids and excreta.

In this review, summaries of these procedures are outlined in a tabular presentation. In conjunction with the tables, considerations of fundamental methodology are discussed.

GENERAL CONSIDERATIONS IN RAT EXPERIMENTATION

The purpose of this section is to guide the reader to general, but essential, information on the use of rats in laboratory science. Scher (25) has written a concise summary of some factors to be considered in designing any laboratory animal experiment. These factors include selection of the appropriate animal species, writing an experimental protocol, and procedures for data analysis.

Anatomy—Several books illustrating the anatomy of the rat are available (6, 26–31). The most recent of these anatomy texts (31) is very useful in the laboratory as it is a photographic atlas in color. A stereotaxic atlas of the rat brain has been assembled (32). The scientist seeking detailed anatomical information is referred to the atlas compiled by Greene (26). Anatomical structures in this review are referred to using the nomenclature of Greene (26).

The anatomy of specific parts of the vasculature has been summarized by several authors. Halpern (33) provided a well-illustrated, detailed manuscript on coronary circulation in the rat, while Chambers and Zweifach (34) studied the capillary beds of the visceral tissues. Several workers have reported on the collection of blood from the so-called orbital plexus (35–37) or orbital sinus (38–44). The anatomy of the orbital vasculature in the rat has been detailed by Timm (45), who demonstrated the presence of an orbital venous plexus (with no anatomical venous sinus) in the eye. Of the various veins, the caudal–dorsal anastomotic vein is defined as the major anatomical site accessible to venipuncture.

The rat differs anatomically from humans in several respects (46). Cardiac (33) and omental circulations (34) differ from their human counterparts. The rat has a relatively smaller thyroid, brain, and lungs than the human (47). The rat has no gallbladder (26), and is capable of liver regeneration following subtotal hepatectomy (48). In addition, Nairn (49) has demonstrated the presence of a di-

rect transdiaphragmatic pathway for fluid transfer between the peritoneal and pleural cavities.

Physiology—Average values in the rat for organ weights (50), hematological parameters (50–52), blood volume (53), several serum enzymes (54), physiological parameters (20), breeding characteristics (55), and interspecies comparative hematology (56a) have been enumerated and are briefly summarized in Table I. The pathophysiology of aging rats has been recently reviewed (57). A wealth of biological data on the rat were compiled by Donaldson (58). Blood flow to various organs of the rat has been detailed (59, 60). Such data, along with organ–blood flow data in humans, have been used in animal-to-human scale-up methods for physiological pharmacokinetic models (61–64).

The usual nutritional requirements of the rat (65–67) are met by a variety of commercial feeds. The rat has proven particularly useful in nutrition research because it is susceptible to dietary induction of various vitamin and amino acid deficiencies. Newberne (68) discussed the possible influence of dietary factors on pharmacological experiments.

The particular susceptibility of the rat to chronic respiratory disease has been well reviewed (69). Although contraction of human disease from laboratory rats seems to be rare, scientists should be aware of rat-borne infectious zoonotic diseases, reviewed elsewhere (70). The allergic respiratory disease commonly contracted by scientists working with various laboratory animals, including the rat, was detailed by Lutsky and Toshner (71).

Housing—Cages and rooms for housing rats must meet two essential requirements. First, the housing must facilitate maintenance of the health and comfort of the animals. Second, the cages must satisfy the requirements of the specific experiment, *e.g.*, metabolism, restraining, or exercise cages. Current space recommendations for rats are cages of 17.8 cm in height for rats of all weights, with floor surface areas of 110, 148, 187, and 258 cm^2 for rats weighing <100, 100–200, 201–300, and >300 g, respectively (72). The following equation (73) has been used in estimating the floor surface area (A, cm^2) needed for one rat of a given weight (w, g):

$$A = 0.7w + 6\sqrt{w} \qquad \text{(Eq. 1)}$$

A well-ventilated animal room should be maintained at a constant temperature within 20–25°, with relative humidity kept at 50–65% (74). Bedding for rat cages must be selected carefully since the use of softwood chips (*i.e.*, red cedar, white pine, ponderosa pine) can result in the induction of microsomal enzyme activity (75). More specific information on cages and animal rooms can be found elsewhere (6, 72, 74). Metabolism cages are discussed later in this review.

Handling—The rat can be lifted easily by the root of the tail. Since rats seem more at ease when their paws are in contact with something, one easy way to move a rat is by holding the root of the tail and resting the rat's body on the mover's hip (Fig. 1). Rats may also be picked up by slipping a hand over the shoulders to restrain its head between the thumb and first finger (Fig. 2). This procedure can be combined with restraint of a hind limb using the fifth finger of the free hand to provide a hold for intraperitoneal injection (Fig. 3). Pregnant rats should not be lifted by the tail, but either with the support of a hand under the body or with a hand over the shoulders, as described above. Gloves may be useful for momentarily restraining a rat by hand (see *Momentary Restraint*).

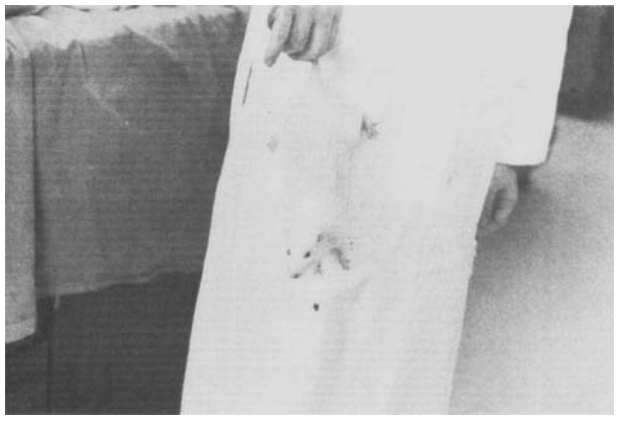

Figure 1—*Illustration of an easy method for moving a rat, without undue animal stress, around the laboratory. The rat usually remains calm while resting against the coat.*

Anesthesia—Surgical anesthesia in rats has been discussed in detail previously (21, 23, 76–82). In addition, laboratory animal scientists are advised to read an informative review of the health risks associated with chronic exposure to various gaseous anesthetics (83a).

In many laboratories, the rat is anesthetized with ether (peroxide-free, anesthetic grade) by the introduction into a large dessicator jar containing an elevated wire-mesh floor and ether, followed by maintenance of anesthesia using a 50-ml beaker containing an ether-moistened surgical sponge placed over the nose of the rat (Fig. 4). More elaborate anesthesia chambers have been described (83b). Ether anesthesia is generally administered for <5 min when brief procedures are performed immediately on the rat. If ether is administered for longer periods of time, the rat is given at least 1 day to recover to avoid experimentation during a time period in which plasma volume may be decreased due to the ether anesthesia (84). Extended (>5 min) exposure to ether vapors will irritate the eyes of the rat. This irritation is indicated in the postoperative conscious rat by increased blinking and grooming of the eyes and head with the forepaws. Topical preoperative application of one drop of an artificial tear preparation[2] to each eye prevents irritation[3].

Although ether is a widely used, reliable anesthetic, maintaining the proper depth of anesthesia requires constant, careful supervision of the rat to avoid respiratory arrest. The depth of ether anesthesia can be difficult to judge, but some useful guidelines have been presented (12). Although ether has been administered by intraperitoneal injection (85), this may cause peritoneal inflammation and adhesions, and therefore is a relatively uncommon route of administration. Several masks and other apparatus for inhalation-anesthetic delivery have been described (86–92). Of these, that described by Stark and Lipman (91) is inexpensive and can be constructed easily from a 135-ml (4.5-oz) plastic specimen jar, a 23-gauge butterfly infusion set, and a 1-ml glass syringe. Such an apparatus is useful

[2] Tears Naturale; Alcon Laboratories, Fort Worth, Tex. Tearisol; Smith, Miller, and Patch, San German, P.R.

[3] Personal communication, David Soda and Gerhard Levy, Department of Pharmaceutics, SUNY at Buffalo, Amherst, N.Y.

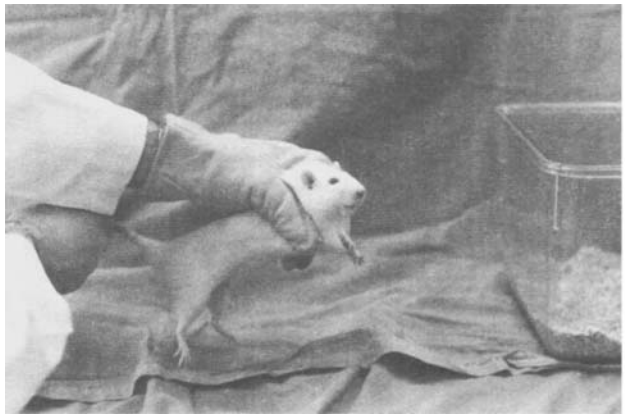

Figure 2—*An over-the-shoulder method for lifting a rat while restricting head movements by crossing the forelimbs under the chin. The leather glove is used in preventing biting by rats not conditioned to this grip.*

for administering halothane[4], ether, or methoxyflurane[5] anesthesia.

Pentobarbital[6] (30–40 mg/kg) administered intraperitoneally is a commonly used anesthetic for the rat (77, 93). Valenstein (94) has provided a table of intraperitoneal injection volumes for anesthesia *via* pentobarbital in conjunction with chloral hydrate. Pentobarbital (21 mg/kg ip) has been advantageously used in conjunction with intramuscular ketamine[7] (60 mg/kg) to induce surgical anesthesia (95). Ketamine has also been used alone (96) for acute drug-induced immobilization to facilitate cardiac puncture (22 mg/kg) and for surgical anesthesia (44 mg/kg), but its use is difficult in view of the highly variable depth of anesthesia induced by a given dose (97). Chloral hydrate (300 mg/kg ip) is still used in some laboratories, despite an ~50% incidence of adynamic ileus after a chloral hydrate administration of 400 mg/kg ip (98). A combination product[8] containing fentanyl (0.4 mg/ml) and droperidol (20 mg/ml) is available for intramuscular injection. Doses of 0.1–0.5 ml/kg have been used to induce surgical anesthesia (99–101). Alfaxalone–alfadolone combination has been used as an intravenous surgical anesthetic (102). Methohexital[9] has proven useful as an intravenous anesthetic for periodic endotracheal intubation of the rat (103).

Figure 3—*Use of the over-the-shoulder grip with the left hand to restrain the head and upper body (note the crossed forelimbs), while restraining via the fifth finger of the right hand the hind limb on the side of the peritoneum to be injected intraperitoneally. The thumb, first, and second finger of the right hand are free to administer the injection (via a syringe of appropriate volume and usually a 23-gauge, 1-in. needle).*

[4] Fluothane; Ayerst Laboratories, New York, N.Y.
[5] Penthrane; Abbott Laboratories, North Chicago, Ill.
[6] Nembutal sodium; Abbott Laboratories, North Chicago, Ill.
[7] Ketalar; Parke-Davis, Morris Plains, N.J. Ketaject; Bristol Laboratories, Syracuse, N.Y.
[8] Innovar-Vet; Pittman-Moore, Indianapolis, Ind.

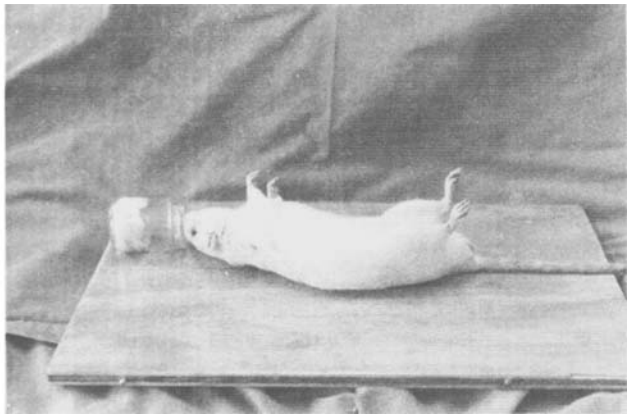

Figure 4—*The rat reclining on a home-built surgical board (41-cm long × 27-cm wide × 1-cm thick plywood painted with polyurethane varnish) while breathing some ether vaporized from an ether-moistened surgical sponge packed in the bottom of a 50-ml beaker. This procedure should be restricted to a fume hood to reduce the flammability risk of ether use.*

Parker and Adams (104) have reviewed the effects of six common anesthetics (α-chloralose, droperidol, fentanyl, halothane, ketamine, and pentobarbital) on cardiovascular function in various laboratory animals. Researchers studying the aspects of cardiovascular function in the rat should find this review helpful in choosing an anesthetic or chemical restraining agent.

Scientists placing animals under surgical anesthesia are advised to keep a resuscitation device available. Two readily constructed resuscitators have been described (105, 106). For rats with an ether overdose, some experimentalists prefer to employ the simple device described by Rassaert (106) as this can be used both to aspirate mucus from the respiratory tract and force air into the lungs. However, the simple two-bulb resuscitator described by Ingall and Hasenpusch (105) has been used successfully. In addition to these resuscitators, Shuer *et al.* (107) devised a simple mask for the artificial respiration of rats *via* a positive-pressure respirator. This procedure avoids the problematic procedures of tracheotomy (78, 108) and endotracheal intubation (103, 109, 110).

Lineberry (111) has recently provided a highly informative review of animal models used in pain research. However, we are not aware of any detailed information on either postoperative assessment or therapeutic management of pain in the laboratory rat. Brief discussions of the general aspects of postoperative care are available (23, 112). Since the majority of scientists working with laboratory rats do not have formal training in veterinary medicine, a detailed review of all aspects of postoperative care would be a valuable contribution to the literature.

Xenobiotic Administration Procedures—Procedures are described elsewhere for administration of substances to the rat *via* the oral, percutaneous, intravenous, intra-

[9] Brevital sodium; Eli Lilly and Co., Indianapolis, Ind.

Table II—Methods of Euthanasia for the Rat

Physical Methods	Chemical Methods
Cervical dislocation	Barbiturates
Decapitation[a]	Carbon dioxide gas
Decompression (oxygen lack)	Carbon monoxide gas
Electrocution	Chloral hydrate
Hemorrhage (aortic transection)[b]	Chloroform
Pneumothorax[b]	*d*-Tubocurarine chloride
Stunning (skull fracture)	Ether
	Gallamine triethiodide
	Hydrogen cyanide gas
	Magnesium sulfate
	Nitrogen gas
	Potassium cyanide
	Strychnine

[a] Rodent guillotines supplied by EDCO Scientific, Inc., Chapel Hill, N.C. and Harvard Apparatus Company, Inc., South Natick, Mass. [b] Method only for use on anesthetized rats.

muscular, intraperitoneal, subcutaneous, intra-arterial, and intracardiac routes (6, 12, 21, 113–115). Shani *et al.* (116) designed a device for the oral administration of gelatin capsule doses to rats. The use of subcutaneously implanted devices (117) and subcutaneous infusions (118) for long-term xenobiotic administration, first applied to rats by Rose and Nelson (117), has been advanced by the commercial availability of uniform osmotic pumps[10]. Novin *et al.* (119) devised an airtight injection system which minimized the need for handling unrestrained rats during injection into an indwelling cannula. Various devices have been constructed to enable intermittent intravenous self-administration of xenobiotics by rats (15, 120–122). Application of this methodology to drug addiction studies in rats has been discussed by Weeks (123).

Three invaluable compendia of drug dosages for the rat are available (124, 125a,b).

Euthanasia Methods—Euthanasia methods are procedures for the painless death of laboratory animals. Useful euthanasia procedures for the rat can be classified as physical methods, *i.e.*, severe body perturbation without administration of a toxic xenobiotic, and chemical methods, *i.e.*, administration of toxic xenobiotics, usually intraperitoneally or by inhalation. The methods outlined in Table II are discussed elsewhere in more detail (126, 127). It is difficult to evaluate the relative humaneness of these different methods. The relative humaneness of gallamine-induced asphyxia and decapitation has been studied in rats by electroencephalographic monitoring (128). Histological changes in the major organs associated with various euthanasia methods (decapitation, carbon dioxide, methoxyflurane, and pentobarbital) and the sympathoadrenal discharge induced in the rat by decapitation have also been documented (129, 130).

If tissues are to be obtained from rats for posteuthanasia study, the scientist is advised to use care in selecting the euthanasia method. For example, *in vitro* drug metabolism studies using various subcellular fractions of rat hepatic homogenates commonly are prepared from liver tissue obtained from rats sacrificed by decapitation (without prior anesthesia) or carbon dioxide asphyxiation. [A chamber for carbon dioxide euthanasia can be readily constructed from a large can (131) or plexiglass[11] (Fig. 5).] Decapitation and carbon dioxide euthanasia avoid the known alterations of microsomal enzyme activity associated with the use of ether (132–136) and other inhalation anesthetics (133, 134). This example illustrates that scientists studying various events in anesthetized rats or in tissues from sacrificed rats must verify the lack of anesthetic or euthanasia-method effect on the experimental events observed.

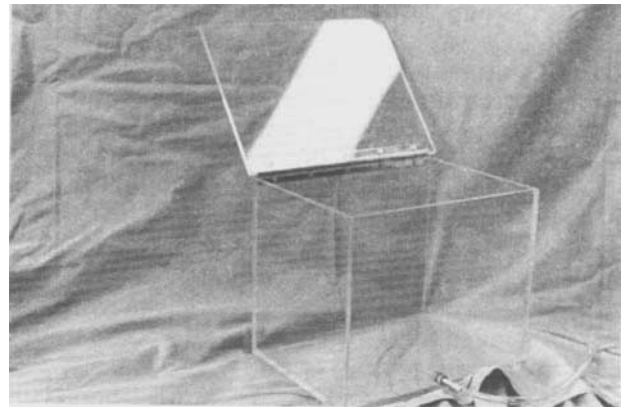

Figure 5—*Plexiglass chamber (0.6-cm thick plexiglass) used for carbon dioxide euthanasia of rats. The base of the chamber is 26-cm long and 19-cm wide, while the chamber is 21-cm high. The top serves as a hinged door and a gas inlet is placed near the bottom of the chamber. The top need not have a gasket seal.*

VASCULAR ACCESS

Methodology Overview—*Cannula Materials*—Vascular cannulas most commonly are constructed of polyethylene[12] or silicone rubber[13] (137, 138). Polypropylene, polyurethane[14], Tygon[15], polytef[16], and Micro-Line[17] tubing have had less use. All of these materials are somewhat flexible, can be molded into desired shapes (139, 140), and have various degrees of physiological inertness. These synthetic materials comprise the main substance of the cannula and are often supplemented by various other materials. Metal connectors are used to link different pieces of tubing or provide a reinforced site to facilitate ligature fixation of the cannula in the vessel (141). An alternative means of joining different pieces of polyethylene tubing consists of welding with a stream of hot air (13, 139). A short piece of flexible, heat-shrinkable polyolefin tubing[18] also can be used to provide reinforcement of the junctions between different tubing pieces or the cannula plug and tubing (13). Although a metal connector is useful for connecting two different types of tubing, such as polyethylene and silicone rubber, it has the disadvantage of promoting thrombotic occlusion of the cannula by both decreasing the cannula lumen diameter (thereby increasing turbulent blood flow distal to the metal connector) and providing a metallic intravascular thrombogenic focus. Therefore, the use of either heat welding or heat-shrinkable tubing is preferred whenever two pieces of the same type of heat-insensitive tubing (polyethylene, polypropylene, Micro-Line) are to be joined.

Methods of Inserting a Cannula in a Blood Vessel—

[10] Alza Corporation, Palo Alto, Calif.
[11] Plexiglass; Rohm and Haas Co., Philadelphia, Pa.
[12] Intramedic polyethylene tubing; Clay Adams, Parsippany, N.J.
[13] Silastic; Dow Corning Corp., Midland, Mich.
[14] Micro-Renathane, Braintree Scientific, Inc., Braintree, Mass.
[15] Tygon; Norton Co., Plastics and Synthetics Division, Akron, Ohio.
[16] Teflon; E. I. DuPont de Nemours and Co., Inc., Wilmington, Del.
[17] Micro-Line; Thermoplastic Scientific, Inc., Warren, N.J.
[18] FPS-Voltrex; Newark Electronics, Chicago, Ill.

Methods of cannula insertion in a blood vessel can be classified three ways: (*a*) two-step puncture and insertion, (*b*) cannula through a needle, and (*c*) cannula attached to a needle. The latter methods are one-step procedures, *i.e.*, puncture of the blood vessel and cannula access to the blood are both accomplished in a single step.

The most commonly used procedures for both arterial and venous cannula insertions are two-step puncture and insertion methods. Ophthalmic Castroviejo iris scissors, microdissecting scissors, or various needles are used to puncture the blood vessel. A needle (25 gauge for aorta, 23 gauge for jugular vein) with a 90° bend ~6 mm from the needle tip is particularly useful. It is inexpensive and readily available, and the tip can be used bevel up to puncture the blood vessel wall with a minimal risk of puncturing the opposite wall (13)[19]. Use of a needle for vessel puncture is often preferable to use of scissors since a needle produces a puncture hole of defined, reproducible size, which cannot be guaranteed using scissors. Using a needle with an outer diameter approximately equal to the outer diameter of the cannula tubing minimizes hemorrhagic leakage from the puncture site. Insertion of the cannula into the puncture hole can be facilitated by gently lifting the lip of the hole with a pair of fine forceps and inserting the cannula into the blood vessel with a slow, gentle rotation of the tip.

Cannula-through-a-needle methods have the advantage of combining the blood vessel puncture and cannula insertion procedures into one step. Physicians and other scientists with clinical training are usually familiar with the various devices marketed to facilitate cannula placement through a needle inserted in a blood vessel.

One device used to cannulate mesenteric veins of the rat was constructed from an ~2.5-cm segment of hollow steel tubing soldered close to the tip of a 1.5-in., 22-gauge needle (the distal third of which was bent at a right angle) (142, 143). Venipuncture with the tip of the 22-gauge needle enabled the cannula to be directed into the vein *via* the steel tubing attached below the needle. Hollow steel tubing[20] of various diameters can be used to accommodate different cannulas. The device can be stabilized to ensure a clean venipuncture by attaching the hub of the 22-gauge needle to a 1-ml syringe. The principle of this device was used in constructing a similar device which consisted of a single needle with the tip bent at a right angle to the remainder of the shaft and a semicircular tunnel in the distal portion (144). Vascular puncture is followed by insertion of the cannula in the lumen of the blood vessel *via* the semicircular tunnel. This has the advantage of facilitating easy removal of the device from the cannula tubing. The previously described device with the fully circular cannulating needle must be passed over the total length of tubing for removal. This creates the inconvenience of having to remove a fluid-containing syringe from the cannula connection to remove the device. Note that in the special case of the caudal (lateral tail) veins, a cannula may be inserted *via* a straight needle used for vessel puncture (145–148).

Cannula-attached-to-needle methods have been useful one-step procedures when placement of a needle within the blood vessel lumen was consistent with the acute nature of the experiment (149–151). The cannula-attached-to-needle method is useful for percutaneous puncture of the caudal artery (152, 153), and external jugular vein (154–156); the former effort may be aided by depilation with sodium sulfide (157). Needle-tipped cannulas have also had limited utility for chronic intermittent injections into the sagittal and transverse sinuses (158) and for chronic blood pressure–multiple blood sampling *via* the caudal artery (159). Due to the increased chance of thrombotic complications associated with the metallic needle tip in the vessel, the cannula-attached-to-needle methods are less preferred than insertion of a conventional metal-free cannula.

One novel cannula-attached-to-needle method warrants attention when the external jugular vein must be cannulated. Harms and Ojeda (160) constructed an implantation needle (20-gauge tip) to which a silicone rubber cannula is attached. The implantation needle (with attached cannula) is passed in and out of the same side of the vein. The implantation needle is disconnected from the cannula tubing, and the cannula tubing is then pulled back into the lumen for advancement to the heart. This method has the advantages of being a one-step procedure, eliminating the need to dissect the vein free from surrounding tissues, and making application of ligatures around the vein unnecessary. This method has proven a useful vascular access for pharmacokinetic studies in rats (161–163).

We are unaware of any application to the rat of the cannula-over-the-needle method used commonly in humans (164).

Methods of Fixing a Cannula in a Blood Vessel—There are four basic methods for fixing the intravascular portion of the cannula within the lumen of the blood vessel: (*a*) ligature to surrounding structures (141, 165, best illustrated in 166), (*b*) ligature around the cannula and through an occluded distal portion of the cannulated blood vessel (167, 168), (*c*) application of cyanoacrylate-type glues for the blood vessel–cannula junction (150, 169–171)[19], and (*d*) fashioning a circular shock-absorbing loop in the cannula tubing between the sites of cannula insertion and ligation to a nearby structure (165)[19]. Use of one or more of these methods should maintain the cannula within the lumen and minimize cannula-mediated abrasion of the intravascular structures. Robinson *et al.* (172) constructed a heating element device for producing a small bead on the outside of polyethylene tubing without altering the lumen. This bead served as an anchor for fixing the cannula in place.

Methods of Exteriorizing the Cannula—Intravascular and other indwelling cannulas (*e.g.*, urinary bladder, bile duct) are commonly exteriorized by subcutaneous tunneling from the vascular incision site to the back of the neck for emergence at a point midway between the clavicles or ears (13, 15, 141, 160, 165, 166, 170, 173–183)[19]. To further secure the exterior portion, the cannula may be passed through a plastic cap or steel button which is sutured to the skin of the neck (166, 177, 180). A modification of exteriorization at the neck is to subcutaneously tunnel the cannula for passage through a cranial pedestal (168, 171, 186–188).

A cannula may also be exteriorized *via* subcutaneous tunneling from the incision site to the root of the tail, where

[19] Methodology notes provided by J. R. Weeks, The Upjohn Company, Kalamazoo, Mich.

[20] Small Parts Inc., Miami, Fla.

the cannula is exteriorized and covered by some type of protective shielding (146, 148, 189–191). Cannulas have also been exteriorized *via* subcutaneous tunneling from the incision site to the side of a rat maintained in a restraining cage (192) or body harness (176).

Methods to Aid in Maintaining Cannula Patency—Various procedures have been used to minimize the incidence of thrombotic occlusion of the intravascular cannulas and to remove existing thrombotic obstructions to prolong the patent lifetime of cannulas. These procedures can be classified into three groups: (*a*) fluid-flushing procedures, (*b*) designs for the intravascular portion of cannulas, and (*c*) procedures for removal of thrombotic obstructions.

Fluid-flushing procedures consist of selection of a solution for instillation in the cannula when it is not in use and a schedule for routine cannula care, *i.e.*, withdrawal of the instilled solution, flushing the cannula to clear it of blood, and refilling the cannula with fresh solution. Six different solutions have been used to fill cannulas: heparin, heparin–povidone mixture, heparin–streptokinase solution, heparin–dextrose solution, isotonic saline, and 50% dextrose in water.

Heparin has been instilled in cannulas in concentrations of 10–1000 U/ml, usually dissolved in isotonic saline (149, 151, 159, 166, 173, 175, 181, 185, 191, 193–204). A localized anticoagulant effect can be obtained with 20 U/ml of heparin. A mixture of heparin–povidone [1 g of povidone plus 2 ml of heparin solution (500 U/ml)] has been used because of its increased viscosity to prevent diffusion of blood into the unused cannula (186). Similar mixtures of heparin–povidone were used without problem by other investigators (168, 187, 205). However, Allsop and Burke (201) found use of this heparin–povidone mixture no better than the more easily handled heparinized (10 U/ml) isotonic saline. Heparin (1000 U/ml) plus streptokinase (200 U/ml) solution was used to maintain patent aortic cannulas for blood pressure monitoring (206). This combination of an anticoagulant and plasminogen-activating agent may be more effective in maintaining patent cannulas than either agent alone. Solutions of heparin (≤500 U/ml) and dextrose (≤10%) have been used in an effort to increase the viscosity beyond that obtained with heparin alone (207, 208). For experiments in which no anticoagulant can be administered to the rat or added to the flushing solution, the two alternatives that have been used are isotonic saline (7, 13, 141, 160, 165, 170, 174, 177, 184, 209–218)[19] and 50% dextrose in water[21].

Schedules for routine cannula care have not been comparatively studied and standardized. In general, increased frequency of cannula care (withdrawal of the instilled solution, flushing the cannula to clear it of blood, and refilling the cannula with fresh solution) should ensure increased long-term cannula patency. Allsop and Burke (201) and Brown and Breckenridge (168) have provided detailed descriptions of a cannula-care schedule consisting of the following:

1. Initial aspiration of fluid from the cannula. This fluid is discarded since reinjection carries the risk of introducing aspirated thrombi.
2. Injection flushing of the cannula with a volume of solution slightly larger than the volume of the cannula as calculated by:

$$V = \pi r^2 l \qquad \text{(Eq. 2)}$$

where V is the volume, r is the radius, and l is the length of the cannula.
3. Sealing the exteriorized end of the cannula with a segment of steel stylet (13) or an epoxy-filled blunt-end needle (201).

These investigators recommended that cannula care be done once a day for the patent lifetime of the cannula. Such daily cannula servicing is useful for other types of aortic and jugular cannulas (204, 207). Based on these proven procedures, cannulas intended for chronic use should be serviced daily when not in use.

Designs for the intravascular portion of cannulas have been proposed as means of minimizing thrombotic occlusion. Simple beveled and nonbeveled cannula tips, a double-mitered cannula tip, a sealed cannula tip with laterally placed holes, and an open cannula tip with laterally placed holes have been designed. Commonly, the open end of the vascular cannula is fashioned with a 90° angle to the tubing wall (no bevel) or a 30–60° angle of the bevel.

While most investigators agree that a beveled end is more easily inserted in the blood vessel, it has been reported (*via* anecdote) that the beveled end can be occluded more readily by a flap of thrombotic material deposited over the bevel and also may rest more readily against the blood vessel wall, thereby inhibiting blood sampling (13). Scientists having difficulty maintaining cannula patency with a beveled or nonbeveled tip may consider using an alternative type of cannula tip. Nicolaidis *et al.* (187) designed a double-mitered silicone rubber cannula tip which is reported to effectively minimize local coagulation. Steffens (186) cut two 0.75-mm holes in the opposite lateral walls 0.75 mm from the tip of a silicone-rubber cannula with an open-lumen tip. Stripling (171) plugged the intravascular end of a silicone rubber cannula with hardened silicone rubber adhesive and made holes near this plugged tip by puncturing through both cannula walls six times with a 25-gauge needle. This type of cannula tip is said to comprise a one-way valve which allows drug infusion, while preventing entry of blood into the cannula (219).

Procedures for removal of thrombotic cannula obstructions can be classified in three categories: (*a*) aspiration of the obstruction, (*b*) forceful injection of the obstruction into the circulation of the rat, and (*c*) use of an obturator that fits in the cannula lumen to mobilize the obstruction. Before using any of these procedures for clearing an obstruction in a venous cannula, one can verify that the cannula is still inside the vein by injecting rapidly 3–6 mg (0.05–0.10 ml of a 60-mg/ml solution) of pentobarbital sodium (13), 1 mg (0.10 ml of a 10-mg/ml solution) of methohexital sodium[19], or thiamylal sodium[22] (15). If the cannula is out of the vein, the rat may struggle or vocalize, or the fluid may accumulate in a subcutaneous pocket with only clear fluid (no blood) aspirated (13). If the barbiturate injection is indeed intravenous, the eyes of the rat will squint closed almost immediately, the rat will lose its righting reflex, and the rat will become ataxic

[21] Personal communication, J. E. Brown, Division of Clinical Pharmacology, Duke University Medical Center, Durham, N.C.

[22] Surital; Parke-Davis, Morris Plains, N.J.

within ~10–15 sec. The rat normally recovers completely within ~5–10 min. When this procedure shows that the cannula is indeed in the vein but blood withdrawal is still difficult, flexing the head and neck of the rat or lifting it by the root of the tail so that only the forelegs touch the cage floor may aid in moving the cannula tip from against the blood vessel wall, thereby facilitating blood withdrawal. If these simple maneuvers do not work, one of the three aforementioned procedures can be tried.

Since thrombotic material can accumulate on the intravascular end of arterial and venous cannulas, it is suggested that blood be withdrawn through the cannula prior to injection to aspirate any small thrombotic obstruction (168, 201). The blood initially withdrawn should not be reinjected since it may contain small thrombi. Forceful injection of 0.10–0.15 ml of isotonic saline *via* a 0.25-ml tuberculin syringe will often break up and clear a thrombotic obstruction in a jugular venous or abdominal aortic polyethylene cannula (13, 165)[19]. The small emboli mobilized as a result of this procedure will usually be trapped in the vasculature of the lungs and legs when ejected from the external jugular vein and abdominal aorta, respectively (13)[19]. Lastly, various homemade wire or nylon obturators can be slid down the lumen of some cannulas to mobilize the obstruction. The utility of thin wire is limited by its relative inflexibility and high potential for cannula puncture. In view of this, use of wire obturators often has been limited to relatively straight cannulas implanted in the caudal vein or artery. Brown and Breckenridge (168) described a very useful and flexible obturator made from 25-pound test monofilament nylon fishing line fashioned with a ball on the end made by holding it close to a hot soldering iron. This seems relatively unlikely to puncture the cannula. Indeed, Brown and Breckenridge (168) used this obturator to clear obstructions from a curved silicone-rubber cannula implanted in the external jugular vein.

Swivel Joints—In chronic rat experiments in which xenobiotics are injected intermittently or continuously *via* the intravenous or intraventricular route, the duration of the experiment is usually limited by cannula damage (twisting and kinking) due to the physical activity of the rat. For reasons discussed elsewhere in this review, such experiments can not be executed during sustained anesthesia or restraint. Therefore, the swivel joint was introduced to prevent such motion-related cannula damage and enable chronic xenobiotic administration and blood sampling *via* indwelling cannulas in the unanesthetized, unrestrained rat. Jacobs (220) appears to be the first investigator to have devised a swivel joint for administration of a constant-rate intravenous infusion to the dog for up to 3 weeks. In principle, this and other swivel joints are fluid-tight, flow-through joints placed into the tubing near the point of emergence from the rat (back of the head or neck) so that the tubing proximal to the rat rotates within the swivel joint as the rat moves, thereby preventing cannula damage by twisting. Rhode *et al.* (221) inserted a circuit of flexible, rotatable tubing between the dog and the infusion bottle. Epstein and Teitelbaum (222) commented that such an arrangement will not work for the rat, a more active species.

Several home-built swivel joints for use with rats have been described in the literature (15, 174, 179, 186, 187, 222–233). The utility of these various devices is based on the comparative ease of construction, availability of components, cost, durability, size, force required to rotate the swivel, tendency to leak, and requirements for periodic cleaning and lubrication.

Several single-channel swivel joints are relatively inexpensive to construct with hand tools from widely available materials. These single-channel swivel joints function well (224, 225, 227, 229, 232). The single-channel swivel of Strubbe (227) was designed to minimize friction for improved utility with small-diameter tubings. While most of the single-channel swivel joints require at least one polytef component, that of Brown *et al.* (229) was constructed solely from parts of plastic syringes, disposable needles, and epoxy adhesive. Khavari (225) constructed a combined intracerebral cannula–swivel unit for use in the rat.

Double-channel swivel joints have been devised to enable either simultaneous infusion of two different fluids into the same rat, simultaneous infusion and blood pressure recording, or simultaneous infusion and blood sampling. Some double-channel swivel joints require special machining during construction (187), a motor to rotate the swivel (234), or apparent supernormal manual dexterity for assembly (233). Smith and Davis (15) described assembly of two single-channel swivel joints from commercially available parts. Blair *et al.* (231) have supplied details for construction of an inexpensive double-channel swivel joint. This construction does not require any machining of parts and uses readily available materials. Testing verified that there were no leaks between the two channels after 4 weeks of continuous use. Another easily constructed double-channel swivel joint has been described, but leak-test information was not provided (232).

Since available swivel joints can not accommodate the arterial blood back-pressure (~100 mm Hg) without leaking, Grantham *et al.* (235) designed a nonswivel spring system for use with a special cage in order to infuse drugs intra-arterially. Where the tubing emerges from the spring at the end distal to the rat, a long unencumbered loop of tubing hangs outside the cage. This absorbs the movements of the rat without kinking for ~1 day. With daily uncoiling of this tubing loop, continuous intra-arterial infusions have been done for 2 weeks.

Complications of Vascular Access—A limited amount of information is available regarding complications in the use of an indwelling vascular cannula or percutaneous vascular access in laboratory animals. Despite minimal gross indications of ocular damage, localized and reversible necrotizing inflammation of the Harderian gland develops following collection of a single blood sample from the retro-orbital venous plexus (236). The degree of thrombogenicity of some artificial (*i.e.*, nonphysiological) substances has been studied using various *ex vivo* models (237–239). Unfortunately, insufficient information is available to enable recommendation of one type of tubing over another based on inertness toward tissues.

In 22 animals (dog, cat, rabbit, and monkey) with an indwelling cannula, Hysell and Abrams (240) identified vegetative deposits on the cardiac valves, septic visceral infarcts of the kidney, spleen, and brain, and fatal hemorrhage due to a ruptured vein or artery. In all three types of cannula-related lesions, systemic bacterial infection

played an important role in the animals' deterioration. Inadequately sterilized cannulas, nonadherence to aseptic technique when injecting or withdrawing from the cannula, and infection *via* the point of exteriorization are the apparent means of bacterial access to the vasculature.

Meuleman *et al.* (241) inserted a polyethylene cannula into the abdominal aorta *via* the carotid artery to study cannula-associated platelet consumption in the rat. Platelet count and the half-life of ^{51}Cr-labeled platelets were both significantly decreased in cannulated rats compared with stressed control rats (cannula inserted, then immediately withdrawn); the degree of decrease was related to the length of the inserted cannula. Platelet turnover did not differ substantially between cannulated and control rats. These investigators have also reported aortic endothelium loss due to the presence of an indwelling cannula (241). Aortic lesions in rats having polyethylene cannulas implanted for 48 hr were also documented, and used to assess antiplatelet drug efficacy (242).

Some investigators using indwelling intravascular cannulas in the rat for various pharmacological studies have reported renal infarcts after cannulation of the carotid artery and abdominal aorta with polyethylene, polypropylene, or silicone rubber tubing (177, 201, 206). Frequency of renal infarction increased with time (177) and with injection flushing of the cannula to force thrombotic blockage into the circulation (201). Polyethylene-tipped cannulas present in the carotid artery for an average of 7.2 days were associated with renal infarcts in 11 rats (n = 15, infarction rate = 10.2% per catheter day), while silicone rubber-tipped cannulas inserted for an average of 11.5 days were associated with small, solitary renal infarcts in 10 rats (n = 25, infarction rate = 3.5% per catheter day) (201). Renal infarcts occurred in association with polyethylene-tipped cannulas even when no injection flushing was done (201). Since arterial cannulation of either the carotid artery or abdominal aorta is used commonly for serial blood sampling in pharmacokinetic studies in the rat, investigators must exercise appropriate caution (in accordance with the following five recommendations) to ensure that data is not collected from rats with impaired renal function. First, a decreased incidence of renal infarction was reported by Engberg (177) when a 12-mm intraaortic segment of polypropylene was inserted into the abdominal aorta at a point 3–4 mm above the iliac bifurcation. Second, the significantly reduced incidence of renal infarcts with silicone rubber tubing suggests that it should be preferred for carotid cannulation (201). Third, thrombi mobilized from the tip of the catheter can deposit in the kidneys. Therefore, it is suggested that blood be withdrawn through the cannula to clear such small thrombi prior to injection (168, 201). Fourth, the anchoring of a polyethylene abdominal aortic cannula alongside the aorta using a circular shock-absorbing loop in the tubing has been reported to virtually eliminate thrombotic infarctions and aortic aneurisms (165)[19]. Fifth, postexperiment necropsy of the kidneys should be done to ensure collection of pharmacokinetic data from rats with noninfarcted kidneys.

Thrombotic deposits on cannulas can also be mobilized as emboli to areas other than the kidneys. Buñag *et al.* (206) observed embolic paralysis of the hind legs of some rats having an indwelling cannula inserted in the abdominal aorta *via* the left iliac artery. Although this is apparently the only published observation, such limb paralysis has also been observed in rats following cannulation of the ipsilateral femoral vein and artery[21]. This complication should be carefully examined with respect to its ability to interfere with the experimental goals. Interestingly, limb paralysis has not been a major problem when the abdominal aorta is cannulated directly, thereby avoiding direct occlusion of the femoral or iliac arteries by a cannula[19].

Investigators using vascular cannulation methods as a means of xenobiotic administration, blood sampling, or cardiac function monitoring must remain aware of the aforementioned complications associated with indwelling intravascular cannulas. Appropriate steps must be taken both to minimize the incidence and verify the absence of such complications *post facto*.

Specific Methods for Vascular Access—To provide a more detailed summary of the numerous methods available for vascular access in the rat, methodological notes, procedural comments, and the demonstrated uses (243–294) were collected into separate tables for arterial (Table III), venous (Table IV), and miscellaneous (Table V) methods. Each table is organized by specific blood vessels, listed alphabetically according to the blood vessel nomenclature presented by Greene (26). It is hoped that scientists seeking information on access to a particular blood vessel will find these tables sufficient to enable them to limit the number of cited papers to be consulted for detail. In addition, this listing should inform scientists about published access methods for infrequently utilized blood vessels (*e.g.*, renal artery, adrenal vein) and thereby eliminate the need for an extensive noncomputerized search of the scientific literature, which is often 20–30 years old.

Although they are not presented in detail, several methods are available for cross-transfusion of blood between a pair of parabiotic rats (295–300). Such methods are useful in specialized studies investigating the association of particular effects with various blood-borne substances.

Investigators must be aware that certain parameters in the rat (plasma glucose concentration, blood coagulation parameters, plasma corticosterone concentration, plasma renin activity, serum hormone concentrations, serum enzyme activities, and drug binding to plasma proteins) may vary depending on the site and method of blood collection (301–307), the extent of rat handling (301, 308), acclimatization time (309), and the presence of another previously treated rat (310).

Vascular Access and Surgical Methods for Studying First-Pass Effect—First-pass effect is defined as the uptake and elimination of a xenobiotic during the absorption process as it passes from the lumen of the GI tract through the GI membranes and the liver for the first time, and into the circulation. Such first-pass elimination of xenobiotics can be studied in the rat by three methods: (*a*) transhepatic blood sampling, (*b*) hepatectomy, or (*c*) surgical construction of a vascular bypass to shunt blood away from the portal venous hepatic inflow (portal–systemic shunt).

Transhepatic Blood Sampling—The hepatic artery carries oxygenated blood from the aorta to the liver and supplies ~25–30% of hepatic blood flow in the cat, dog, and

Table III—Methods for Arterial Access

Arterial Site	Reference	Methodology Notes[a]	Demonstrated Uses[b]	Procedural Comments
Aorta:	Browning *et al.* (197)	PE, U, W	BP, CO	Cannula filled with heparin
	Buñag *et al.* (206)[c]	PR, TE/TY, U	BP	Cannula filled with heparin (1000 U/ml) plus streptokinase (200 U/ml)
	Carvalho *et al.* (215)[c]	PE, U, UR, W	M	Cannula filled with isotonic saline, used for 9 days
	Cocchetto & Bjornsson (unpublished)	Aortic puncture	T	Direct puncture of surgically exposed aorta just above the iliac bifurcation using a 20-ml syringe with a 20-gauge, 1.5-in. needle; anticoagulant can be in the syringe; collect up to 15 ml of blood from adult rats
	Garthoff & Towart (243)	PE, U, UR	BP	Continuous recording of BP and heart rate; data shown for 8-hr recording; requires special data collection equipment
	Kleinman *et al.* (213)	PE, U, UR	I, M	Cannula filled with isotonic saline, patent for up to ~22 days
	Laffan *et al.* (244)	PE, U, UR, W	BP	Continuous recording of BP; data gathered for 10 hr; requires special data collection equipment
	Lushbough & Moline (245)	Aortic transection	T	Reported blood yield 3% of body weight
	McIlreath *et al.* (214)	PE, U, UR, W	BP	90% surgical survival rate; cannula patent for weeks, filled with saline
	Popper *et al.* (207)	PE, TR, U, UR, W	BP, M	Cannula filled with 10% dextrose solution plus heparin (500 U/ml); used up to 10 days
	Purdy & Ashbrook (208)	PE, U, UR, W	BP	Cannula filled with 5% dextrose solution plus heparin (10 U/ml); spring attachment screwed into skull; cannula failure by 3 weeks
	Still (209); Still & Whitcomb (210, 212); Still *et al.* (211)	PE, TR, U, UR	BP, I, M	Cannula filled with isotonic saline; used up to 2 weeks
	Weeks & Jones (165)	PE, TR, U	BP, I	Cannula filled with isotonic saline, used up to 6 months; 80% surgical success rate, excellent photographs of surgical field by Stanton (501)
	Weeks (170)[19]	PE, TR, U, UR	BP, I, M	Extensive modifications of original method (165); 50 and 10 of 55 cannulas patent for 4 and 12 months, respectively; cannula fixed in vessel using cyanoacrylate glue
Carotid:	Allsop & Burke (201)	PE/SI, U, UR	M	Cannula filled with heparinized (10 U/ml) isotonic saline; 10 of 25 rats had renal infarcts after an average of 11.5 cannula-days
	Bullard (193)	PE, TR, U	BP, CO, M	Heparin given to inhibit clotting in cannula
	Buñag *et al.* (206)	PR, U	BP	Cannula failed after 7–14 days
	Burt *et al.* (204)	PE, U, UR	M	Surgery done aseptically; cannula filled with heparinized (20 U/ml) isotonic saline, patent for 3 weeks for five test rats; special swivel assembly
	D'Amour *et al.* (194)	PE		Cannula filled with heparin (1000 U/ml); excellent photographs of procedure
	Garthoff & Towart (243)	PE, U, UR	BP	Continuous recording of BP and heart rate; data shown for 8-hr recording; requires special data collection equipment
	Grantham *et al.* (235)	PE, PR, U	I	Continuous intra-arterial infusion for up to 2 weeks; cannula filled with heparinized saline
	Popovic & Popovic (173)	PE	M	Cannula filled with heparinized physiological solution; average cannula lifetime of 22 days
	Scharschmidt & Berk (246)	A, PE	M	Used a needle-three-way stopcock device for drawing blood samples up to every 30 sec
	Staub & Coutris (218)	PE, U, UR	M	Cannula filled with isotonic saline, consists of an exteriorized shunt of carotid arterial blood; 80% patent for 2–4 days, 20% patent for 5–7 days
	Waeldele & Stoclet (205)	PE/SI	BP, I, M	Cannula filled with 40% povidone plus 10% heparin; used for 10 days
Caudal:	Agrelo & Dawson (159)	N/PE, U, UR	BP, M	Cannula filled with heparinized (1000 U/ml) isotonic saline; used for up to 4 weeks
	Chiueh & Kopin (202)	PE, U, UR	BP, I, M	Cannula filled with heparinized (500 U/ml) isotonic saline; patent up to 2 weeks
	Fujita & Tedeschi (196)	PE, TR, U	BP	Cannula filled with heparinized (10 U/ml) isotonic saline; used up to 48 hr
	Hurwitz (152)	A, N, P	M	Percutaneous puncture using a clear-hub 21-gauge needle; each sample was 420 μl
Femoral:	Ekelund & Olin (247)	PE		Radiopaque cannula inserted in the aorta, superior mesenteric artery, or renal artery *via* the femoral artery; requires angiographic verification of cannula location
Hepatic:	Leivestad & Malt (248)	PE, PR, U	I	Cannula inserted with aid of magnification, exteriorized after subcutaneous tunnel to tail; rear of the animal is restrained to protect the cannula; patent up to 6 weeks
Pulmonary:	Carrillo & Aviado (249)	A, PE	BP	Cannula inserted *via* the right jugular vein
	Forrest *et al.* (250)	A, PP	BP	Cannula inserted *via* the right jugular vein; cannula is a No. 4 French gauge, balloon-tipped, Swan-Ganz flow-directed catheter; used in the rabbit and guinea pig, perhaps adaptable to the rat
	Hayes & Will (251)	A, TE	BP	Specific bends must be made in cannula; cannula inserted *via* the jugular vein in a closed-chest rat
	Herget & Paleček (252)	A, PE	BP	Cannula inserted *via* right jugular through an introducer; illustrates the use of blood pressure waves to monitor catheter tip position; cannula filled with heparinized saline
	Rabinovitch *et al.* (253)	SI, U, UR	BP	Cannula flushed with heparin (20 U/ml) and animal heparinized (150 U/kg) daily; inserted *via* a 19-gauge needle introducer
	Stinger *et al.* (254)	A	BP, CO	Cannula was a commercially available No. 3-½ French umbilical vessel catheter, inserted *via* the jugular vein in a closed-chest rat

continued

Table III—*Continued*

Arterial Site	Reference	Methodology Notes[a]	Demonstrated Uses[b]	Procedural Comments
Renal:	Beuzeville (195)	A, PE	I	Cannula filled with heparinized (10 U/ml) isotonic saline; patent up to 48 hr
	Evans & Nikitovitch (255); Evans (256)	PE, U, UR	I	Cannula exteriorized at the back of the neck *via* a plastic cap sutured to the skin

[a] Methodology key: (A) postoperative experiment on anesthetized rats; (N) needle inserted into blood vessel, with tubing attached to needle; (P) percutaneous method; (PE) polyethylene cannula; (PP) polypropylene cannula; (PR) postoperative experiment on partially restrained rats; (SI) silicone rubber cannula; (TE) polytef cannula; (TR) postoperative experiment on totally restrained rats; (TY) Tygon cannula; (U) postoperative experiment on unanesthetized rats; (UR) postoperative experiment on unrestrained rats; (W) modification of the method of Weeks and Jones (165). [b] Key for demonstrated uses and procedural comments: (BP) blood pressure determination; (CO) cardiac output determination; (I) injection; (M) multiple blood sampling with rat survival; (S) single blood sample with rat survival; (T) terminal blood sample. [c] Cannula threaded into the aorta *via* the iliac artery.

human (311). In addition to this arterial blood flow, the liver also receives blood from the visceral organ vasculature *via* the portal vein. Portal venous blood comprises 70–75% of hepatic blood flow in the cat, dog, and human (311). Blood is returned to the inferior vena cava *via* the hepatic vein. Hepatic hemodynamics and blood pressures at various locations in the inferior vena cava of the rat have been determined (312).

To study the first-pass effect with respect to a specific orally administered xenobiotic, the concentration of the xenobiotic in both portal and hepatic venous blood must be determined. Since the hepatic venous blood concentrations of a xenobiotic and associated metabolites are equivalent to that in venous blood collected from other peripheral veins (*e.g.*, external jugular, femoral, caudal) if there is no interposed organ that further metabolizes these substances, a technically easier procedure for transhepatic blood sampling is cannulation of the portal vein plus a femoral or caudal vein. Several methods (Table IV) have been described for collection of blood from each of the above vessels. An excellent example of such transhepatic blood sampling methodology is in the first-pass metabolism studies of clonazepam in the rat (313). Transhepatic blood sampling after oral xenobiotic administration is, of course, sensitive to first-pass effects due to the gut contents and GI wall as well as the liver. By infusing a xenobiotic directly into the portal vein, the first-pass effect due to the liver alone can be quantified. Several exemplary applications of this method are available for review (283, 285, 314, 315).

Hepatectomy—Hepatectomized laboratory animals have been used to construct animal models for liver disease-associated coagulopathies (316), abnormalities in amino acid transport (317), alterations in the brain uptake of glucose (318), abnormalities in drug disposition (319), and evaluation of the existence of extrahepatic drug elimination processes (320). Surgical procedures have been described for the excision of part or all of the liver (subtotal and total hepatectomy, respectively) and the production of acute functional exclusion of the liver from its normal vascular connections while leaving the nonfunctional liver in the body (exclusionary hepatectomy).

Higgins and Anderson (48) surgically excised the large median and left lateral lobes of the liver in rats, removing ~65–75% of the total liver mass (thus the name two-thirds hepatectomy) in a one-step procedure. Illustrations of this procedure are available (12). Interestingly, the remaining right lateral and caudate lobes of the liver underwent compensatory hypertrophy so that the liver essentially was restored to its preoperative weight after 10–14 days. Approximately 16 and 24% of the excised liver mass is restored within 24 and 48 hr of removal, respectively. Therefore, rats used within 48 hr after two-thirds hepatectomy can be useful in studies of drug disposition in the presence of impaired hepatic function (319). Selye and Dosne (321) extended this subtotal hepatectomy by excising the median, left lateral, and right lateral lobes in order to remove ~85% of the total liver mass. Ryan *et al.* (322) performed two-thirds hepatectomy in rats between noon and 3 pm to avoid variation in mitotic activity due to diurnal rhythm (323). Higgins and Anderson (48) calculated the ratio of total liver–whole body weight equal to 0.0358 for 30 rats weighing from 125–225 g. This number is particularly useful in estimating the number of rats in this weight range needed to supply a required amount of hepatic tissue for *in vitro* hepatic microsomal metabolism studies.

Total hepatectomy in laboratory animals can be performed using two- and three-stage surgical procedures where a portion of the total procedure is performed at each stage and the animal is allowed to partially or totally stabilize between stages. Such multiple-stage procedures allow a period of time after constriction of the portal vein and inferior vena cava for development of collateral circulation, thus minimizing engorgement of the abdominal organs during the last stage of total hepatectomy. Although the three-stage procedure (portal vein-to-inferior vena cava anastomosis, portal vein ligation proximal to the liver, and total hepatectomy) has proven useful in the dog (324), it has not been used in the rat.

Two-stage hepatectomy (portal vein and inferior vena cava constriction, then total hepatectomy) can be performed in the rat using silk ligatures as per the method of Meehan (325). Alternatively, Bollman and Van Hook (326) used a strip of cellophane in place of the silk ligature placed around the portal vein. The cellophane progressively constricts over a period of ~2 months, at which time total hepatectomy can be done. In each method, the minimum size of the portal venous constriction produced in the first stage is crucial in preventing rapid, fatal congestion of the visceral vascular bed. With an intravenous infusion of glucose following hepatectomy (150 mg/kg/hr for 8–10 hr then 250 mg/kg/hr, using a solution of glucose in isotonic saline infused at 1.25 ml/hr), ~90 and 60% of the rats survive at least 8 and 24 hr, respectively (326). Glucose can also be administered by intermittent subcutaneous injections (325). Rats treated with glucose rarely live >30 hr after hepatectomy; those not receiving glucose rarely survive >2 hr (325).

Exclusionary hepatectomy is useful for acute studies in

Table IV—Methods for Venous Access

Venous Site	Reference	Methodology Notes[a]	Demonstrated Uses[b]	Procedural Comments
Adrenal:	Schapiro & Stjarne (257)	A, PE, S	M	Uses an artificial adrenal vein–renal vein shunt, for up to 6 hr; heparin used to inhibit clotting
	Singer & Stack-Dunne (258)	A, S	T	Adrenal vein blood collected continuously for 1.5 hr; no nephrectomy; cannula construction and insertion procedure not described
Brachial:	Young & Chambers (259)	S	T	Brachial artery and vein are severed with blood collection in a pipet; developed for the mouse, probably useful for the rat
	Slusher & Browning (260)	PE, S, U, UR	M	Cannula inserted through a 19-gauge needle, exteriorized and protected in a metal button sutured to the skin; used for up to 3 weeks
Caudal:	Agrelo & Miliozzi (149)	N/PE, P, TR, U	II	Cannula filled with heparinized (100 U/ml) isotonic saline; used for 6 hr
	Born & Moller (146)	PE, PR, S, U	CI	Cannula threaded into a vein through a 20-gauge needle that is then removed; protected by electrical shielding
	Cotlove (261)	N, P, PE, TR, U	CI	A 23-gauge needle with attached tubing is inserted into the vein
	Eve & Robinson (223)	P, PE, PR	CI	Cannula threaded into the vein through a 21-gauge needle that is then removed; uses a special swivel–fluid conduit; used for >6 months continuous infusion without anticoagulants
	Kellogg *et al.* (262)	S, TR, U	C	Cannula inserted with aid of dissecting microscope
	Little *et al.* (189)	P, PE, PR, U	C	Cannula protected by plastic tubing attached to tail; 2 of 9 rats infused for 90 days
	Plum (263)	A, S	M	Blood collected with suction after cutting the distal segment of the tail
	Rhodes & Patterson (148)	P, PE, PR, U	CI, I	Cannula threaded into the vein through a 19-gauge needle that is then removed; protected by electrical shielding; 32 of 34 rats had patent cannulas for 10 days (one rat chewed through the protective shielding)
	Saarni & Viikari (147)	A, P	C	Cannula threaded into the vein through a needle that is then removed (needle gauge and cannula type not specified); heparin (40 U/kg/hr) and droperidol (0.16 mg/kg/hr) infused throughout the experiment
	Stuhlman *et al.* (264)	S, TR, U	M	Blood collected with suction after cutting the distal segment of the tail, up to 10 samples per animal; rat is restrained in a cage on a heating pad
	Videm (265)	A, P	S, SI	Used a 21-gauge needle for collection of up to 4 ml of blood or injection into the vein at the root of the tail
	Wright (266)	S, TR, U	S	Up to 3 ml of blood collected in a capillary device[c] after cutting the distal segment of the tail; rat is restrained in a cage near a heating element
Dorsal Metatarsal:	Nobunga *et al.* (267a)	P, TR, U	S, SI	Injection *via* a 27-gauge needle into the right hind leg; collection of up to 0.2 ml of blood *via* a 22- or 23-gauge needle; useful in docile rats for acute venous access without anesthesia
Dorsal Penile:	Salem *et al.* (41)	P, TR, U	SI	Injection *via* a 26–30-gauge needle; method extended to rats by Nightingale and Mouravieff (267b)
	Virolainen (268)	P, TR	II	Injection *via* a ≤20-gauge needle; each rat received up to 14 injections in 1 week; good photograph of the procedure
Femoral:	Benzman-Tarcher (269)	A, S, SI	CI	Cannula advanced into inferior vena cava, used to infuse a fatty acid emulsion for 6 hr; no anticoagulant used
	D'Amour *et al.* (194)	PE, S		Cannula filled with heparin (1000 U/ml); excellent photographs of the procedure
	Jones & Hynd (191)	NY, PR, S, U	CI, M	Cannula filled with heparinized isotonic saline; exteriorized *via* the tail and protected by a plastic sheath; up to 7 days infusion in 300 rats
	Nishihara *et al.* (270)	PE, S	SI	Cannulation of the femoral artery and vein on one side, followed after time by the same procedure on the other side to study drug interactions
Femoropopliteal[d]:	Pearce (271)	P, TR, U	SI	A 27-gauge needle is used for injection
Hepatic:	Suzuki *et al.* (272)	PE, S	M	One cannula is passed down the external jugular vein into the inferior vena cava; the blood sampling cannula is inserted in the inferior vena cava *via* an abdominal incision
	Wernze (273)	A, PE, S	II, M	Cannulation aided by a microscope; cannula filled with heparinized (10 U/ml) isotonic saline; isobutylcyanoacrylate glue used to fix the cannula in the vein
	Yokota *et al.* (150)	A, N, PE, S	M	Direct vein cannulation with the bent tip of a 25-gauge needle connected to tubing; cannula was filled with heparin and fixed to the vein using a surgical glue; 0.25 ml of blood drawn slowly over 4 min to prevent reflux; blood drawn for up to 45 min
Hypophysial portal:	Ben-Jonathan & Porter (274)	A, PE, S	M	Modified method of Porter and Smith (275) to use an embolator apparatus to enable collection of blood samples
	Porter & Smith (275)	A, PE, S	M	Collected blood is free of CSF contamination; collected continuously for up to 6 hr; requires a microscope for surgery
Inferior vena cava:	Kaufman (185)	S, SI, U, UR	II, M	Cannula filled with heparinized saline and exteriorized at the back of the neck; patent for many months
	Welch *et al.* (276)	A, S	T	Direct venipuncture of the surgically exposed vein

continued

Table IV—*Continued*

Venous Site	Reference	Methodology Notes[a]	Demonstrated Uses[b]	Procedural Comments
External jugular:	Allsop & Burke (201)	PE/SI, S, U, UR	II	Cannula filled with heparinized (10 U/ml) isotonic saline; an epoxy-filled needle used to plug the end
	Brandstaetter & Terkel (234)	DL, S, U, UR	II, M	Cannula filled with heparin (20 U/ml); rat attached to a pulley assembly for continuous vascular access; cannulas in 46 rats patent for 12–21 days
	Brown & Breckenridge (168)	S, SI, U, UR	II, M	Cannula filled with an air bubble plus heparin–povidone mixture, sutured into the vein and exteriorized *via* a cranial pedestal; aseptic surgical methods used; patent for >1 year
	Bullard (193)	A, PE, S	VBP	Rats were heparinized; acute study
	Burt *et al.* (204)	S, SI, U, UR	CI	Surgery done aseptically; cannula filled with heparinized (20 U/ml) isotonic saline; patent cannulas for 3 weeks in five test rats; special swivel assembly
	Cox & Beazley (180)	PE, S, U, UR	CI	Cannula exteriorized at the back of the neck and passed through a spring that is sutured to the rat; uses a ball swivel, requires daily catheter straightening; cannula used up to 6 weeks (gives useful technical hints concerning cannula placement)
	Dalton *et al.* (176)	H, PP, S, U	CI	Cannula threaded into the vein through a 19-gauge needle and exteriorized through a rat body harness; used for up to 3 weeks
	D'Amour *et al.* (194)	PE, S		Cannula filled with heparin (1000 U/ml)
	Edmonds & Thompson (178, 179)	PP, S, U, UR	CI	Cannula secured *via* a subcutaneous collar around the neck; used for up to 3 months; rat prevented from biting cannula by electrical-shock device
	Engberg (177)	PP, S, U, UR	CI	Cannula exteriorized *via* a plastic cap sutured to the back of the neck; filled with isotonic saline; used up to ~3 weeks
	Ensminger *et al.* (190)	PE, PR, S, U	CI, SI	Cannula exteriorized at the root of the tail and passed through a spring enclosing the tail; infusion for up to 3 days; a larger diameter cannula can be used for serial blood sampling
	Harms & Ojeda (160)	S, SI, U, UR	M	Uses an implantation needle that is passed in and out of the vessel lumen, leaving the attached cannula tubing in the lumen; anchored in place *via* an attached silicone rubber sheet; cannula filled with isotonic saline; patent for 4–8 weeks for six test rats
	Mayer *et al.* (277)	A, PE, S	II	Jugular vein shunt constructed to enable serial injections without cannula flushing; prevents undesirable blood volume expansion; used acutely for bioassay
	Nicolaidis *et al.* (187)	S, SI, U, UR	CI	Uses a double-mitered cannula tip to prevent thrombotic blockade of the cannula; exteriorized at the head *via* a cranial pedestal; cannula filled with heparin–povidone mixture; uses a special swivel assembly; cannula patent for up to 5 months
	Popovic & Popovic (173)	PE, S, U, UR	II, M	Cannula filled with heparinized isotonic saline; exteriorized at the back of the neck; average cannula lifetime of 22 days
	Popovic *et al.* (175)	PE, S, U, UR	ECG, II, M	Cannula is advanced into the right ventricle; filled with heparinized isotonic saline; exteriorized at the back of the neck; cannula patent up to 124 days (with heparin) or 50 days (without heparin flushing)
	Renaud (155)	A, P, S	II, M	Uses a 20-gauge needle for direct venipuncture of the surgically exposed vein or percutaneous rostral puncture *via* depilated skin; collected up to 2 ml of blood, up to five samples per week
	Smith & Davis (15); Davis (278)	PE/SI, S, U, UR	CI, II	Describes two cannulas for jugular vein implantation; used up to 6 months; contains a wealth of practical information on cannula construction, surgical procedures, and infusion apparatus; excellent procedural photographs
	Steffens (186)	S, SI/TE, U, UR	CI, M	Cannulation of one tributary (for infusion) or two tributaries (for simultaneous infusion and blood sampling) of the external jugular vein; cannula filled with air bubble and heparin–povidone mixture; exteriorized *via* a cranial pedestal; sampled blood may be contaminated with infused substance
	Stripling (171)	S, SI, U, UR	CI, II	Cannula has intravascular end plugged, but delivers drug *via* lateral holes that prevent blood from entering the cannula; fixed in vein using cyanoacrylate glue and exteriorized *via* a cranial pedestal; patent in 61 and 60 of 65 rats for 2–3 and 5–9 weeks, respectively
	Terkel & Urbach (166)	S, U, UR, V	M	Cannula filled with heparin (250 U/ml); anchored in vein by seven sutures and exteriorized at the back of the neck into a protective plastic cylinder sutured to the neck; cannulas in 20 rats were patent for an average of 28.5 days (maximum 88 days)
	Upton (181)	S, SI, U, UR	II, M	Cannula filled with heparinized (10 U/ml) isotonic saline; exteriorized at the back of neck; serial blood samples collected up to once per min, with up to 5 weeks usage

Continued on next page

Table IV—*Continued*

Venous Site	Reference	Methodology Notes[a]	Demonstrated Uses[b]	Procedural Comments
	Weeks (7, 13, 170)[19]; Weeks & Davis (141); Weeks & Compton (184)	PE/SI, S, U, UR	CI, II, M, SI	Cannula filled with isotonic saline; exteriorized at the back of the neck; cannula patency [injection, blood sampling] for 10 rats tested after 3 months: [8, 7], 4 months: [8, 5], 6 months: [5, 4], and 8 months: [2, 2]: methodological details are best presented in one paper (13)
Internal jugular:	Mouzas & Weiss (279)	A, N, S	M, S	Direct needle puncture of the surgically exposed vein; collects up to 5 ml of blood
Mesenteric	Rappaport *et al.* (143)	PE, PR, S, U	CI	Uses an easily constructed device to facilitate cannulation of small vessels; cannula filled with heparinized saline
	Zammit *et al.* (203)	PE/SI, S, U	II, M	Cannula filled with heparinized saline; 17 of 20 rats had patent cannulas for 3 weeks
Palpebral:	Anderson *et al.* (280)	P, TR, U	SI	Injection *via* a 30-gauge needle to the palpebral venous blood supply of the eye of newborn rats
Portal:	Cassidy & Houston (151)	A, N, S	SI	Inserted a 27-gauge butterfly infusion needle into the vein; cannula flushed with heparinized isotonic saline
	Gallo-Torres & Ludorf (282)	PE, S, TR, U	CI, M	(*a*) Splenectomy followed by threading of a cannula *via* the splenic vein into the portal vein; portal blood continuously sampled for 60 min; patent for up to 1 week; (*b*) splenectomy followed by insertion of a T-shaped cannula into the portal vein; patent for several months
		PE, S, TR, U	M	Direct cannulation of the vein followed by fixing the cannula in the vein with a piece of plastic and cyanoacrylate adhesive; patent for 3–4 days; excellent surgical field diagrams
	Hyun *et al.* (281)	A, PE, S	M	Cannula inserted in the portal vein *via* the splenic vein; filled with heparin (1000 U/ml) or distilled water
	Pelzmann & Havemeyer (198)	A, PE, S	M	A shunt of tubing was inserted in opposite directions in the portal vein; cannula filled with heparin
	Sable-Amplis & Abadie (199)	PE, S, U, UR	M	A T-shaped cannula inserted in the portal vein; cannula was siliconized, filled with heparinized (4 mg/ml) saline, and exteriorized at the back of the neck; 8 and 5 of 10 rats had patent cannulas at 1 week and 1 month, respectively
	Suzuki *et al.* (283–285)	A, PE, S	CI	Cannula threaded into the portal vein *via* the pyloric vein; methylene blue injected into the cannula after the procedure to verify location by blue staining of the liver; portal infusions given for 50 min
Retro-orbital venous plexus:	Lapeyrac (40)	A	S	Up to 0.6 ml of blood drawn into a glass pipet
	Lim *et al.* (44)	A	M	Collected up to six serial blood samples of ~0.5 ml each; diagram of ocular anatomy
	Nöller (36)	U	M	Drew up to several milliliters at once; glass capillary tube used as a collection device
	Riley (37)	U	M	Collected blood samples of ~0.2 ml each
	Salem *et al.* (41)	U	M	Collected up to 30 serial blood samples of up to 0.5 ml each; excellent description and photographs of method
	Sanders (42)	A	M	Adapted method of Riley (37) to germ-free animals
	Sorg & Buckner (43)	A/U	S, T	Up to 8 ml of blood collected into a test tube *via* the posterior canthus; claims less likelihood of nose bleeds and eye trauma
	Stone (39)	U	M	Collected up to 1 ml of blood per sample; used 2 drops of a 2% cocaine solution applied to the eye before sampling
Sagittal/Transverse Sinus:	Davis & D'Aquila (286)	N, PE, S, U, UR	II	Requires stereotaxic techniques; not used as a chronic infusion method
Saphenous:	Everett & Sawyer (287)	P, TR, U	II	Shaved the area, then dilated the vein with 70% alcohol and snapping with a finger; injection *via* a 26-gauge needle; good illustration of the injection site
	Grunt *et al.* (288)	A, N, P	II	Used an indwelling 27-gauge needle for maintenance of venous access during anesthesia
	Rusher & Birch (289)	P, U	M	Collected up to 0.2 ml of blood on each of 3–5 venipunctures per rat; used a 20-gauge needle
Sublingual:	Greene & Wade (290)	A	SI	Access to the sublingual vein is facilitated *via* a fine suture passed through the tip of the tongue; injection *via* a 26- or 27-gauge needle

[a] Methodology key: (A) postoperative experiment on anesthetized rats; (CSF) cerebrospinal fluid; (DL) double lumen cannula (1.5 mm o.d., 0.71 mm i.d.; DVE-8, Dural Plastics, Dural, NSW, Australia); (H) postoperative rat maintained in a body harness; (N) needle inserted into blood vessel, with tubing attached to needle; (NY) nylon cannula; (P) percutaneous method; (PE) polyethylene cannula; (PP) polypropylene cannula; (PR) postoperative experiment on partially restrained rats; (S) surgical method; (SI) silicone rubber cannula; (TE) polytef cannula; (TR) postoperative experiment on totally restrained rats; (TY) Tygon cannula; (U) postoperative experiment on unanesthetized rats; (UR) postoperative experiment on unrestrained rats; (V) vinyl cannula. [b] Key for demonstrated uses and procedural comments: (CI) continuous infusion; (ECG) electrocardiogram recording; (II) intermittent injections; (M) multiple blood sampling; (S) single blood sample with rat survival; (SI) single injection; (T) terminal blood sample; (VBP) venous blood pressure. [c] Unopette, Becton-Dickinson, Rutherford, N.J. [d] Lateral marginal vein.

anesthetized rats. Pang and Gillette (320) supplied evidence contrary to the hypothesis of extrahepatic elimination of acetaminophen in the rat using exclusionary hepatectomy *via* ligation of the hepatic artery and portal vein at points proximal to the liver. The absence of blue discoloration of the liver following a 0.5-ml iv injection of blue ink was used to verify the complete cessation of blood flow to the liver. Rats treated in this manner survived for ~85 min.

Portal–Systemic Shunts—A portal–systemic shunt is a surgically constructed connection between the side or end of the portal vein and a systemic vein (inferior vena cava or renal vein, a portacaval or portarenal shunt, respectively) to divert portal venous blood away from the liver.

Table V—Miscellaneous Methods for Vascular Access

Method	Reference	Demonstrated Uses[a]	Procedural Comments
Cardiac puncture:	Burhoe (5); Gupta (291); Kraus (21); Moreland (292); Waynforth (12)	M, S, T	Rats are anesthetized; generally uses a 25- or 26-gauge needle (0.5–0.75 in. long); Burhoe (5) collected up to 12 ml of blood at once without killing the rat and up to 5 ml of blood weekly for 3 months; procedure can be terminal, Stuhlman *et al.* (264) reported 12% mortality from cardiac puncture in rats; photographs and excellent procedural descriptions by Burhoe (5) and Waynforth (12); Gupta (291) used cardiac puncture for neonatal rats
Decapitation:	Bush & Bush (293); Moreland (292)	T	Particularly useful for terminal blood collection when anesthesia must be avoided; provides mixed arterial–venous blood; although aesthetically unpleasant, it is a humane procedure when done by experienced personnel using commercially available guillotines[b]
Tail amputation:	Enta *et al.* (294); Kraus (21)	M, S	Tail may be warmed before procedure; distal part (2–3 mm) of tail of unanesthetized rat is amputated to collect up to 4 ml of arterial blood (if lateral caudal veins are occluded by direct pressure) or mixed arterial–venous blood; bleeding is stopped by applying pressure or cauterization
Toe clip[c]:	Kraus (21); Moreland (292)	S	Rats are anesthetized; clipping the toe can yield up to 0.3 ml of mixed arterial–venous blood; due to postprocedural pain to the rat, this procedure is not recommended

[a] Key for demonstrated uses: (M) multiple blood samples per rat; (S) single blood sample per rat; (T) terminal procedure for blood sampling. [b] Harvard Apparatus, South Natick, Mass. and EDCO Scientific, Chapel Hill, N.C. [c] Plantar and dorsal digital arteries plus the plantar veins are clipped.

Procedures for portal–systemic shunting in the rat were introduced in 1946 by Whitaker (327) and Reinhardt and Bazell (328). Laboratory animals with a portal–systemic shunt have provided, due to conditions resulting from the shunt, useful models for the study of altered drug disposition in the dog (329, 330), altered pharmacodynamics in the cat (331), hemostatic abnormalities (thrombocytopenia, hypofibrinogenemia, impaired platelet aggregation) in the rat (332–334), hyperuricemia in the rat (335), and potentially altered blood–brain barrier permeability in the rat (336–338). A number of other shunt-caused metabolic abnormalities have yet to be exploited as animal models (339). Bircher (339) has provided a useful summary of the animal model utility of the portacaval shunt rat.

It is impractical to consider here the detailed methodologies available for portal–systemic anastomosis in the rat. Lee *et al.* (340) have presented an outstanding review of the relevant history and methodology, along with a thorough discussion of the hepatic and metabolic alterations associated with portal–systemic anastomosis. Steiner and Martinez (341) studied systematically the cytological and gross changes associated with ligation of the portal vein, hepatic artery, and bile duct. Although Bircher (339) has recently proposed routine use of the portacaval shunt rat for evaluation of first-pass effect, no study using such methodology in the rat has yet been published. The need for vascular surgical skills and an operating microscope undoubtedly hinder the widespread use of portacaval shunt methodology for studying the first-pass effect in the rat. Nonetheless, the portacaval shunt rat offers the opportunity for studies of a longer duration than can be accommodated using transhepatic blood sampling methodology. Perhaps this advantage will be exploited in the near future.

One portacaval shunt procedure requires neither an operating microscope nor vascular surgical skills, yet produces a rat useful for short-term experiments. Bernstein and Cheiker (342) completed a portacaval shunt without a vascular anastomosis by obliterating the arterial blood supply to the liver (*via* ligation of the coeliac, superior hepatic, hepatic, and gastroduodenal arteries and diverting the blood flow through a polyvinyl tube (with needle tips at both ends) connecting the portal and iliolumbar veins. Rats so prepared were used in experiments for several hours while anesthetized.

COLLECTION OF OTHER BODY FLUIDS

Urine Collection Methods—Studies of xenobiotic disposition often require sampling or total collection of excreted urine. Some investigators have developed detailed methods for renal clearance determination in the rat (343). Urinary excretion studies may often be impeded by the relatively low urine output (1.20 ± 0.24 ml/200 g/hr, mean ± *SEM*, $n = 10$) of healthy adult rats (344). Urine flow rate was increased four- to fivefold by oral gavage administration of a single volume of warm distilled water (0.05 ml/g body weight) to a maximum urine flow rate of 2.8 ± 0.2% of body weight/hr (mean ± *SEM*, $n = 4$; equal to 5.6 ml/200 g/hr) (345). Urine flow returned to the basal rate ~3 hr after the water load. Three administrations of warm distilled water at half-hour intervals (0.05 ml/g body weight each) further increased the urine flow rate to a maximum of 6.8 ± 0.4% of body weight/hr (mean ± *SEM;* equal to 13.6 ml/200 g/hr) (346, as cited in 345). Jeffers *et al.* (347) induced diuresis in the rat by oral gavage administration of a single volume (0.05 ml/g of body weight) of an aqueous ethanol solution (12% v/v of 95% ethanol in water) followed 30 min later by oral gavage administration of a volume (0.03 ml/g of body weight) of warm tap water. This regimen resulted in a stable increased urine flow rate (3.1–8.1 ml/hr) in male rats (180–220 g) achieved 90–120 min after the first gavage. However, the amount of ethanol used has a sedative effect.

Kraus (21) has classified urine collection methods as the free catch method, reflex emptying following periodic stimulation or massage, bladder puncture, cystostomy, urethral catheterization of female rats, and external drainage catheter for male rats (Table VI). For all methods, urine is collected in some type of ice-cooled receptacle. An antioxidant and a metal ion-complexing agent should be added when collecting oxidizable xenobiotics. After esti-

Table VI—Methods for Urine Collection.

Method	Advantages	Disadvantages
Free catch: Nelson *et al.* (349)	Useful in all age groups and both sexes; nonsurgical method	May not elicit complete emptying of bladder
Reflex emptying[a]: Adolph *et al.* (350)	Useful in all age groups and both sexes; nonsurgical method	Requires restraining cage; may not elicit complete emptying of bladder
Suprapubic pressure[b]: Hayashi & Sakaguchi (351)	Can collect urine in a capillary tube; useful in all age groups and both sexes; nonsurgical method	Elicits small urine volume.
Bladder centesis: Heller (352)	Useful in all age groups and both sexes; bladder can be emptied completely	Used only at necropsy
Cystostomy: Hoy & Adolph (353)	Useful in all age groups and both sexes; useful in conscious rats; provides continuous urine collection	Surgical method
Urethral catheterization: Cohen & Oliver (354)	Provides continuous urine flow; serial catheterizations are possible; nonsurgical method	Useful in adult female rats only; rats must be anesthetized
External drainage catheter: White (355)	Provides continuous urine flow; nonsurgical method	Useful in adult male rats only; rats must be anesthetized

[a] Raising and lowering the rat. [b] Applied with the fingers.

mating the volume of urine to be collected during the designated period (based on a conservative urine output of ~0.3 ml/200 g body weight/hr), an appropriate volume of stock antioxidant solution (1.0*M* α-thioglycerol plus 0.1*M* EDTA) is added to the urine to achieve final urinary concentrations of 0.01*M* α-thioglycerol and 0.001*M* EDTA (348a)[23]. Mulder *et al.* (348b) recommended addition of sodium azide to the urine receptacle to inhibit bacterial growth. For anaerobic collection and to prevent evaporation of urine, the receptacle can be partly filled with mineral (261) or paraffin oil (343).

Free Catch Method—Rats will frequently urinate when touched, picked up around the midsection, raised with their hind legs out of contact with the cage floor, or a small cotton ball moistened with diethyl ether is waved under the nose (349).

Reflex Emptying—Kraus (21) provides a description of the salient features of a restraining cage used by Adolph *et al.* (350). With a rat in this cage over a receptacle, urination was elicited by quickly raising and lowering the frame and animal. Reflex urination can also be elicited by applying digital pressure to the suprapubic region (351). A capillary tube can be used to collect urine obtained in this manner (351).

Bladder Puncture—Urine can be withdrawn *via* a needle and syringe from the bladder at the necropsy of the rat (352).

Cystostomy—Hoy and Adolph (353) surgically implanted a plastic cannula in the urinary bladder to obtain a continuous flow of urine. This cannulation method is useful in both male and female rats of all ages. Hoy and Adolph (353) observed a continuous urine flow rate of ~0.3–0.5 ml/hr in healthy, conscious adult rats.

Urethral Catheterization of Female Rats—Repeated short-term catheterization of the female rat urethra can be done using a No. 4 coude ureteral catheter (354). A continuous urinary flow for up to 4 hr while the rat was maintained under anesthesia was reported. This method apparently has not been used in conscious rats.

External Drainage Catheter for Male Rats—A modified polyethylene catheter[24] can be slipped over the tip of the penis of the anesthetized male rat, tucked under the foreskin, and secured in place by tying the foreskin around the catheter with a single silk ligature (355). Urine flow rates of 6–10 ml/hr were observed after fluid loading *via* an orogastric tube. This method cannot be used in either female or conscious rats.

Bile Collection Methods—*Introduction*—Experiments on hepatobiliary excretion of drugs often necessitate short-term, intermittent, or chronic collection of bile from the rat. Eight salient facts must be considered when collecting rat bile. First, the rat does not have a gallbladder to serve as a bile reservoir, and therefore bile flows continuously through the common bile duct into the duodenum (26). Second, the pancreatic duct empties pancreatic fluid into the common bile duct distal to the place where the bile enters. This anatomical arrangement enables the experimenter to collect either bile, pancreatic fluid, or a mixture of the two. The identity of the collected fluid must be ensured by close inspection of these anatomical features and correct placement of the ligatures. Since collection of pancreatic secretions in the rat has had limited application in the area of pharmaceutical sciences (356, 357), the interested reader is referred elsewhere for detailed methods on pancreatic secretion collection (11, 356–361). Third, electrolytes depleted by chronic bile diversion must be replenished by parenteral or oral fluid administration (362, 363). Free access to drinking water containing 2.5–5% glucose plus isotonic sodium chloride (192, 363), 0.85–1.0% sodium chloride (182, 364, 365), 5% glucose plus 0.9% sodium chloride plus 0.05% potassium chloride (366), or Ringer's solution (188) reportedly supply adequate electrolyte replacement. Intravenous saline infused at a rate of 1 ml/hr has also proven effective (367). Addition of glucose to oral fluids seems to encourage fluid intake in the rat (366). Fourth, bile flow is dependent on body temperature (368, 369). Therefore, maintenance of constant rectal temperature *via* use of a heat lamp is necessary for comparative studies of hepatobiliary excretory function (319, 369, 370). Fifth, phenobarbital (75 mg/kg/day for 4 days) can be used as an experimental tool to induce an ~50% increase in bile flow rate (371–373). Alternatively, cycloheximide reduces bile flow rate to ~33–50% of the flow rate in control rats (374). Sixth, although the rate of bile flow does not differ significantly in males compared with female rats, lactating female rats (7–10 days postpartum) and female rats at their 20th gestational day have an ~50% greater bile flow rate. On

[23] Concentrations of α-thioglycerol and EDTA specified here were modified from those stated in ref. 348a in accordance with methodology notes provided by W. D. Conway *et al.*

[24] Leur-End Intramedic polyethylene catheter; Clay Adams, Parsippany, N.J.

removal of the rat pups from the lactating female, this elevated bile flow rate decreases to that of normal female rats (370). Seventh, interruption of enterohepatic circulation by bile duct cannulation alters bile composition by lowering the ratio of cholic acid–cholesterol (375). This alteration must be considered in evaluation of the results of biliary excretion studies. Eighth, there is a circadian variation of both bile production (average nighttime production being 50 and 38% higher than daytime production in female and male rats, respectively) and bile composition which could markedly alter studies of xenobiotic disposition (376).

The physiology of bile secretion and the anatomical structures involved have been reviewed (11, 377, 378). Colwell (363) has provided a useful photograph of a bile duct injected with india ink. Mechanisms of hepatobiliary excretion and enterohepatic recycling of xenobiotics have been reviewed (379–381), as have some theoretical pharmacokinetic principles (320, 382).

Substantial research has been done to discern the general characteristics of xenobiotics undergoing hepatobiliary excretion in the rat. Sperber (383) suggested that compounds with molecular weights >400 (in the unconjugated form) are excreted efficiently in the bile. Millburn *et al.* (384) studied a group of different xenobiotics and concluded that compounds with a molecular weight of ~300 or greater containing a highly polar anionic moiety are likely to undergo significant hepatobiliary excretion. The reader interested in further details on this topic is referred to the extensive original research (192, 382, 385–390).

Concurrent with their studies of the types of compounds undergoing hepatobiliary excretion, Abou-El-Makarem *et al.* (386) compared eight species (rat, dog, hen, cat, sheep, rabbit, guinea pig, and rhesus monkey) with respect to their ability to excrete eight different xenobiotics in the bile. This excellent study revealed that the rat and dog are two of the better hepatobiliary excretors, while the rabbit, guinea pig, and rhesus monkey are poor hepatobiliary excretors. This information is valuable when evaluating interspecies differences in drug disposition characteristics.

Methods—Bile collection methods can best be considered by classifying them into three categories:

1. Intracorporeal reservoir methods, *i.e.*, cannulation of the common bile duct with total continuous diversion of bile flow into a reservoir surgically implanted in the peritoneal cavity (363, 366, 391) or abdominal wall (362).

2. Extracorporeal reservoir methods, *i.e.*, cannulation of the common bile duct with total continuous diversion of bile flow through the cannula to an extracorporeal collection reservoir (182, 188, 192, 364, 374, 376, 390, 392, 393).

3. T-cannula method, *i.e.*, implantation of a T-shaped cannula in the common bile duct to enable intermittent collection of bile (394).

In each of these three categories, various methods exist for acute sampling in anesthetized rats or chronic continuous collection in unanesthetized, unrestrained rats. Table VII provides procedural comments relevant to the advantages and disadvantages of available bile collection methods.

Continuous bile collection has been reported for up to 54 days in chronically cannulated, unrestrained rats (364).

Table VII—Methods for Bile Collection

Method	Procedural Comments[a]
Intracorporeal reservoir methods: Johnson & Rising (366)	PP-10 cannulation of the common bile duct which then drains into a 12-ml glass reservoir implanted in the peritoneum; bulb can be drained *via* an extracorporeal segment of tubing attached to the reservoir; bile collected for up to 6 days from unanesthetized, unrestrained rats
Sawyer & Lepkovsky (391)	An 18- or 19-gauge stainless steel cannula is inserted into the common bile duct and drains into a 6-ml glass bulb implanted in the peritoneum; bulb can be drained *via* an extracorporeal segment of tubing attached to the bulb; no cannulated rat survived >90 hr
Extracorporeal reservoir methods: Abou-El-Makarem *et al.* (192)	Polythene (0.4-mm i.d., 0.8-mm o.d.) cannulation of the common bile duct; cannula exteriorized *via* a subcutaneous tunnel to an extracorporeal reservoir; bile collected from unanesthetized rat in a Bollman (395) restraining cage for up to 24 hr
Balabaud *et al.* (188)	PE-50 cannulation of the common bile duct; cannula exteriorized *via* a subcutaneous tunnel to a cranial pedestal connected to an extracorporeal reservoir *via* a swivel; bile collected for up to 6 days from an unanesthetized rat in a small metabolism cage
Enderlin & Honohan (182)	PE-50 cannulation of the common bile duct; cannula exteriorized *via* a subcutaneous tunnel to an interscapular incision connected to an extracorporeal reservoir *via* a swivel; bile collected for up to 4 days from an unanesthetized rat in a small metabolism cage
Friedman *et al.* (392)	PE-10 cannulation of the common bile duct; cannula exteriorized *via* a subcutaneous tunnel exiting at the foot [see figure in Nakayama (396)]; bile collected for 24 hr from unanesthetized, restrained rats
Fisher & Vars (364)	PE-50 cannulation, of the common bile duct; cannula exteriorized *via* a subcutaneous tunnel to a midline back incision into a piggyback cylindrical collection reservoir; bile collected for up to 54 days from unanesthetized rats
Knapp *et al.* (393)	PE-50 part of the cannula is inserted into the common bile duct, then the cannula is firmly anchored in place *via* a PE-250 cannula jacket; long-term bile collection from unanesthetized rats
Lock *et al.* (374)	PE-10 cannulation of the common bile duct; cannula exteriorized *via* a subcutaneous tunnel out of the back of the head to an extracorporeal reservoir; bile collected for 14 hr from an unanesthetized rat in a small metabolism cage
Takada *et al.* (390)	PE cannulation of the common bile duct for retrograde infusion studies in anesthetized rat
Vonk *et al.* (376)	Silicone-rubber cannula inserted into the common bile duct; cannula exteriorized at the skull *via* stainless steel tubing glued to the skull
T-Cannula method: Klauda et al. (394)	Silicone-rubber T-shaped cannula inserted into the common bile duct; permits periodic sampling of bile; a rubber collar around the neck was used to prevent chewing of the cannula

[a] Key for procedural comments: (PE) polyethylene; (PP) polypropylene.

Table VIII—Methods for Lymph Collection.

Reference	Procedural Comments[a]
Bollman *et al.* (404)	0.1 ml of 0.5% Evans blue dye was injected to enable identification of the lymphatic vessels; lymph collected continuously from unanesthetized, restrained, postoperative rats; 1–1.5 mm diameter plastic cannula used for lymphatic vessels of small intestine, liver, and abdomen; lymph flow rates of 5, 20, and 25 ml/24 hr from hepatic, intestinal, and thoracic lymphatics, respectively; lymph was collected for up to 3, 10, and 10 days from hepatic, intestinal, and thoracic lymphatics, respectively
Gallo-Torres & Miller (405, 406)	PE-50 cannula inserted into the abdominal thoracic duct; common bile duct was double cannulated for bile collection and saline infusion into the intestine; used cyanoacrylate adhesive to fix the cannula in the thoracic duct; lymph flow rate was proportional to the saline infusion rate; diverted bile can be reinfused *via* the intestinal cannula
Gowans (407)	Cannulation of the abdominal thoracic duct using the method of Bollman *et al.* (404); designed a pumping apparatus to reinfuse lymph and lymphocytes *via* the femoral vein
Reinhardt (408)	0.5 ml ip of 1% trypan blue enabled identification of the lymphatic vessels; rats anesthetized throughout procedure; cannula was tapered glass with a 25–27-gauge tip; thoracic duct cannulated in the neck; lymph collected for up to 6 hr; mean flow rate was 0.45 ml/hr (range of 0.13 to 0.70 ml/hr, n = 10)
Tasker (409)	Lymph collected continuously from unanesthetized, unrestrained, postoperative rats; a plastic cannula drained an intestinal lymphatic vessel into a glass reservoir (10–20 ml) placed in the peritoneum; lymph is collected *via* an extracorporeal reservoir access tube; collection for up to 10 days; flow rates of 0.5–1.0 ml/hr in fasting rats and 2–5 ml/hr in fed rats

[a] Key for procedural comments: (PE) polyethylene.

Cannulation of the common bile duct[25] with exteriorization at an interscapular incision for flow into an extracorporeal reservoir while housing the rat in a low-ceiling metabolism cage[26] has proven a useful procedure for relatively short (≤24 hr) bile collection. For more prolonged bile collection, passage of the extracorporeal portion of the cannula through an appropriate swivel joint will facilitate maintenance of a patent, unkinked cannula.

A unique procedure for the total exclusion of bile from the GI tract of male rats was described in 1936 (362). This procedure entails construction of a fistula from the bile duct to the vas deferens so that bile is eliminated in the urine. Rats prepared by this method remained apparently healthy for 3–4 months.

Bile collection methods are currently used in drug disposition and pharmacokinetic studies (366, 367, 397–403). Johnson and Rising (366) allowed at least 16 hr for rats to recover from bile duct cannulation, then included each rat in experiments only if (a) reasonable bile flow was established, *i.e.*, ~2–3 ml/kg/hr (366, 374, 391, 392), (b) the rat seemed to defecate normally (*i.e.*, no evidence of intestinal blockage), (c) the rat appeared healthy and active, not listless or lethargic, and (d) adequate food and fluid intake was maintained. These criteria are very useful guidelines for the postoperative and periodic evaluation of the rat with a cannulated bile duct.

Lymph Collection Methods—In 1945, Reinhardt (408) reported the first method for cannulation of the thoracic duct (at its location in the neck) and collection of lymph in the anesthetized rat. A single injection (0.5 ml ip) of 1% trypan blue administered 30 min prior to cannulation facilitated identification of the main lymphatic trunks. These investigators observed a lymph flow rate of 0.13–0.70 ml/hr (mean = 0.45 ml/hr, n = 10). Methodological considerations for lymph collection (404–409) are summarized in Table VIII.

Bollman *et al.* (404) extended lymph collection methodology to unanesthetized rats by insertion of a plastic cannula in the hepatic, intestinal, and thoracic duct lymphatic vessels. Postoperative, restrained rats provided continuous collection of lymph for up to 10 days. These investigators noted development of hypoprothrombinemia within 18 hr of lymphatic cannulation (316). Subcutaneous administration of vitamin K (4-amino-2-methyl-1-naphthol) both prevented and corrected hypoprothrombinemia development.

Tasker (409) collected intestinal lymph from unanesthetized, unrestrained rats by inserting a plastic cannula into an intestinal lymphatic vessel that drained into a glass reservoir (10–20 ml volume) placed in the peritoneum. Lymph was collected periodically by emptying the peritoneal reservoir *via* rubber tubing passing from the reservoir to a point of exteriorization at the right flank. This method seems useful for continuous lymph collection when restraint is undesirable.

Gowans (407) cannulated the abdominal thoracic duct using the method of Bollman *et al.* (404) to continuously collect lymph from restrained, unanesthetized rats. The collected lymph was reinfused into the femoral vein of the same cannulated rat *via* a pump apparatus. Such continuous reinfusion of lymph and living lymphocytes prevented the marked decrease in lymphocyte output from the thoracic duct previously noted when lymph flow was diverted for >2 days (410).

Finally, Gallo-Torres and Miller (405) introduced some modifications to simplify the method of Bollman *et al.* (404). A polyethylene cannula was inserted into the abdominal thoracic duct, while two other cannulas[27] were inserted into the common bile duct to enable bile collection with simultaneous infusion of isotonic saline (1.2–4.6 ml/hr) into the intestine. In rats fed a fatty meal 1 hr prior to surgery, the thoracic duct was recognized by its white coloration. Lymph flow rate was proportional to the saline infusion rate and lymph was collected for up to 10 days (405, 406). The integrity of the hepatobiliary excretory circuit can be reestablished by connecting the bile collection cannula to the intestinal infusion cannula. These investigators used a cyanoacrylate ester adhesive[28] to fix the cannula in the thoracic duct. Several excellent diagrams of the relevant anatomical regions are provided (19, 405).

Gallo-Torres (19) has provided a well-illustrated, integrated discussion of the application of lymphatic vessel,

[25] Using a polyethylene PE-10 cannula.
[26] Maryland Plastics, Inc., Federalsburg, Md.
[27] Each of the three cannulas were polyethylene PE-50.
[28] Permabond 910 adhesive; Permabond International Corp., Englewood, N.J. Duro Super Glue; Woodhill Chemical Sales Corp., Cleveland, Ohio. Eastman 910 adhesive; Eastman Kodak Co., Rochester, N.Y.

bile duct, and portal vein cannulation in evaluation of the bioavailability of xenobiotics in the rat. Since it provides a thorough review and examples of the experimental utility of lymph collection in the setting of other cannulas, the reader contemplating use of lymph collection methods is encouraged to refer to that work.

Cerebrospinal Fluid Sampling—The development of methods for ventricular access has been a critical contribution to the effort to advance knowledge in the neuropharmacology and psychopharmacology areas. Collection of cerebrospinal fluid and intraventricular administration of xenobiotics are highly specialized, intricate procedures that usually require experience in stereotaxic methods, knowledge of the anatomy of the brain, increased attention to aseptic procedures, and a high level of manual dexterity. Stereotaxic atlases of the rat brain are available to aid efforts to gain access to specific anatomical sites (32).

The majority of methods for ventricular access were designed for administration of either a single dose, multiple intermittent doses for up to 60 days, or a continuous infusion of very small volumes of xenobiotic-containing fluids (411–419). These methods recently have been reviewed by Myers (419) and illustrated by Avery (416). An easily constructed cannula originally used for intracerebral injections into cats and rabbits has proven useful in rats as well (420, 421). Wagner and DeGroot (422) constructed a single intracerebral cannula from two hypodermic needles and a small plexiglass plate. Hayden *et al.* (423) described detailed procedures for constructing and implanting in the lateral ventricle a relatively simple, inexpensive plexiglass cannula that enabled intraventricular injection for up to 3 months. The cannula constructed by Myers *et al.* (413) has proven to have great durability through the use of vinyl tubing[29] as an external protective shield. Khavari (225) has devised a combined intracerebral cannula–swivel unit to facilitate chronic studies in unrestrained rats. Although devised to enable intraventricular injection, some of these methods undoubtedly could be modified to enable sampling of cerebrospinal fluid.

Larger laboratory animals (cat, monkey) are preferred for chronic sampling of cerebrospinal fluid. Nonetheless, serial sampling over a 2-hr period has been accomplished in the rat. Serial small-volume samples (~5 μl) of cerebrospinal fluid were collected for ~2 hr *via* cisternal–lumbar (424) and ventriculocisternal perfusions (425) in order to monitor transport of seven amino acids into the cerebrospinal fluid. The artificial cerebrospinal fluid used by Merlis (426) for perfusion of the spinal subarachnoid space of the dog was useful in these perfusion studies in the rat (424, 425).

A single sample of cerebrospinal fluid can be obtained from the carefully recovered brain of a decapitated rat by inserting a glass microcannula (0.15 mm o.d.) into the mammillary recess of the third ventricle (427). By capillary action, 0.3–0.5 μl of cerebrospinal fluid was collected. Clemens and Sawyer (428) collected *via* cisternal puncture ~0.1 ml of cerebrospinal fluid from each anesthetized rat prior to sacrifice. No details (needle size, surgical preparation, exact site of puncture) of the method were given. Chou and Levy (429) described in detail a procedure for cisternal puncture of the anesthetized rat and collection of 50–100 μl of cerebrospinal fluid. Since this procedure requires only a 25-gauge needle and a piece of silicone rubber tubing, it should prove to be a valuable technique when only a single sample is required.

Saliva Collection Methods—Saliva remains a seldom-exploited fluid for collection in studies of xenobiotic absorption, distribution, and elimination. Welch *et al.* (276) accurately determined the elimination half-life of antipyrine in rats by the serial determination of salivary antiyrine concentrations. It was necessary to administer 2.0 mg/kg sc pilocarpine to some rats to collect (by capillary tube) 5 μl of saliva per sample. DiGregorio and Kniaz (430) monitored salivary amphetamine and methamphetamine concentrations in rats receiving a constant infusion of pilocarpine (0.125 mg/min) *via* the brachial artery. Saliva was continuously collected in a piece of polyethylene tubing over discrete time intervals *via* a polyethylene cannula placed in the parotid salivary duct. This technique of saliva collection was a modification of methodology developed for the mouse (431).

Additional details, illustrations, and pharmacokinetic applications of saliva drug concentration monitoring have been presented (432, 433). A number of other methods used for collecting rat saliva have been described in the dental research literature. These methods have been reviewed by Kraus (21).

METABOLISM CAGES AND RESTRAINING DEVICES

A number of commercial and home-built cages and devices, primarily constructed of wire or plexiglass, have been designed for short-term restraint with strict spatial confinement (434–444), restriction of certain body motions without spatial confinement (10, 445–450), and housing (metabolism cages) to facilitate intravascular xenobiotic infusion, quantitative collection of urine and feces, and/or measurement of food and water intake (235, 451–464).

Cage design and selection are commonly assumed to be relatively unimportant factors in designing animal experiments. However, molybdenum toxicity is increased in rats housed in zinc-coated cages (465) and the acute toxicity of various respiratory depressants in rats is markedly affected by cage design (466). Serum creatine phosphokinase activity in rats housed in cages with large spacings in the floor grating was approximately double that observed in rats housed in cages with small spacings in the floor grating (467). The effect on physiological variables of individual as opposed to group housing of rats has been recently reviewed (468). In view of these effects of cage design and individual housing, investigators should consider carefully the experimental impact of the metabolism cages and restraining devices described in the following sections.

Restraining Devices—*Short-Term Restraint*—Short-term restraint with strict spatial confinement is provided by restraining devices. These devices are used to achieve immobilization to facilitate administration of injectable substances, application of topical medication, collection of a blood sample, artificial insemination by vaginal smear, inhibition of access to indwelling cannulas, or induction of stress in studies of stress-associated effects in rats. The old restraining method of tying the conscious rat to a board (469, 470) is clearly excessive and unneces-

[29] Biraco vinyl tubing, number 318-B, size 8 Birnbach; Terminal Radio, New York, N.Y.

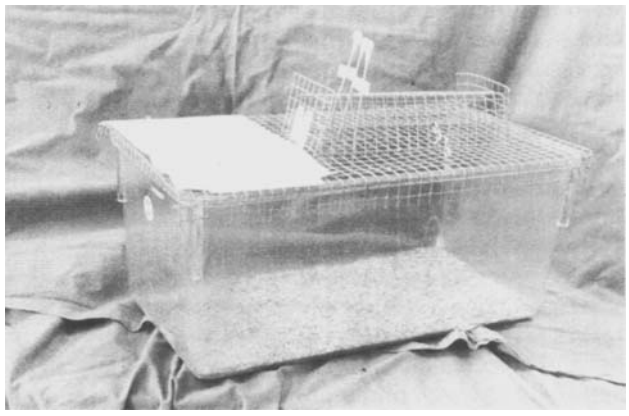

Figure 6—*A home-built restraining cage constructed from galvanized steel-wire hardware cloth. The rat fits inside the wire tunnel which has dimensions of 23-cm long, 7-cm wide, and 5.5-cm high. The size of the support wire grating can be chosen to fit on top of an available plastic animal bin.*

sary. Most commercially available restraining devices[30] resemble the Bollman-type confining tubular devices (261, 262, 395, 436–439, 471).

Each of these confining tubular devices: (a) is able to accommodate only a limited-size range of rats, (b) can accumulate feces in the rear of the cage, (c) imposes total restraint on rat movements, and (d) does not allow access to the rat for periodic oral gavage administration of xenobiotics. The novice will quickly discover that the conscious rat can not be removed effortlessly from any very confining device.

Scheline (471) modified the Bollman cage to make it adjustable to rat size and to allow urine collection. Similar characteristics are provided by a plastic stall restraining device (262). Davis and Coleman (442) used an adjustable, rectangular, plastic restraining cage for holding instrumented 200–400-g rats for up to 8 hr. Cotlove (261) used a tubular restraining cage from which the hind feet and tail protruded to collect urine during intravenous infusion into the lateral tail (caudal) vein. An adjustable cable-type restraining device was designed to restrict motion to facilitate undisturbed percutaneous absorption of substances applied topically to 200–300-g rats for $\leq$ 72 hr (115). Wilson (439) cut an opening in the top of a cardboard mailing tube to facilitate subcutaneous injection in the back of a restrained rat and access to the protruding tail for injection or blood sampling. To facilitate rapid removal of the rat from this tube, the distal end opened into a standard rectangular cage.

In designing an alternative to the Bollman cage to facilitate lymph collection, Baker *et al.* (472) placed the rat in a plexiglass rectangular cage with only the buttocks and tail restrained. This cage enabled the rat to move all but the hindquarters, allowed urine and feces to fall freely into a collecting vessel, enabled collection of lymph *via* a cannula exteriorized in the inguinal region, allowed access for oral gavage administration without removal of the rat from the cage, and accommodated rats ranging from 200 to 450 g.

As another alternative to the confining tubular devices, persons with minimal skill in the use of common hand tools (pliers and wire cutters) and a supply of inexpensive galvanized steel-wire cloth (available in most hardware stores) can readily construct the simple and durable restraining device described by Girardet (440). Figure 6 illustrates a modified version of this device, which was constructed to lie on top of a widely available plastic cage. This restraining device was constructed in ~1.5 hr and cost <$1.00 for materials (excluding plastic cage). By locating the wooden stick applicators at the buttocks of the rat, one device will accommodate rats of various body sizes. Similar characteristics are provided by a recently described restraining device which is inexpensively constructed from one small plexiglass plate (444). This device offers the advantage of enabling optional separate collection of urine and feces during restraint, but has the disadvantage of restraining the rat by greater immobilization of the limbs than used with the device illustrated in Fig. 6. Prolonged exposure to excessive restraint has been associated with increased incidence of pathological events as described in a later section.

Momentary Restraint—Momentary restraint is often essential for injection, blood sampling, collection of a vaginal smear, or artificial insemination. Such acute restraint can be accomplished by properly holding the rat in one hand (Fig. 2), using a protective glove when animal biting is a problem. Since various individuals prefer leather gloves (available in hardware stores), butcher's metal-mesh boning gloves[31] (473), or gloves made of 29 aramid[32] (474), each animal handler should try different gloves and use that which proves personally preferable.

Two alternatives to holding the rat are allowing the animal to crawl into a close-fitting restraining sack (435), polythene tube (441), or plastic cone[33], and wrapping the rat in a towel with the head, neck, and tail exposed (6). Use of these methods is restricted by the limited region of the body which remains accessible. The use of tubes into which the rat will crawl enables convenient collection of blood or injection *via* tail blood vessels by a single experimenter (441). Transillumination of the tail may also be helpful in visualizing the blood vessels (475).

Restricting Motion—Restriction of certain body motions without spatial confinement is often useful in preventing the rat from disturbing indwelling cannulas, irritating surgical wounds, or consuming fecal material (coprophagy). Graham (450) described a small box which allows the rat to hide its head for up to 1 hr, thereby facilitating investigator access to a cannula emerging from the back of the neck. Coprophagy-preventing devices and Elizabethan collars are described below.

Coprophagy and Its Prevention—The degree of coprophagy by the rat is recognized as a major variable in certain nutritional studies, particularly those involving vitamins B and K, since these are ingested by the rat following synthesis by intestinal bacteria (476–479). In fact, this behavioral drive is so strong that healthy rats consume on the average 35–65% of their total fecal output (480–482). The extent of coprophagy was most dramatically demonstrated by Greaves (483), who observed that unrestrained

[30] Model 700R rat restrainer; Braintree Scientific, Inc., Braintree, Mass. No. 56-4500 rat restrainer; Harvard Apparatus Company, Inc., South Natick, Mass. Model H2284 restraining cage; Hazelton Systems, Inc., Aberdeen, Md.

[31] Model MMG-100 metal-mesh gloves; Braintree Scientific, Inc., Braintree, Mass.

[32] Kevlar; E. I. DuPont de Nemours and Co., Inc., Wilmington, Del.

[33] Model DC-200 DecapiCones; Braintree Scientific, Inc., Braintree, Mass.

rats fed a vitamin K-free diet for 180 days remained healthy and still excreted appreciable amounts of vitamin K in their feces. Unfortunately, many scientists in areas other than the nutritional sciences are unaware that coprophagy is essential for the normal growth and development of young rats (484).

Since the rat primarily consumes feces immediately after extrusion from the anus (482), the raised wire-mesh floors in many metabolism cages are ineffective in preventing coprophagy (482). Clearly, this fecal–oral recycling may be critical to mass balance studies, quantification of drug excretion routes, and pharmacokinetic studies. Three methods have been devised to inhibit coprophagy (485): (*a*) tubular cages restricting flexion and lateral motion (481, 486), (*b*) a tight-fitting leather jacket restricting flexion (448), and (*c*) placement of a plastic cup over the tail and anus of the rat to catch fecal pellets, thus rendering them inaccessible to the rat (480, 487, 488). In addition, the Elizabethan collar may be useful in inhibiting coprophagy.

Elizabethan Collars—The Elizabethan collar is easily constructed and very useful for restricting motion to prevent the rat from disturbing indwelling cannulas, irritating surgical wounds, or consuming fecal material (Fig. 7). The collar consists of an adjustable plastic or reinforced cardboard cone which fits snugly around the neck and encloses the head in the body of the cone, thereby preventing scratching of the head, licking or biting other parts of the body, and coprophagy (10, 446, 449). The desired collar size can be calculated by:

$$\text{collar i.d.} = \frac{\text{neck circumference}}{\pi} \qquad \text{(Eq. 3)}$$

and

$$\text{collar o.d.} = \text{collar i.d.} + \text{desired depth} \qquad \text{(Eq. 4)}$$

Metabolism Cages—A metabolism cage is a unit used to house an individual rat to facilitate quantitative separate collection of urine and/or feces, measurement of food and/or water intake, quantitative collection of expired respiratory carbon dioxide, and/or containment of radiolabeled substances administered to the rat. Since most of these cages confine the animal to a relatively small defined space without immobilization, metabolism cages also provide some degree of cannula protection when housing rats with indwelling cannulas. Generally, rats will tolerate well a relatively short period in any metabolism cage. However, experiments with durations >12 hours necessitate the use of metabolism cages that enable adequate air flow to the rat, ready access to food and water, and easy retrieval of the collected urine and feces. In addition, a slightly higher room temperature (23–26°) may help prevent respiratory disease during confinement (456).

Grantham *et al.* (235) noted that rats rest easier when maintained in square, rather than circular, metabolism cages since this shape allows the rat to sleep in a corner. Lazarow (456) has provided an extensive review of the many aspects involved in designing and building metabolism cages for the rat, mouse, dog, and monkey. The basic aspects of metabolism cages have not changed much over the years (except for the availability of various polymeric construction materials such as plexiglass and Nalgene[34])

[34] Nalge Company, Rochester, N.Y.

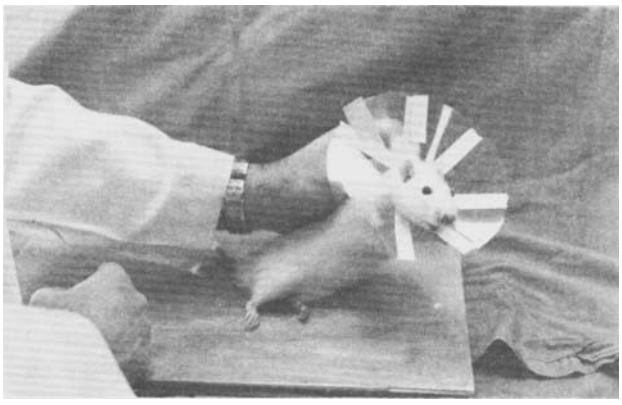

Figure 7—*An adult rat fitted with a plastic Elizabethan collar (highlighted with white tape strips for photographic clarity) around the neck. This collar (3.0-cm i.d., 16.0-cm o.d.) was cut from a rectangular plastic sheet usually used for transparencies.*

and the individual interested in home-built metabolism cages will find the detailed illustrations in this review extremely useful despite their age. A round wire-mesh cage using a funnel which contains a screen or shield for urine and feces separation, and a frame holding six such cages is described. Similar inexpensive wire cages have been described by others (452, 453, 458, 487). Suspended steel cages and a rack accommodating 36 such cages have been described (456). This unit is similar to many commercially available racks of suspended steel metabolism cages. Lazarow (456) also described cages which could be easily disassembled following studies with radioisotopes for thorough cleaning and decontamination, as well as a cage used for the quantitative collection of expired respiratory carbon dioxide.

Meticulous Urinofecal Separation—Cross contamination of urine and feces must be meticulously prevented in studies of elimination routes when the excreted substance can appear in both excretory products or when a drug excreted in the urine could be biotransformed by enzymes (such as β-glucuronidase) in the feces (348b). These situations commonly arise when studying the excretory routes of radiolabeled substances (454) and when assaying urinary activity of enzymes also present in large quantities in the feces (460a). The plastic stockade type of metabolism cage (454, 456, 460a) has been shown to meticulously prevent such cross contamination. Unlike most metabolism cages, it appears that this cage would prevent cross contamination even in the presence of diarrhea.

Leathwood and Plummer (460a) quantified the degree of fecal contamination of urine collected *via* three conventional metabolism cages, which used the following means of urinofecal separation: (*a*) a hollow, somewhat ellipsoidal glass device over which the urine flows for transfer into a collection beaker while the feces bounce off into another path (type I separator), (*b*) a urine collecting gutter, which diverts the falling urine into a collection beaker while the feces fall downward into a beaker (type II separator), and (*c*) a stockade-type metabolism cage (454, 456). Fecal contamination of the urine was most severe for the type I separator (which is used in several commercially available metabolism cages[35]). The type I

[35] Maryland Plastics, Inc., Federalsburg, Md. Model number 650-0100 metabolic cage; Nalge Company, Rochester, N.Y.

separator also failed to separately collect ~60% of the voided urine. The type II separator was fairly efficient in separating the urine and feces and in collecting urine. Efficient urinofecal separation was afforded by the stockade-type cage. However, the adverse effects (anorexia, weight loss) of strict restraint for >2 days necessitates only brief confinement of the rat to this type of cage.

Low-Temperature Urine Collection—The metabolism cage designed and illustrated by Lartigue *et al.* (463) is useful for the collection and rapid freezing of urine in cases where a voided substance is subject to temperature-sensitive degradation. This cage uses a urine collection dish immersed in propylene glycol to enable rapid freezing (−19°) of urine. Since propylene glycol is nonflammable and relatively nontoxic, it is preferred to other methods using flammable and irritating heptane and dry ice as the coolant (489, 490).

Miscellaneous Metabolism Cages—A number of metabolism cages necessitate only moderate confinement of the rat while allowing visual observation of rat behavior and providing separate collection of urine and feces, with reduced possibility of urinofecal cross contamination for the animal excreting well-formed feces (456, 457, 459, 461–463). One of these metabolism cages (459) is inexpensive to construct from widely available materials. This cage has the advantages of being easy to disassemble for cleaning and readily mountable on multi-cage racks. Lambooy (459) has provided detailed step-by-step directions for construction.

Jensh *et al.* (491) described a plexiglass cage useful for observing unrestrained pregnant rats in an anechoic, microwave-transparent chamber, which does not alter a number of potentially environment-dependent variables. Grantham *et al.* (235) built a square cage from plexiglass and wire mesh to facilitate intra-arterial infusion in relatively unrestrained, unanesthetized rats *via* a nonswivel spring attachment.

Accurate quantification of food and fluid intake is often difficult due to spillage of the food pellets and fluid into various regions of the cage. In addition, it is sometimes desirable to strictly minimize food contamination of the collected urine and feces. Guest *et al.* (455) described a metabolism cage constructed from an inverted 11.4-liter (3-gallon) narrow-necked glass bottle into which a wire-mesh floor was inserted. A fine mesh screen positioned below the floor enabled separation of urine from feces. A special detachable feeder was readily constructed from sheet metal, a 50-ml beaker, and wire mesh. This feeder minimized the amount of food dropped outside the feeder (<1% of food intake per day) and, therefore, was useful in quantifying daily food intake. In addition, this feeder minimized food contamination of the collected feces and urine. This basic type of feeder design has been incorporated into one commercially available metabolism cage[36]. To aid in accurate quantitation of fluid intake, Robbins (492) fit a modified plastic syringe with a sipper spout made of 8-mm o.d. glass tubing. Use of an appropriate size plastic syringe enables achievement of the desired degree of accuracy with respect to fluid consumption measurement.

Recently, Toon and Rowland (464) described a tubular plexiglass cage that allowed longitudinal movement by the rat, separate urine and feces collection, and access to various cannulas for up to 8 hr of confinement. Although the virtual necessity of workshop construction by skilled personnel and the relatively high cost are disadvantages, the unique features of this cage may warrant the investment by some laboratories.

Effects of Restraint—Often, observation of hyperactivity, sitting in a corner of the cage, scratching at a harness, decreased curiosity and exploratory behavior, free micturition and defecation, and/or passage of soft stools may suggest stress in a rat (460a, 493). The stress associated with various physical restraints (tying the conscious rat to a surgical board, housing in a restraining cage, application of a body cast, placement in a harness) or periodic shocks from an electrified cage has been shown in the rat to be associated with neutrophilia (approximately a sevenfold increase after 6 hr of restraint) (494), decreased whole blood clotting time (495), diminished altitude tolerance (496), occurrence of stomach lesions after as little as 48 hr of restraint with a greater frequency and severity noted in restrained rats housed in groups (497, 498), increased gastric acid concentration (499), decreased growth rate (493, 498), decreased daily food intake (493), and hyperactivity (493). Of particular interest is a study documenting the stress response associated with placement in a body harness (500) for chronic intravenous infusion (493). These stress-associated pathophysiologic events must be considered in designing experiments requiring extended restraint or harnessing of the rat.

CONCLUSIONS

The vast literature on methods of vascular access and collection of various body fluids has been reviewed. The investigator new to these methodologies is advised to gather a minimal but essential resource library consisting of papers providing highly detailed and illustrated accounts of cannula construction and implantation (13, 15, 501); aseptic surgical methods (23); rat resuscitator assembly (105, 106); a general review providing information on animal handling, anesthesia, and euthanasia (6, 10, 12, 21, 22); a well-illustrated anatomy text (31); and information on metabolism cages (456). It is hoped that this literature survey provides a useful summary of practical information and a valuable description of alternative methodologies to experimental scientists[37].

ADDENDUM

Since the submission of this manuscript, a textbook of experimental techniques for the rat has been published (C. Petty, "Research Techniques In The Rat," Charles C Thomas, Springfield, Ill., 1982). This textbook includes a broad range of experimental methods and a multitude of useful illustrations. This book is well worth its price for both the novice and experienced researcher.

Two additional publications of interest are a useful tabular compilation of LD_{50} values in several species of

[36] Model no. 650-0100 metabolic cage; Nalge Company, Rochester, N.Y.

[37] On a very limited basis, the authors will make available a photocopy of these references complete with each citation's title, all authors, and full pagination. This offer can be extended only to those scientists and information services with a serious need for such information.

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