transonic

THE MEASURE OF BETTER RESULTS.

Tools & Techniques for Hemodynamic Studies in Rodents

www.transonic.com

This workbook presents protocols, tools, tips of the trade and application support for successful hemodynamic measurements in rodent models. The information in the workbook synthesizes and updates material of two earlier workbooks, **Tools and Techniques for Hemodynamic Studies in the Rat** and **Tools and Techniques for Hemodynamic Studies in the Mouse**.

Included is introductory material about experimental design, acute versus chronic models, anesthesia, aseptic techniques, post-surgical care, and study models. A comprehensive chapter of direct blood volume flow measurements with gold standard transit-time ultrasound technology follows. The chapter includes equipment, technical notes and numerous acute and chronic protocols for measuring volume blood flow in rat and mouse vessels. A brief chapter on pressure measurements follows. The final chapter focuses on tissue perfusion blood flow measurements using laser Doppler technology.

We gratefully acknowledge the paramount financial assistance of the National Institutes of Health (NIH) under whose grants the miniaturized sensors and measurement techniques used to measure flow and pressure in small animal models were developed.

We also wish to thank Thomas L. Smith, PH.D. and Michael F. Callahan, Ph.D from Wake Forest University School of Medicine, Department of Orthopaedic Surgery, Winston-Salem, NC. It is their unparalleled small animal surgical expertise and gracious information sharing that makes it possible to present you with these “tools and tricks of the trade” for direct blood volume flow measurement with transit-time ultrasound technology in small animal models. Their collaborative protocols and data are copywritten by Wake Forest University School of Medicine, Department of Orthopaedic Surgery and are used with permission.

We appreciate the feedback of our many customers whose studies form the foundation for the included application protocols and whose quest for solid scientific data continues to stimulate ongoing product improvements.

transonic
THE MEASURE OF BETTER RESULTS.
www.transonic.com

Transonic Systems Inc. is a global manufacturer of innovative biomedical measurement equipment. Founded in 1983, Transonic sells “gold standard” transit-time ultrasound flowmeters and monitors for surgical, hemodialysis, pediatric critical care, perfusion, interventional radiology and research applications. In addition, Transonic provides pressure and pressure volume systems, laser Doppler flowmeters and telemetry systems.

AMERICAS
Transonic Systems Inc.
34 Dutch Mill Rd
Ithaca, NY 14850
U.S.A.
Tel: +1 607-257-5300
Fax: +1 607-257-7256
support@transonic.com

EUROPE
Transonic Europe B.V.
Business Park Stein 205
6181 MB Elsloo
The Netherlands
Tel: +31 43-407-7200
Fax: +31 43-407-7201
europe@transonic.com

ASIA/PACIFIC
Transonic Asia Inc.
6F-3 No 5 Hangsiang Rd
Dayuan, Taoyuan County
33747 Taiwan, R.O.C.
Tel: +886 3399-5806
Fax: +886 3399-5805
support@transonicasia.com

JAPAN
Transonic Japan Inc.
KS Bldg 201, 735-4 Kita-Akitsu
Tokorozawa Saitama
359-0038 Japan
Tel: +81 04-2946-8541
Fax: +81 04-2946-8542
info@transonic.jp

Table of Contents

transonic
THE MEASURE OF BETTER RESULTS.

Table of Contents Cont.

transonic
THE MEASURE OF BETTER RESULTS.

Table of Contents Cont.

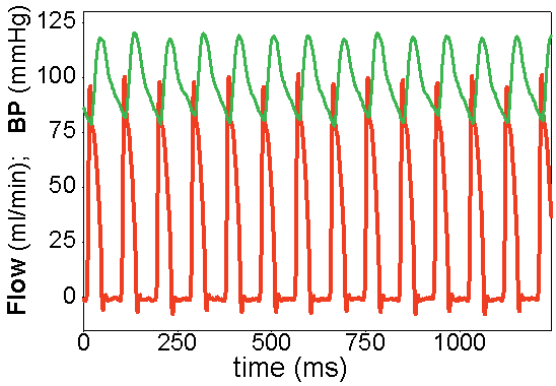
transonic
THE MEASURE OF BETTER RESULTS.

Introduction

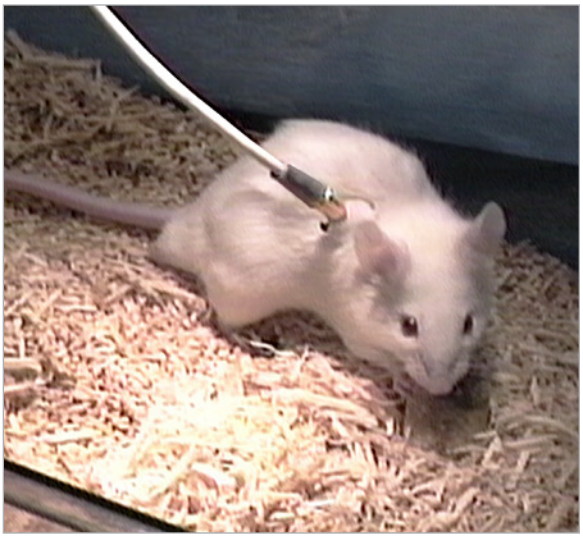
Over the past quarter century, small rodent models have become the animal models of choice for much life science research. Genetic manipulations to alter cardiovascular function have made mice particularly attractive models and has underscored the need for accurate hemodynamic measurements. A mouse's small size makes it difficult to assess its hemodynamic parameters. This has propelled the development of miniaturized sensors and techniques to measure direct volume flow and pressure in the mouse.

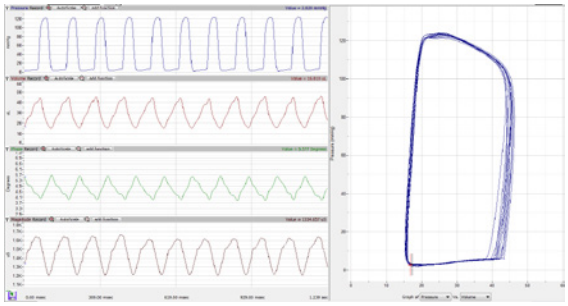
As the leading supplier of blood flow instrumentation, Transonic® has collaborated with the scientific community and shares the responsibility to document and make available procedures for the effective use of its transit-time ultrasound blood flow measurement technology. With our incorporation of Scisense into the Transonic® family we have expanded our product line to include pressure and pressure-volume measurements using solid-state Catheters.

In this updated edition of the "Rodent Workbook" we address some of the challenges of working with these scientifically powerful rodent models and expand our pressure section for a more complete discussion of hemodynamic measurements. We are pleased to present you with a composite of these New Tools and Techniques for Hemodynamic Studies in Rodents and invite your feedback and contributions for the next edition of this workbook.



Ascending aortic blood flow & pressure in a conscious mouse 7 days after implantation. Courtesy, B. Janssen, University of Maastricht, Maastricht, The Netherlands





Mouse baseline Admittance PV Loop with Pressure, Volume, Phase and Magnitude signals.

Experimental Design: Overview

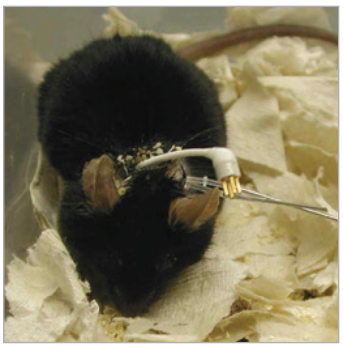
By Thomas L. Smith, Ph.D.

Answers to experimental questions are developed according to results from experimental protocols. The challenge is to address a question so that the answer is valid. Analysis of these results must, therefore, take into account the experimental conditions under which they were obtained. Moreover, an interpretation of results is always limited to the confines of the experimental conditions. There are several approaches to experimental design to try to realize the most valid answers.

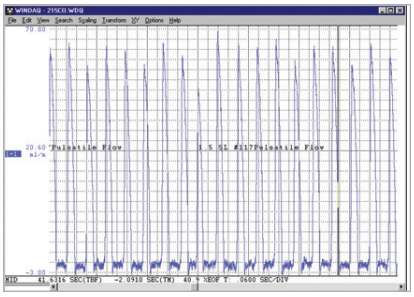
The simplest designs to execute are acute experiments in which all variables are controlled except for the variable under examination. In experiments with animals this usually means that anesthesia is used. If the experimental question involves the cardiovascular system, then the effects of anesthesia on cardiovascular parameters have to be taken into account.

Truly long-term, chronic experiments to study the cardiovascular system can be designed to examine the variables in question in intact conscious animals. These experiments can be elegant but there are many new variables which must now be controlled such as light/dark cycles, environmental stimuli such as temperature and noise, olfactory stimuli and potential long-term stressors such as cage bedding, social interactions and other behavioral factors. One can also quickly appreciate that in a design in which repetitive measures are made in each animal over time that loss of a data point in a specific animal can effectively remove that animal from the study, at a great expense in lost time.

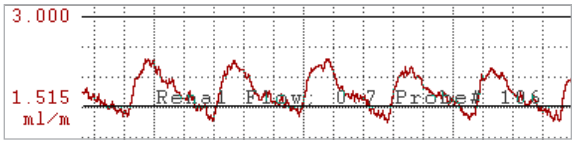
Acute experiments in chronically instrumented mice: These experiments take advantage of short-term observations but avoid anesthetics and acute surgical stress.



Flowprobe connector and catheter exit from C57BL6 Mouse. Courtesy, B. Janssen, University of Maastricht, Maastricht, the Netherlands



Chronic CO in the mouse: 1.5PSL Probe on Ascending Aorta

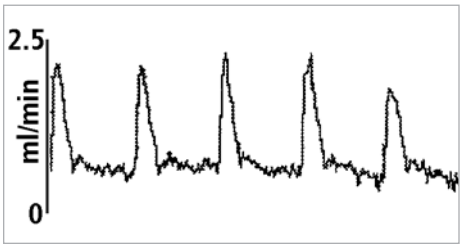


Conscious mouse renal arterial blood flow 1 day after implant

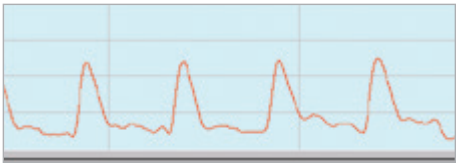
Experimental Design: Overview Cont.

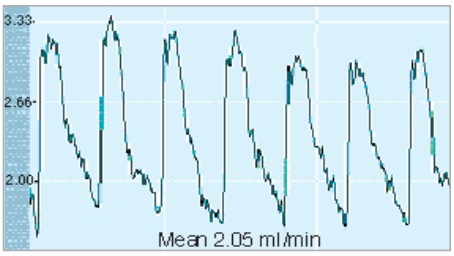
Approaches: Acute Versus Chronic Design

1. Acute Experiments:
   - Simplest to execute but often the most difficult to design
   - In the perfect design all variables are controlled except for the variable being examined
   - In acute animal experiments, the design usually calls for the use of anesthesia which often leads to components of the experiment which are difficult to explain, because anesthesia can have powerful effects on the cardiovascular system.
   - Ether, pentobarbital sodium and clorose urethane affect mean arterial pressure, cardiac output, heart rate and total peripheral resistance
   - Anesthesia also has strain dependent effect, producing differing cardiovascular effects on different strains with different anesthetics
   - Therefore, the challenge is to design an acute experiment so that anesthesia is less important
2. Chronic Experiments: An Alternative to Acute Experiments
   - Instrument – 5-7 days recovery – follow over time
   - 24-hour hemodynamic considerations such as diurnal variation which must be taken into account by monitoring lights and other environmental stimuli
   - Primary consideration: how many days the experiment should last; although one can think of many elegant experiments, they can end up being costly if there is failure on day 5 or after.
   - Perhaps the most practical type of experiment is one which performs acute observations on chronically instrumented animals. Chronic studies in mice necessitate the availability of instruments appropriately scaled for small implants.



Acute mouse carotid artery flowtrace


Typical mouse chronic femoral artery trace



Conscious mouse renal arterial blood flow
4 days after implant of 0.5PSL Flowprobe

Anesthetic Guidelines

Considerations for Choosing Anesthetic Agents

A. Considerations Related to the Procedure

1. The type of procedure
2. The projected length of the procedure
3. The amount and type of pain/distress anticipated
4. Study goals are important parameters influenced by certain drugs?)
5. Survival or terminal study (agents associated with prolonged recovery or delayed effects may be approved for terminal studies while deemed inappropriate for survival procedures)

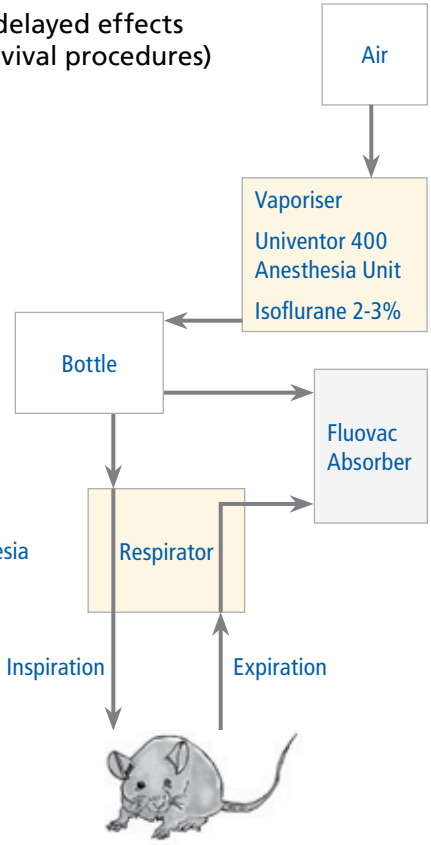
B. Considerations Related to the Animal

1. Species and strain
2. General condition and underlying health problems
3. Age
4. Sex
5. Weight
6. Previous Drug Exposure
7. Nutritional Status
8. Time of day as related to circadian rhythm
9. Numbers of animals to be anesthetized simultaneously

Note: There can be remarkable variation in response to anesthesia. Investigators should monitor anesthesia closely in each animal and make appropriate modifications in the anesthetic regimen when necessary

C. Considerations Related to the Drug(s) Used:

1. Drug Safety and ease of use
2. Appropriateness for the procedure
3. Appropriateness for the animal
4. Side effects
5. Equipment and training required for safe use
6. Previous experience using the agent(s)
7. Cost and status as controlled or uncontrolled drug



Isoflurane inhalation system for mouse anesthesia.

Summary: Anesthetic Agents Should:

- Provide an appropriate depth and length of anesthesia and analgesia without affecting important study parameters
- Be appropriate for the animal given its species, medical history and physical condition
- Have minimal side effects
- Be safe for both the animal and the personnel administering anesthesia

transonic
THE MEASURE OF BETTER RESULTS.

Anesthetic Guidelines Cont.

Anesthetics

Several anesthetic agents have been taken off the market or are no longer in use. Anesthetic agents must be balanced with proper analgesics. The best advice is to check what is currently available and allowed with your Institutional Animal Care & Animal Use Committee and know what affects the drugs will have on the parameters you are interested in studying.

Many common anesthetics have a significant effect on cardiovascular measurements and can obscure or confound study results; sometimes over a longer period of time than anticipated. A study by Janssen, Callahan, Smith, et. al. (Effects of anesthetics on systemic hemodynamics in mice. Janssen BJ, et al., Am J Physiol Heart Circ Physio. 2004 Oct;287(4):H1618-24.) examined and compared the direct systemic hemodynamic effects of isoflurane, ketamine/xylazine, pentobarbital Na and urethane on cardiac output, heart rate, stroke volume and arterial pressure in conscious mice implanted with ascending aorta Flowprobes. Their study confirmed that isoflurane preserved cardiac function better than other anesthetic regimens, and that ketamine/xylazine mixtures had "potent cardiodepressive effects" suppressing cardiac index by as much as 68±2%. These data underscore the importance of knowing the effects of the anesthetic and analgesic that will be used and whether these will impact the measurement results you will be recording.

CATEGORY	AGENT	DOSE (MG/KG)	ROUTE
Anticholinergics	Atropine	0.04 - 0.1	SQ
Tranquilizers	Acepromazine	1 - 10	IM
	Chlorpromazine (ThorazineR)	1 - 20	IM, P
Analgesics (do not put in water)	Acetaminophen	100 - 300	PO
	Aspirin	100 - 120	PO
	Meperidine (Demerol HC1R)	5 - 40	SQ, IM
	Morphine	5 - 10	SQ, IM
	Pentazocine (Talwin-VR)	8 - 12	SQ
Anesthetics - Injectable	Chloral hydrate	300 - 400	IP
	Alpha-chloralose[1]	55	IP
	Fentanyl & droperidol (Innovar-VetR)	2	IM
	Ketamine HCl	44	IM
	Ketamine HCl/Acepromazine	40 - 80/2.5	IP
	Ketamine HCl/Diazepam (ValiumR)	40 - 80/5 - 10	IP
	Ketamine HCl/Xylazine (RompunR)	80/12	IP
	Pentobarbital sodium (NembutalR[2])	30 - 50	IV, IP
	Telazol	20 - 30	IM, IP
	Thiamylal (SuritalR)	25 - 50	IV, IP
	Thiopental sodium (PentothalR)	20 - 40	IV, IP
	Urethane[1]	780 - 1250	IP
Anesthetics - Inhalent[3]	Isoflurane (ForaneR)	To effect	
	Methoxyflurane (MetofaneR)	To effect	
Euthanasia	CO_2 Pentobarbital	150 - 200	IP

1. Terminal Studies only.
2. Dilute stock solution to accurately dose animals
3. These agents should be used only in ways that prevent exposure to personnel. Induce anesthesia in a closed container and maintain with a nose cone in an appropriately ventilated hood.

ANESTHESIA

An anesthetic mixture of ketamine and xylazine works well in mice. The margin of safety and rapid recovery are positive features.

Isoflurane is also an excellent anesthetic but the cost of the equipment necessary to safely use this volatile anesthetic is high. The surgeon should be careful to work in a vented hood to reduce exposure during procedures.

IDEAL ANESTHETIC AGENT

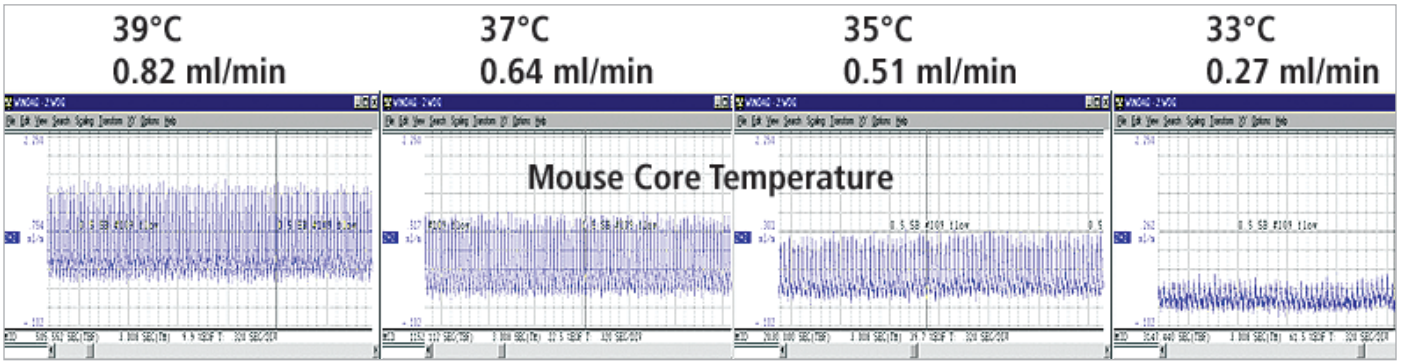
- Reliable
- Wide safety margin
- Rapid onset/rapid recovery
- Easy to administer & control
- Nontoxic
- Causes no physical impairment
- Produces analgesia and muscle relaxation

Anesthetic Guidelines Cont.

Body Temperature

The high metabolic rate and high surface-to-volume ratio of mice means that they lose heat very quickly. It is therefore imperative to avoid anesthetics such as barbiturates, which alter the animal's ability to maintain core temperature.

Similarly, the animal should be warmed during operative procedures which open a body cavity and expose even greater surface area to ambient temperatures for heat loss. Body temperature should be monitored during heating to avoid increasing body temperature above 38°C.



Effect of Core Temperature On Femoral Blood Flow in a 22 gram CD-1 Mouse: As the effect of progressive lower core temperatures in the respective flow traces demonstrates, temperature has a profound effect on femoral blood flow and must be monitored.

Data, courtesy of M.F. Callahan, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC

ACKNOWLEDGEMENT

Thomas L. Smith, Ph.D., Bowman Gray School of Medicine, Dept. of Orthopedic Surgery, Wake Forest University, Winston-Salem, NC.

Aseptic Surgical Guidelines

The following protocol used at Bowman Gray School of Medicine is consistent with the Public Health Service guidelines.

1. Room Preparation

a. The surgery table surface should be wiped with a disinfectant before and after use. A bluepad/ drape should cover the table.

b. The use of a heating pad is recommended to prevent hypothermia and to aid in a quicker, uneventful recovery.

2. Animal Preparations

a. Remove hair from surgical site with clippers or a depilatory.

b. The surgical site should be cleaned with an antiseptic scrub followed by an antiseptic solution (e.g. chlorhexidine or povidone iodine scrub and solution, respectively).

3. Instrument Sterilization

a. All instruments must be sterilized. The method of choice will be determined by the surgical instruments or devices being used.

4. Surgeon Preparation

a. The surgeon and all others in the operating room must wear a surgical face mask prior to initiation of animal prep. The surgeon is required to wear sterile gloves; a cap and sterile gown is also recommended.

b. The surgeon must wear a scrub shirt. Non-surgeon personnel in the room must wear a lab coat or a gown over their street clothes.

5. Draping the Animal

a. Draping material for the rodent includes a 15"x 15" reinforced paper drape and 3"x 3" gauze sponges.

6. Closure of the Animal

a. Abdominal/thoracic body wall should be closed with absorbable suture material in a simple interrupted or similar interrupted pattern.

b. Skin should be closed with non-absorbable, monofilament suture material in a simple interrupted or similar interrupted pattern.

c. Sutures must be removed 7 to 10 days after surgery to prevent inflammation and other postoperative complications.

7. Animal Recovery

a. Recovery should occur in a warmed environment.

b. Animals should be observed closely until they are able to maintain a sternal position and then every 6-8 hours until fully recovered.

c. Post-operative antibiotics should be given after surgery when justified by the investigator and the veterinary staff.

8. Multiple Surgical Procedures

a. After the first surgery, the sterilized instruments must be kept in a sterile tray containing 70-90% ethyl or isopropyl alcohol or other acceptable solution.

b. Sterile gloves must be changed between surgeries.

ASEPTIC SURGERY

- Follow recommended guidelines & common sense.

Healthier animals yield better science.

- Sterility: follow aseptic techniques; all instruments and gloves, mask, drapes, implants, sutures should be sterile.
- Use top quality surgical instruments: buying the instruments constitutes an initial investment, but the instruments will last for a long time if properly maintained.

REFERENCE

Principles of Proper Laboratory Use in Research and Teaching, Wake Forest University, Bowman Gray School of Medicine, 1992

Surgical Recovery

Principles of Proper Laboratory Use in Research and Teaching,
(Wake Forest Univ., Bowman Gray School of Medicine, 1992)

During the post-surgical period, a record must be kept in the room where the animal is housed. It should include a brief description of the surgical procedure, anesthetic used, time of induction, duration of surgical anesthesia, and time returned to cage. It should also include the findings of each physical examination during the recovery period. The post surgical medical record will be retained as a part of the animal's permanent medical record. It is best to keep experimental notations in a separate location.

For medical monitoring purposes, it is helpful to stage animals according to extent of recovery from surgery and anesthesia. The animal should be examined and the findings recorded according to the following schedule:

Stage 4 - Animal unconscious or semiconscious and unable to sit or maintain sternal recumbency.

a. Examine and record findings no less frequently than every 2 hours. More frequent examination is recommended. Examples of notations include:
   i. Body temperature
   ii. Heart rate
   iii. Respiratory rate
   iv. Capillary refill time (record in seconds)
   v. Jaw tone (record resistance or no resistance)
   vi. Response to toe pinch (record withdrawal or no withdrawal)
   vii. Time of extubation
b. Animal should be turned from side to side frequently to prevent dependent pulmonary congestion & edema.
c. Ambient temperature should be adjusted (heat lamp or warming board) to bring body temperature to normal. Take care to not burn or over heat animal at this stage of recovery. The animal should be kept dry.
d. The state of hydration should be assessed and fluids should be provided as necessary.

Stage 3 - Animal conscious & can maintain sternal recumbency to sit, but can not stand

a. Examine and record findings so less frequently than every 6-10 hours depending on the nature of the surgery and the status of the animal.
   Examples of notations include:
   i. Body temperature until it becomes normal+ 2°F.
   ii. Capillary refill time
   iii. Condition of the operative site
b. Examine closely for other abnormalities
c. Keep the animal dry and adjust the ambient temperature to bring the body temperature to normal
d. Consider use of analgesic medication
e. Professional judgement should be exercised in those cases in which there is difficulty in examinations every 6-10 hours. We recommend that one should be cautious in prolonging examinations of animals in stage 3.

transonic
THE MEASURE OF BETTER RESULTS.

Surgical Recovery Cont.

Stage 2 - Animal can stand and move about but is not eating and drinking normally

a. Examine daily and record findings. Examples of notations include:
   i. Body temperature
   ii. Hydration
   iii. Attitude (alert or depressed)
   iv. Activity (active or inactive)
   v. Food consumption
   vi. Water consumption
   vii. Condition of operative site
b. Examine closely for other abnormalities
c. Consider use of analgesic medication

Stage 1 - Animal active, alert, eating and drinking normally; skin sutures are in place

a. Examine daily and keep a post surgical record of surgical site care until the sutures are removed.
b. Sutures should be removed within 10-14 days of surgery.

Stage 0 - Animal normal and skin sutures removed.

a. Specific post surgical care and record are no longer required.

IDEAL ANESTHETIC AGENT

- Reliable
- Wide Safety Margin
- Rapid onset/rapid recovery
- Easy to administer & control
- Nontoxic
- Causes no physical impairment
- Produces analgesia and muscle relaxation

SUGGESTED ANESTHETIC PROTOCOL FOR MICE

Mix

- 5 ml ketamine (100 mg/ml)
- 1.6 ml xylazine (20 mg/ml)

Administer:

- 0.09 ml solution / 100 g body weight IM (thigh)
- Duration: @ 40 minutes; re-administer at 1/3 dosage

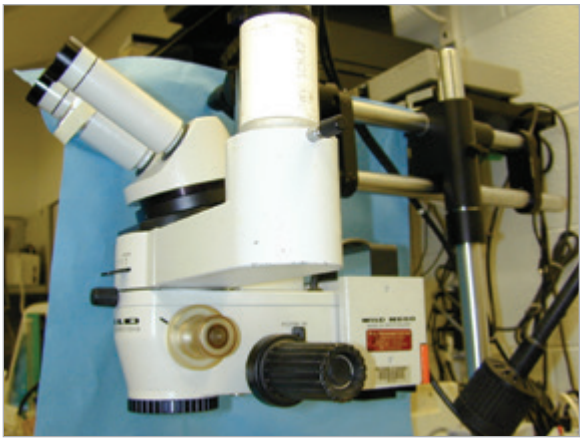
ACKNOWLEDGEMENT

Thomas L. Smith, Ph.D., Bowman Gray School of Medicine, Dept. of Orthopedic Surgery, Wake Forest University, Winston-Salem, NC.

Special Surgical Equipment Needs

Microscope

Equipment needs for mouse surgery vary with the procedures undertaken. Vascular catheterizations are much easier to perform using a stereo micro-scope. Try the microscope in the application you intend to use it in before you purchase it. Working distances and heights vary a great deal between microscopes and not all configurations are appropriate for everyone. Very good surgical loupes can be used for some procedures, but these require adaptation by the user. The focal length is fixed and images are brought into focus by moving one's head. This movement can be distracting until one gets accustomed to it.



A good stereo microscope is recommended for achieving successful measurements in small animals.

Respirator

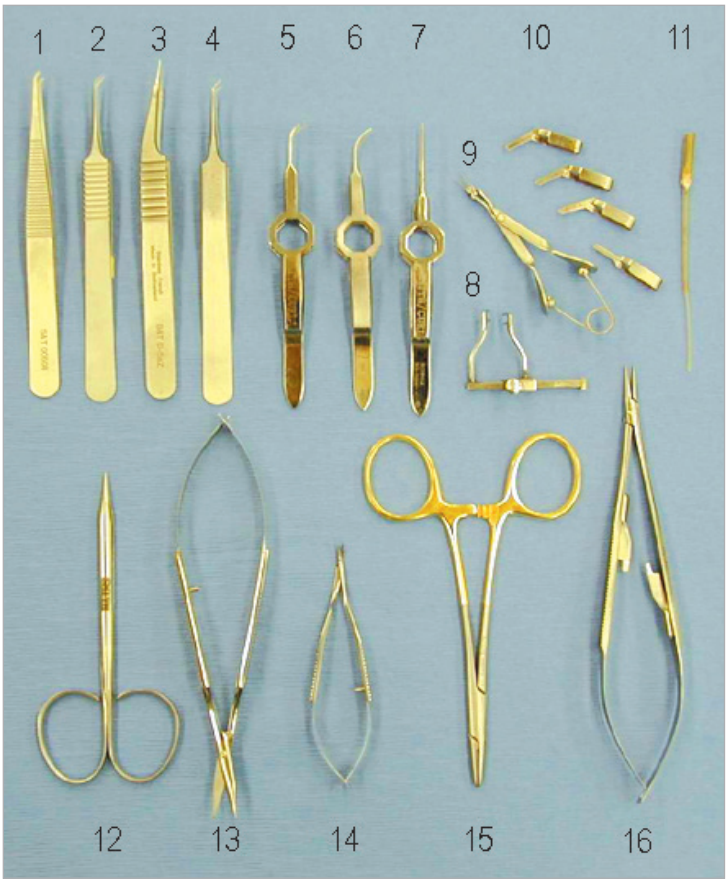
A good rodent respirator is essential for performing mouse thoracotomies. The respirator should provide pressure limits to avoid over infusion of the lungs and should be adjustable (inflation rates, respiratory rates, PEEP).

Surgical Instruments

Finally, surgical instruments for mice are a little smaller, a lot more expensive and very delicate. A common mistake made by investigators is to use instruments that are too big or really cheap. Good advice is to look at a lot of instruments before buying (at scientific conventions), select instruments carefully putting need first, and take extra special care of your instruments once purchased. They should always be cleaned, dried and oiled (if they have moving parts) immediately after use. They should be stored in a safe place. They should not be loaned to others. Good instruments will last many years and are an investment. They allow you to do the work you are trying to perform and pay for themselves in time saved and years of service.

Special Surgical Equipment Needs Cont.

	SURGICAL INSTRUMENT	FINE SURGICAL TOOLS CAT #
[1]	Vessel Cannulation Forceps- mouse catheter	FST # 00608-11
	Vessel Cannulation Forceps- for rat catheter	FST # 00574-11
[2]	#5 45° angle microblunted Dumont	FST # 11253-25
[3]	D-5aZ S&T Vessel Dilator forcep	FST # 00125-11
[4]	#5 45° angle microblunted, atraumatic Dumont altered very blunt tips	FST # 11253-25
[5]	Foerster octagonal: Curved - no teeth	FST # 11041-08
[6]	Foerster octagonal: Curved- with teeth	FST # 11043-08
[7]	Foerster octagonal Straight - no teeth	FST # 11040-08
[8]	Goldstein Micro-Dissecting Retractors 3 x 3 prong (altered to 2 x 2 prong)	FST # 17002-12
[9]	Kleinert-Kutz microclip applying forceps	Pilling Weck
	2 Kleinert-Kutz vessel clips - curved blade 1 x 6 mm (rat)	Pilling Weck
	2 Kleinert-Kutz vessel clips	Pilling Weck
[10]	Schwartz micropserrefines (straight)	FST # 18052-01
[10]	Schwartz micropserrefines (bent)	FST # 18052-03
[11]	Chest Tube	
[12]	Fine Iris Scissors with oversize finger loops - blunt/blunt	FST # 141-08-0908
[13]	Spring Scissors with fine small blades	FST # 141-08-0908
[14]	Extra delicate mini-Vannas (curved)	FST # 15000-10
	Extra delicate mini-Vannas (streight)	FST # 15000-00
[15]	Halsey Micro Needle Holder, extra light and delicate	FST # 12500-12
[16]	Castroviejo with lock and TC jaws	FST # 12565-14
	(Not shown) Edelstein-suture removal	Storz # 5008



Instruments displayed are available from: Fine Science Tools. Courtesy of TL. Smith, Dept. of Orthopaedics, Wake Forest University, Winston-Salem, NC.

Research Equipment Sources

RESPIRATORS

Harvard Apparatus, Inc.
Holliston, MA
ww.harvardapparatus.com

Kent Scientific Corporation
Torrington, CT
www.kentscientific.com

MICROMANIPULATORS / STANDS

Fine Science Tools, Inc.
Foster City, CA
www.finescience.com

World Precision Instruments
Sarasota, FL
www.wpiinc.com

Techni-Tool Inc.
Worcester, PA
www.techni-tool.com

Stoelting Co.
Wood Dale, IL
www.stoeltingco.com

LANGENDORFF APPARATUS

Hugo Sachs Electronik: Harvard Apparatus (see above)

Rattus (Kent Scientific)
(see above)

Radnoti Glass Technology, Inc.
Monrovia, CA
www.radnoti.com

BLOOD PRESSURE

Millar Instruments, Inc.
Houston, TX
www.millarinstruments.com

Data Sciences International
(Telemetry)
Saint Paul, MN
www.datasci.com

INFUSION PUMPS

Razel Scientific Instruments, Inc.
Stamford, CT
www.razelscientific.com/

Rattus (Kent Scientific)
(see above)

SWIVELS

Airflyte Electronics (Aeroflex)
(electronic swivels)
Plainview, NY
www.aeroflex.com

Dragonfly Inc. (electronic swivels)
Ridgeley, WV
www.dragonflyinc.com

Lomir Biomedical, Inc.
Malone, NY / Perrot, Quebec, Canada
www.lomir.com

Harvard Apparatus, Inc.
(see above)

SONOMICROMETERS

Sonometrics Corporation
London, Ontario, Canada
www.sonometrics.com

FLUID INFUSION

Instech Laboratories, Inc.
Plymouth Meeting, PA
www.instechlabs.com

TUBING FOR CATHETERS

VWR Scientific
Radnor, PA
www.VWR.com

Braintree Scientific
Braintree, MA
www.braintreesci.com

CATHETERS

ReCathCo, Inc
Allison Park, PA
www.recathco.com

ADHESIVES

World Precision Instruments
(see above)

Patterson Veterinary
Devens, MA
www.pattersonvet.com

SURGICAL SUPPLIES

Covidien (Kendall)
Mansfield, MA
www.covidien.com
www.kendallhq.com

BD
Franklin Lakes, NJ
www.bd.com

Harvard Apparatus (see above)

CLEANING / DISINFECTING AGENTS

Alconox Inc.
White Plains, NY
www.alconox.com

Ruhof
Mineola, NY
www.ruhof.com

Advanced Sterilization Products (J & J)
Irvine, CA
www.aspjj.com

transonic
THE MEASURE OF BETTER RESULTS.

Renovascular Hypertension Model

INTRODUCTION

The advantages of this rat model over a genetic model are that one can start the hypertension process at the investigator's convenience. Animal costs are lower and one can produce gradations in hypertension by adjusting clip size.

MATERIALS

Prepare arterial clip from pure (0.999) silver (not sterling).

May use silver sheet (0.005 inches thick) or solid silver block.

1. For silver sheet, cut into 2 mm wide strips, 8 - 10 mm long
2. Bend silver strip across thickness gauge (for example, an automotive feeler gauge) of desired size (0.2 mm is conventional) (Fig. 1).
3. Smooth edges.
4. Leave one side slightly longer than the other to facilitate implantation

ANIMAL PREPARATION

Fast animal the night before surgery. Using sterile instruments, prepare animal for aseptic surgery.

SURGICAL PROTOCOL

Expose renal artery through laparotomy. Dissect from renal vein. Apply clip with open end away from renal vein (Fig. 2). Abdominal pressure catheter may be implanted during same operative procedure. Closure is conventional.

RESULTS

Hypertension develops over time with severity and speed of onset determined by sodium in diet and degree of renal artery constriction.

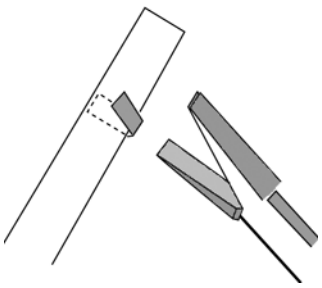


Fig. 1: Clip

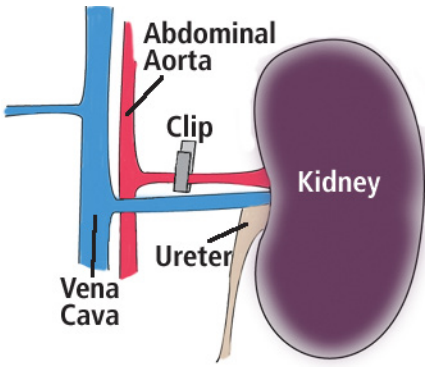


Fig. 2: Renal hypertension model: 2 kidneys, 1 clip (2K1C)

1 Kidney, 1 Clip (1K1C)

- Initial increase in plasma renin activity (PRA)
- Returns to normal in established phase
- Blockade of RA system reduces but does not eliminate hypertension
- 4 weeks after clipping, MAP varied with clip size

CLIP SIZE	MAP
0.3 mm	133
0.25 mm	161
0.20 mm	189

Increasing constriction with decreasing clip size

2 Kidneys, 1 Clip (2K1C)

- Plasma renin activity (PRA) acutely elevated, some find that it stays elevated, others that it returns to normal
- Chronic converting enzyme inhibitor blocks development of 2K-1C and reverses existing hypertension
- 4 weeks after clipping, MAP varied with clip size

CLIP SIZE	MAP
0.3 mm	123
0.25 mm	129
0.20 mm	172*

* Only in 2K1C, 0.2 mm group was PRA increased at 4 wks.

REFERENCE

These techniques were taught by William Murphy at the University of Mississippi.

Aortic Coarctation Hypertension Model

INTRODUCTION

This is an interesting model because it causes hypertension proximal to coarctation but normotension below coarctation in two days.

ANIMAL PREPARATION

Fast animal the night before surgery. Prepare animal for aseptic surgery using sterile instruments.

SURGICAL PROTOCOL

1. Expose abdominal aorta through a laparotomy. Dissect free from the vena cava.
2. Place a 10 x 8 mm piece of sterile Handi-Wrap or Saran Wrap around the aorta at the site of the coarct. This prevents the aorta from growing around the ligature and effectively bypassing the coarctation.
3. Place a blunt hypodermic needle (20 gauge needle for 200 gram rat) along the axis of the aorta over the Handi-Wrap. Tie a length of 3-0 suture around the aorta, the Handi-Wrap and the hypodermic needle (Fig. 1).
4. Tie the suture (3-0 or 3-0 silk) snugly but not so tight as to crush the aorta (Fig. 2).
5. Remove the hypodermic needle, leaving a stenosis in the aorta which is the same size as the hypodermic needle (Fig. 3).

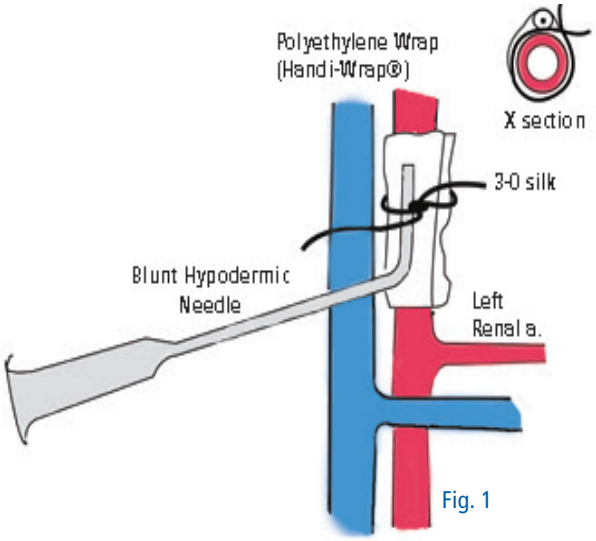


Fig. 1

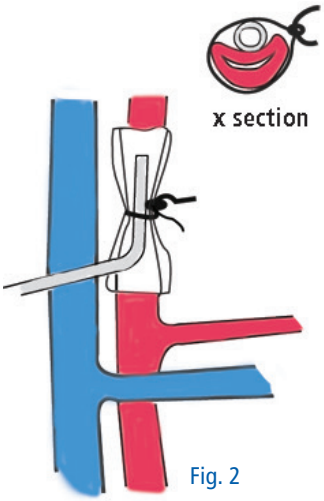


Fig. 2

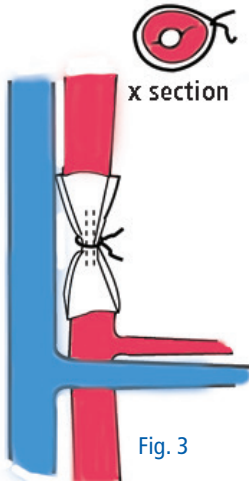


Fig. 3

RESULTS

Hypertension develops in 2 days

Suprarenal - normal renin

MAP 150 (mmHg) in a few days
160 (mmHg) in 1 month
170 (mmHg) in 2 months

Between kidneys - high renin

Subtotal Nephrectomy Hypertension Model

ANIMAL PREPARATION

Fast animal the night before surgery. Using sterile instruments, prepare animal for aseptic surgery.

SURGICAL PROTOCOL

Expose left kidney via laparotomy.

Remove top 1/3 and bottom 1/3 of left kidney (Fig. 1):

a. Have two pieces of gel foam cut to cover the cut surface of the kidney.

b. Cut off upper or lower 1/3 in one stroke and immediately cover with gel foam, exerting mild pressure.

c. After cutting off second pole of kidney, maintain mild pressure on gel foam pads for approximately 1 minute.

Close laparotomy.

1 WEEK LATER

Perform right side nephrectomy by retroperitoneal approach (Fig. 2):

d. Shave back over kidney area.

e. Palpate right kidney.

f. Make longitudinal incision 2 cm long parallel to spine and 1 cm lateral.

g. Blunt dissect through back muscle to expose kidney.

h. Ligate all vessels to right kidney and extirpate.

i. Close skeletal muscle with absorbable sutures.

j. Close skin.

Severity and speed of onset of hypertension can be increased by adding sodium to water or diet.

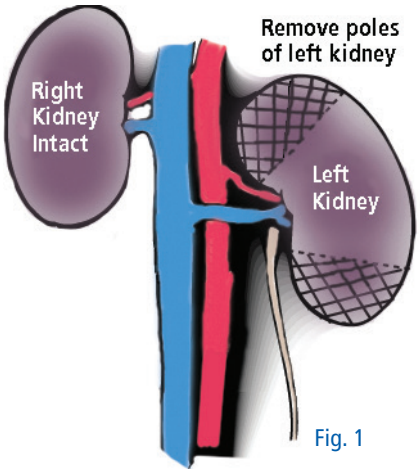


Fig. 1

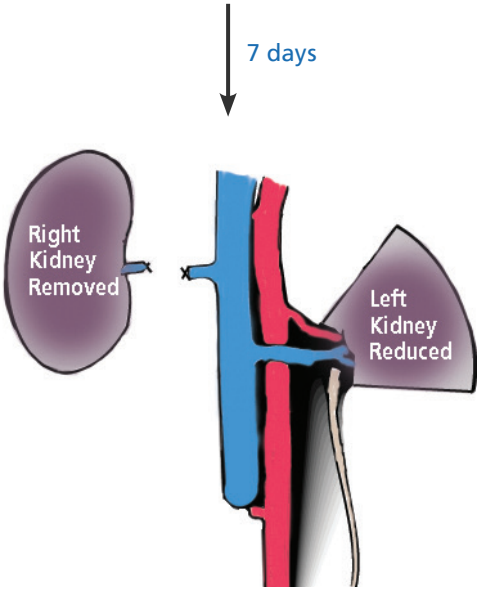


Fig. 2: Subtotal Nephrectomy

Myocardial Infarction Model

Generally this procedure is performed in adult mice > 10 weeks of age. However, it is possible to perform the surgery on younger animals.

PRE-SURGERY

1. The mouse is weighed under aseptic conditions.
2. Pre-operatively buprenorphine is given at 0.1 mg/kg s.c.
3. Anesthesia is induced by placing the mouse in an induction box filled with isoflurane 4-5%. When the anesthetic state is achieved, the mouse is intubated using a blunt needle (19 gauge) and connected to a Hugo Sachs rodent ventilator (type: Minivent, 250µl stroke volume, 4 Hz) while breathing isoflurane (1.5-2%). If gas anesthesia is not possible the mouse can be anesthetized by ketamine (100 mg/kg i.m.) xylazine (5 mg/kg s.c.). Sodium pentobarbital is not used because the use of this agent is associated with higher mortality.
4. The anterior thorax is shaved and disinfected with iodine.
5. If available, two electrodes are placed subcutaneously to monitor the ECG during the procedure.

SURGERY

1. The skin is incised (parasternal) at the level of the left third and fourth ribs.
2. The pectoral muscles are dissected with two fine forceps and retracted gently with 6-0 silk to free the location where the thoracotomy will be made.
3. The thorax is entered about 2 mm lateral of the sternum with small forceps by making a hole through the intracostal muscles and pleura. The opening is enlarged by cutting the intracostal muscles a bit further. For this purpose, the muscles are lifted with small forceps. Care should be taken to not damage the lungs.
4. The wound is opened about 6 mm with a mouse retractor (Fine Science Tools). An absorption triangle is wet in sterile 0.9% NaCl and used to push the lungs gently aside. With two fine forceps the pericardium is opened.
5. Using a microscope, the left anterior descending coronary artery is identified.

ACKNOWLEDGEMENT

J. Debets, A. Brouns, Dr. B. Janssen, Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht, Universiteit Maastricht, Maastricht, The Netherlands

SUMMARY

- Weigh mouse
- Induce anesthesia
- Shave anterior thorax
- Intubate mouse (19-gauge tube)
- Connect to rodent ventilator (200 ml per stroke, 240 strokes per minute)
- Open skin and retract muscles
- Enter thorax between 3rd-4th rib
- Cut intracostal muscles
- Pull lungs away with absorption sponge
- Place and pull ligature 6-0 prolene ligature around the coronary artery
- Close the thorax
- Press chest to restore negative pressure and release muscles
- Close the skin
- Keep the mouse warm while recovering.
- Give buprenorphine (0.4 mg/kg i.p) when mouse is waking up.

Myocardial Infarction Model Cont.

6. A prolene 6-0 ligature is placed around the coronary artery just above the site where the artery splits into two smaller branches (see drawing). When the ligature is tightened, the apex of the heart should lose some of its red color. The ECG should also show marked differences now. If the ligature is placed too high upstream, then the circumflex artery can also be occluded and this may be associated with increased acute mortality. In our experience, lidocaine is not effective as anti-arrhythmic agent here.
7. The wound is closed as follows:
   a. Two ligatures (silk 5-0) are placed around the third and fourth ribs.
   b. The absorption sponge is taken out and the chest is closed with these ligatures.
   c. The 6-0 silk ligatures that were placed around the muscles are now released. Make sure that the muscles cover the wound.
   d. The chest is pressed to restore negative pressure.
   e. The skin is then closed with 5-0 silk.

RECOVERY

1. The ECG is monitored regularly during the first 5-10 minutes after the end of the surgical procedure. Then the isoflurane is stopped and the tube is removed from the trachea.
2. The mouse is allowed to recover for 24 h in a warm environment (28°C). Extra oxygen may help too.
3. 6-24 hours later the s.c. injection of buprenorphine is repeated for additional analgesia (0.1 mg/kg).

In general about 30% of the MI mice die within the first 24 hrs. Sham-operated animals do recover quickly. Cardiac wound healing lasts about 2 weeks.

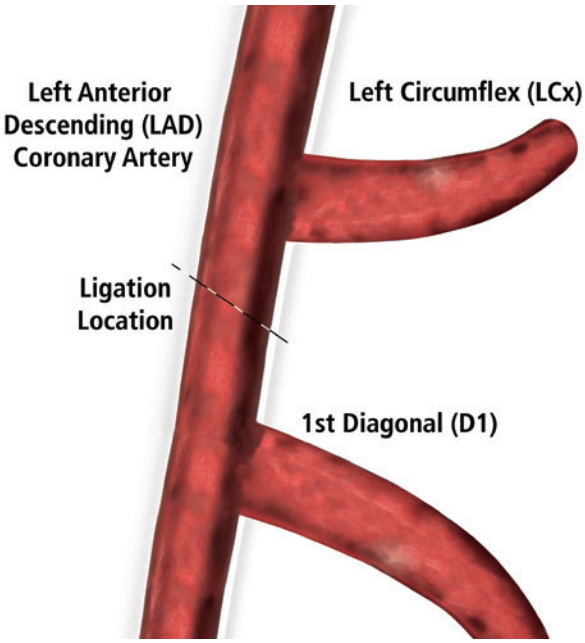


Fig. 1: Schematic drawing of the mouse heart viewed from the ventral site. The ligation is placed just above the bifurcation of the LAD into the 1st Diagonal Branch but after the Circumflex artery originates. When the ligation is placed too high LCx will be ligated too and most of the animals will die.

REFERENCES

The functional and structural consequences of this procedure including the ischemia / reperfusion variant as well as effects of strain differences are described in more detail in:

Lutgens et al. Cardiovascular Research 41: 586-593, 1999

Janssen et al. Am J Physiol Regulatory Integrative Comp Physiol 282: R928–R935, 2002;

De Celle et al. Exp Physiol 89: 605– 615, 2004

van den Borne et al. Cardiovascular Research 84: 273–282, 2009

Negative Pressure System

Materials

Container A - ungraduated cylinder; Fisher cat. no. 08-530G; 300 high x 38 mm OD;
Stopper is #10 1/2 rubber

Container B - 250 Erlemeyer

Container C - any small bottle with stopper

Establishing Proper Negative Pressure

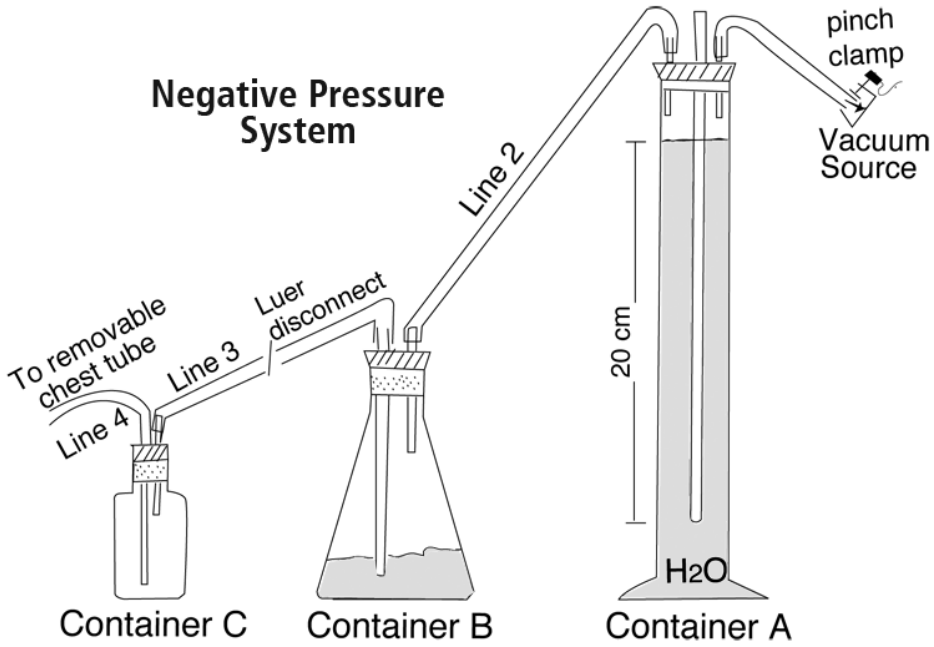
1. Turn on the vacuum source
2. Clamp line 3
3. Adjust pinch clamp on line 1 until bubbles appear at bottom of Z tube in container A; bubble rate of 1/2 sec. is adequate
4. Release line 3-bubbles should now appear in Container B.

Check for Leaks by Clamping Line 4

Bubbles should appear only in Container A.

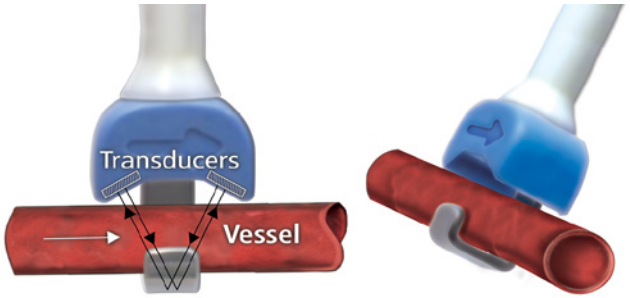
Pneumothorax

If animal has pneumothorax, bubbles will be produced mainly in container B. Once the chest is sealed, container B will have bubbles only infrequently.



Transit-Time Ultrasound Theory of Operation

A Transonic® Perivascular Flowprobe consists of a probe body which houses ultrasonic transducers and a fixed acoustic reflector. The transducers are positioned on one side of the vessel under study and the reflector is positioned at a fixed position between the two transducers on the opposite side. Electronic ultrasonic circuitry directs a Flowprobe through the following cycles:



Schematic views of a Transonic® Perivascular Flowprobe. Using wide beam illumination, two transducers pass ultrasonic signals back and forth, alternately intersecting the flowing liquid in upstream and downstream directions. The Flowmeter derives an accurate measure of the "transit time" it takes for the wave of ultrasound to travel from one transducer to the other. The difference between the upstream and downstream integrated transit times is a measure of volume flow rather than velocity.

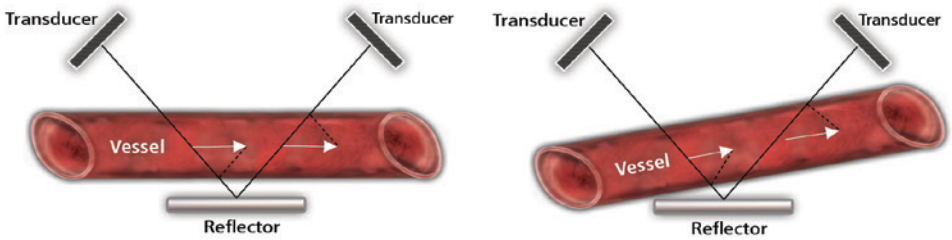
UPSTREAM TRANSIT-TIME MEASUREMENT CYCLE

An electrical excitation causes the downstream transducer to emit a plane wave of ultrasound. This ultrasonic wave intersects the vessel under study in the upstream direction, then bounces off the fixed "acoustic reflector." It again intersects the vessel and is received by the upstream transducer where it is converted into electrical signals. From these signals, the Flowmeter derives an accurate measure of the "transit time" it takes for the wave of ultrasound to travel from one transducer to the other.

DOWNSTREAM TRANSIT-TIME MEASUREMENT CYCLE

The same transmit-receive sequence is repeated, but with the transmitting and receiving functions of the transducers reversed so that the flow under study is bisected by an ultrasonic wave in the downstream direction. The Flowmeter again derives and records from this transmit-receive sequence an accurate measure of the transit time it takes for the wave of ultrasound to travel from one transducer to the other.

Just as the speed of a swimmer depends, in part, on water currents, the transit time of ultrasound passing through a conduit is affected by the motion of liquid flowing through that vessel. During the upstream cycle, the sound wave travels against flow and total transit time is increased by a flow-dependent amount. During the downstream cycle, the sound wave travels with the flow and total transit time is decreased by the same flow-dependent amount. Using wide beam ultrasonic illumination, the Flowmeter subtracts the downstream transit times from the upstream transit times. This difference in the integrated transit times is a measure of true volume flow.



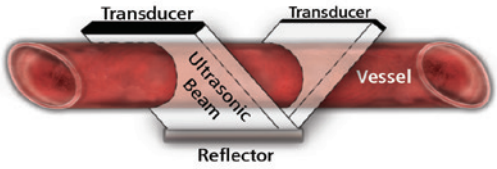
The ultrasonic beam intersects the vessel twice on its reflective path. With each intersection, the transit time through the vessel is modified by a vector component of flow. The full transit time of the ultrasonic beam senses the sum of these two vector components. With misalignment (bottom), one vector component of flow increases as the other decreases, with little consequence to their sum.

transonic
THE MEASURE OF BETTER RESULTS.

Transit-Time Ultrasound Theory of Operation Cont.

WIDE BEAM ILLUMINATION

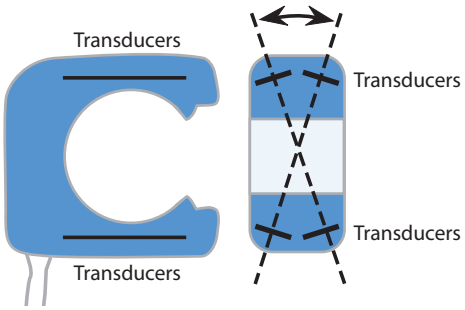
One ray of the ultrasonic beam undergoes a phase shift in transit time proportional to the average velocity of the liquid times the path length over which this velocity is encountered. With wide-beam ultrasonic illumination, the receiving transducer integrates these velocity-chord products over the vessel's full width and yields volume flow: average velocity times the vessel's cross sectional area. Since the transit time is sampled at all points across the vessel diameter, volume flow measurement is independent of the flow velocity profile. Ultrasonic beams which cross the acoustic window without intersecting the vessel do not contribute to the volume flow integral. Volume flow is therefore sensed by Perivascular Flowprobes even when the vessel is smaller than the acoustic window.



The vessel is placed within a beam that fully and evenly illuminates the entire blood vessel. The transit time of the wide beam then becomes a function of the volume flow intersecting the beam, independent of vessel dimensions.

X-BEAM ILLUMINATION

PAU-Series COnfidence Flowprobes® and XL Tubing Flowsensors use four transducers in X-beam illumination to accomplish the same volume flow measurements as the standard Perivascular Flowprobes. Ultrasound waves are transmitted in both the upstream and downstream directions by each pair of transducers. This provides two upstream and two downstream transit times which the Flowmeter combines into a single true volume flow measurement. The X-beam pattern of ultrasonic illumination provides the same advantages as wide beam illumination: measurement independence from velocity profile and vessel orientation.



COnfidence Flowprobes® use four transducers to create an X-beam ultrasonic illumination pattern to achieve a full vessel volume flow measurement.

Drost, C.J., "Vessel Diameter-Independent Volume Flow Measurements Using Ultrasound", Proceedings San Diego Biomedical Symposium, 17, p. 299-302, 1978.
U.S. PATENT 4,227,407, 1980.

transonic
THE MEASURE OF BETTER RESULTS.

T400-Series Consoles & Modules for Research

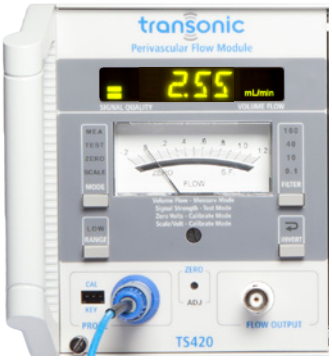
Transonic's ultrasonic transit-time Flow Modules attained "gold standard" status for volumetric blood flow measurements in acute and chronic animal protocols. First introduced in the mid 1980's with Flowprobes for large animal studies, the technology has been honed and adapted for much smaller and more challenging animal models such as rats and mice. Throughout the 1990's Transonic® Flow Modules laid the groundwork for many hypertension studies in SHR rats by enabling direct volume flow measurement in the rat renal artery with a 1 mm Perivascular Flowprobe. In the year 2000, great strides were made in the technology to achieve the same high resolution quality on even smaller vessels, enabling direct flow measurements in mice with 0.5 mm Flowprobes. The protocols documented in this 2013 edition of "Tools and Techniques for Hemodynamic Studies in Rodents" are the product of years of innovative research, development, collaborative product use and validation. As a result, Transonic® Flow Modules offer the cardiovascular research scientist state-of-the-art, reliable flow measurement technology that is referenced in thousands of peer-reviewed journals.

Transonic's T402 & T403 Consoles allow mix & match Module capability in a single bench-top unit. The TS420 Perivascular Flow Module operates Flowprobes for in vivo blood flow measurements. The Flowprobes are configured for either acute/anesthetized or chronic/conscious protocols and are available for arteries, veins or ducts from 0.25 mm to 36 mm diameter. Inline and Clamp-on style Flowsensors are used on the TS410 Tubing Flow Module for volume flow measurements in tubing. The new SP430 Pressure Amplifier Module adds two channels of pressure using Scisense Pressure Catheters (or other pressure sensors including fluid-filled catheters). All modules output analog signals in the range of ± 5 volts ready for data acquisition.

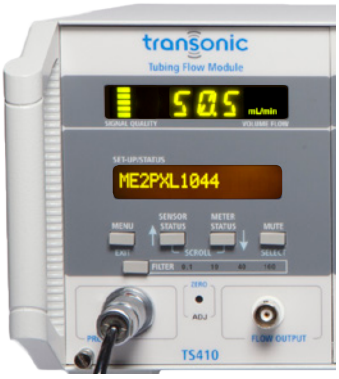
Transonic Gold Standard Flow Modules:

- Validated ultrasonic transit-time technology
- Direct volumetric blood flow measurement
- High resolution and zero baseline stability
- Continuous beat-to-beat flow data
- Non-constrictive Perivascular Flowprobes for vessels as small as 250 micrometers
- Inline extracorporeal Flowsensors for low flow isolated heart studies

T403 Console with TS410 Tubing Flow Module and TS420 Perivascular Flow Module and a blank panel.



TS420 Perivascular Flow Module measures volume flow in arteries, veins or ducts in laboratory animals.



TS410 Tubing Flow Module measures volume flow of liquids in flexible plastic tubing.

SP430 Pressure Amplifier Module provides two channels of pressure measurements.

transonic
THE MEASURE OF BETTER RESULTS.

Perivascular Flowprobe Connectors & Accessories

Connectors

CRA10 ACUTE REDEL

MA- Standard for Acute Use Probes: Plugs directly into Flow Module or extension cable.

CM4B (STRAIGHT) & CM4S (RIGHT ANGLE) 4-PIN

Implantable plastic mini connectors come with a threaded cap with suture hole, sealing gasket, and Probe ID key. Use with 4-pin extension cables. CM4B has a straight cable entry to ease tunneling subcutaneously. The CM4S connector has a right angle cable entry and is used with silicone or rigid cuffs to stabilize connector in mid-scapular region.

CA4B (STRAIGHT) & CA4S (RIGHT ANGLE) 4-PIN

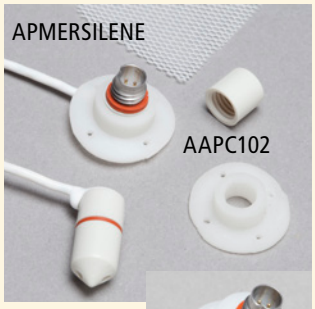
Minimal impact micro connectors, the 4-pins terminate the Probe cable with a bare minimum of housing. Use with 4-pin extension cables. Comes with Probe ID key. CA4B (straight cable entry) can be threaded into spring stock for use with electrical swivels. CA4S connectors have a right angle cable entry and can be stabilized in the mid-scapular region with a rigid or silicone cuff.

CONNECTOR DIMENSIONS IN MM			
CODE	HEIGHT	CAP DIAMETER	SHANK DIAMETER
CRA10	44.5	N/A	14
CM4B	15	8	8
CM4S	15	8	8
CA4B	10	N/A	4.1
CA4S	10	N/A	4.1

EXTENSION CABLES		
CATALOG #	TYPE	DESCRIPTION
CRA10-S-CRA10	10-pin	1.25 meter
CM4-S-CRA10	4-pin	1.8 meter with spring
CM4-M3-CRA10	4-pin	3 meter (not for use with nanoprobes)
CM4-X-CRA10	4-pin	Custom length; no spring
CA4-S-CRA10	4-pin	1.8 meter; mouse cable; no spring

Cuffs for Chronic Probe Implants

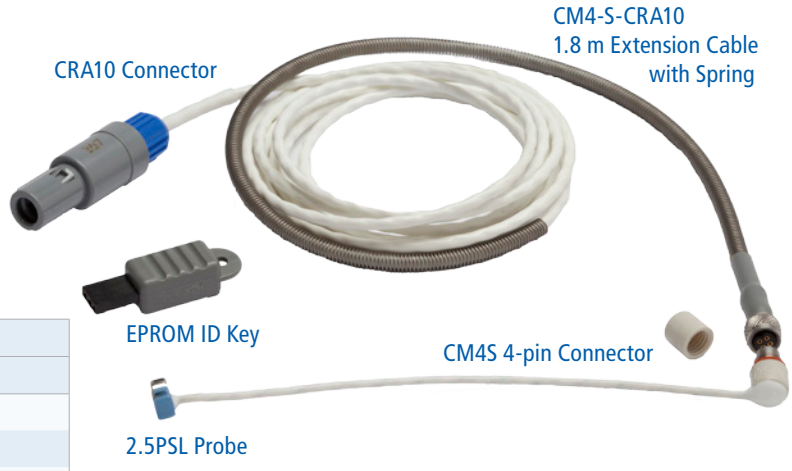
Soft silicone cuffs aid stabilizing mini connectors for short term implant. Delrin rigid cuffs convert mini connectors to skin buttons for long-term or multiple use. Suture holes in base of flange.



CODE	DESCRIPTION	COMPATIBLE CONNECTOR	HEIGHT (MM)	DIAMETER (MM)
AAPC102	Silicone cuff	CM4S	18.9	5.4
AAPC103	Silicone cuff	CA4S	15	5.4
AAPC104	Rigid cuff	CM4S	19	7.1
AAPC105	Rigid cuff	CA4S	9	3.7

MERSILENE MESH (APMERSILENE)

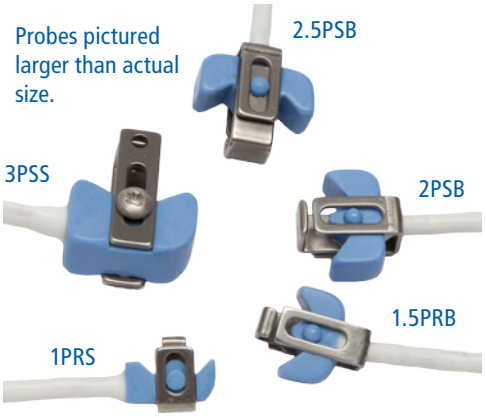
6" square soft interlocked polyester fiber mesh implanted under connector fortifies thin skin for cuff or button sutures.



transonic
THE MEASURE OF BETTER RESULTS.

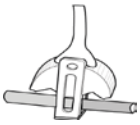
Precision Flowprobes for Rats & Rodents

- Validated, Referenced, Trusted
  - Cited in over 500 studies in rats & rodents
  - Gold Standard accuracy
  - Ultrasonic Transit-time Technology
- Blood Flow Applications
  - Continuous real-time volume flow measurements for numerous applications including: cardiac output, renal or portal hypertension, hemorrhagic shock, ischemia/reperfusion, carotid thrombosis and stroke models.
- Functional & Flexible
  - Compatible with electrical swivels and tethers
  - Wide range of customizable features
  - Acute and chronic use in vessels from 0.25 mm diameter and larger

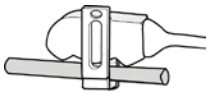


STANDARD MAX CABLE LENGTH			
CODE	DESCRIPTION	PS-SERIES	PR-SERIES
WCxx	Up to 60 cm	Nanoprobes	1 - 1.5
WCxxx	Up to 100 cm	2 - 20*	N/A

CABLE ORIENTATION			
CODE	ORIENTATION	PS-SERIES	PR-SERIES
B	Back	0.5 - 20	1 - 1.5
S	Side	0.5 - 20	1 - 1.5
L	Lateral	0.5, 1.5 - 2.5	N/A



B- Back: perpendicular to vessel



S- Side: parallel to vessel

L- Lateral: for thoracotomy

RAT BLOOD FLOW (wgt. 250-350 g)	
VESSEL	SUGGESTED PROBE
Ascending Aorta	2.5PS, 3PS
Abdominal Aorta	2PS
Pulmonary Artery	2PS
Carotid Artery	1PR, 0.7PS
Femoral Artery	1PR, 0.7PS
Mesenteric Artery	1PR
Renal Artery	1PR, 0.7PS
Portal Vein	1.5PR, 2PS
Hepatic Artery	0.5PS
Vena Cava	2PS, 2.5PS

PROBE SIZE & SERIES	VESSEL OD		BIDIRECTIONAL FLOW OUTPUTS				ACCURACY SPECIFICATIONS			ULTRASOUND
	MA-ACUTE APPLICATION	MC-CHRONIC APPLICATION	RESOLUTION	LOW FLOW (¼ SCALE)	STANDARD FLOW	MAX FLOW (STD FLOW)	ZERO OFFSET	ABSOLUTE ACCURACY	RELATIVE ACCURACY	FREQUENCY
	mm	mm	ml/min	ml/min	ml/min	ml/min	ml/min	%	%	MHz
0.5PS	0.3 - 0.5	0.3 - 0.48	0.03	1.5	6	30	± 0.12	± 15	± 2	14.4
0.7PS	0.5 - 0.7	0.4 - 0.7	0.05	2.5	10	50	± 0.2	± 15	± 2	9.6
1.5PS	1.2 - 1.5	1.2 - 1.5	0.075	10	40	200	± 0.8	± 15	± 2	4.8
1PR	0.7 - 1.2	0.7 - 1.0	0.05	5	20	100	± 0.2	± 10	± 2	7.2
1.5PR	1.2 - 1.8	1.0 - 1.5	0.075	10	40	200	± 0.4	± 10	± 2	4.8
2PS	1.5 - 2.0	1.3 - 1.8	0.1	25	100	500	± 1	± 10	± 2	3.6
2.5PS	1.8 - 2.5	1.5 - 2.4	0.1	25	100	500	± 1	± 10	± 2	3.6
3PS	2.5 - 3.7	2.4 - 3.4	0.4	50	200	1 L	± 2	± 10	± 2	3.6

transonic
THE MEASURE OF BETTER RESULTS.

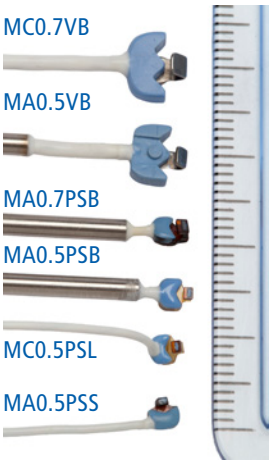
Precision Nanoprobes for Mice

- Sized to Fit Mouse Anatomy
- Unprecedented Capability
  - Continuous real-time volume flow measurements for calculating cardiac output, stroke volume and vascular resistance.
  - Applications include: renal blood flow, autoregulation, carotid thrombosis occlusion & femoral hindlimb studies.
  - For acute or chronic applications with a range of customizable features.
- Gold Standard Accuracy
  - Ultrasonic Transit-time Technology
  - Waveforms demonstrate signature zero baseline stability and high resolution even with flows less than 1 mL/min.

MURINE BLOOD FLOW MAPPING	
VESSEL	**SUGGESTED PROBE**
Ascending Aorta	1.5PSL
Lower Thoracic Aorta	1PR
Pulmonary Artery	1PR
Carotid Artery	0.5PS, 0.5V
Femoral Artery	0.5PS
Mesenteric Artery	0.7PS
Renal Artery	0.5PSL
Portal Vein	1PR

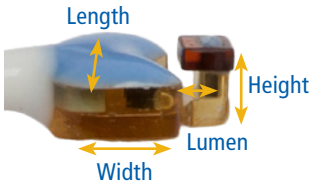
MA- prefix: standard acute configuration; specify size and cable orientation. Acute 0.5PS & 0.7PS and V-Series Probes supplied with 5 cm handle.

MC- prefix: custom or chronic configuration; specify size, cable orientation & length, connector type, & calibration option.



Nanoprobes (PS-Series) are very much smaller than V-Series Flowprobes.

	VESSEL OD		BIDIRECTIONAL FLOW OUTPUTS				ACCURACY SPECIFICATIONS			ULTRASOUND
PROBE SIZE & SERIES	**MA-ACUTE APPLICATION**	**MC-CHRONIC APPLICATION**	**RESOLUTION**	**LOW FLOW (¼ SCALE)**	**STANDARD FLOW**	**MAX FLOW (STD FLOW)**	**ZERO OFFSET**	**ABSOLUTE ACCURACY**	**RELATIVE ACCURACY**	**FREQUENCY**
	mm	mm	ml/min	ml/min	ml/min	ml/min	ml/min	%	%	MHz
0.5PS	0.3 - 0.5	0.3 - 0.48	0.03	1.5	6	30	± 0.12	± 15	± 2	14.4
0.7PS	0.5 - 0.7	0.4 - 0.7	0.05	2.5	10	50	± 0.2	± 15	± 2	9.6
1.5PS	1.2 - 1.5	1.2 - 1.5	0.075	10	40	200	± 0.8	± 15	± 2	4.8
1PR	0.7 - 1.2	0.7 - 1.0	0.05	5	20	100	± 0.2	± 10	± 2	7.2
0.5V	0.25 - 0.5	Acute use only	0.05	2.5	10	50	± 0.25	± 15	± 3	7.2
0.7V	0.35 - 0.7		0.075	5.0	20	100	± 0.5	± 15	± 3	4.8



PROBE SIZE & SERIES	CABLE ORIENTATION	PROBE BODY					CABLE	
		WEIGHT	**LENGTH**	**WIDTH**	**HEIGHT**	**LUMEN**	**LENGTH**	**DIAMETER**
0.5PS	any (B, S or L)	0.09 g	3.2 mm	2.3 mm	1.0 mm	0.47 mm	60 cm	1.0 mm
0.7PS	back only	0.12 g	3.2 mm	2.7 mm	1.2 mm	0.70 mm	60 cm	1.0 mm
1.5PS	lateral only	0.23 g	4.25 mm	3.75 mm	2.0 mm	1.65 mm	60 cm	1.25 mm
1PR	back or side	0.2 g	6.5 mm	4.0 mm	1.1 mm	1.5 mm	60 cm	1.5 mm
0.5V	back only	0.2 g	6.5 mm	4.0 mm	1.1 mm	1.5 mm	60 cm	1.5 mm
0.7V	back only	0.25 g	7.6 mm	3.5 mm	1.8 mm	1.7 mm	60 cm	1.5 mm

transonic
THE MEASURE OF BETTER RESULTS.

Choosing Mouse Probes: Nanoprobes vs V-Series

CHOOSING A FLOWPROBE FOR SMALL VESSELS

Transonic® Nanoprobes and V-Series Probes produce repeatable, high resolution volumetric blood flow measurement data on vessels as small as 250 micron diameter. Both styles are cited in the literature for flow measurement studies in the mouse.

PS-Series Nanoprobes

- Acute and chronic use Flowprobe (Fig. 1) may be configured for acute anesthetized studies or for chronic implantation with short cables and small connectors. The subjects can then be recovered and measurements taken while the animal is conscious over a period of days, weeks or months.
- Smaller Probe body: the Probe occupies minimal space in the surgical field and fits small anatomical spaces such as the mouse renal cavity.
- Measurements are less sensitive to vessel position within Probe lumen. The smaller rectangular lumen of Nanoprobes requires only general vessel position for proper ultrasonic illumination (Fig. 3). The vessel should fill 75% or more of the Probe lumen for best accuracy.
- Small amount of coupling gel needed to fill air space between the Probe and vessel
- Smaller measurement scale; more appropriate range for small vessel flow rates.
- Stainless steel handle is standard for acute use Probes.
- Delicate construction.
- Can be difficult to place vessel within Probe lumen because the reflector is thicker than the metal V-Probe reflector.

Fig. 1: Nanoprobes: Acute 0.5PSB and Chronic 0.7PSL

V-Series Flowprobes

- Acute use only: suppled with stainless steel handle (non-handle versions may be custom ordered) (Fig. 2).
- Larger physical Probe size for small diameter vessel; occupies more space in surgical field and requires a longer isolated vessel segment.
- Not a major problem on mouse carotid application because vessel is long and without branches.
- Is a problem on mouse renal artery where space is limited and the vessel has many small branches.
- Position sensitive; gives erroneous readings if used incorrectly. Vessel must be positioned in bottom of the V (Fig. 3) defined by the reflector even though the Probe lumen is much larger.
- Requires more coupling gel to fill up large air space.
- Rugged construction.
- Thin metal reflector: easier to place vessel within Probe.

Fig. 2: Acute 0.5VB

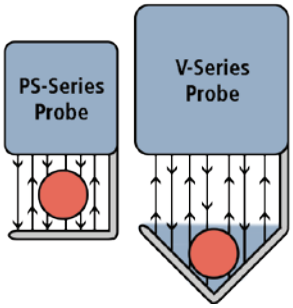


Fig. 3: The full height of the PS-Series Probe's ultrasonic window has the same flow sensitivity, so that the vessel can be positioned anywhere within the Probe. Only within the triangle (shaded) portion of the V will the V-Series Probe reach its full flow sensitivity.

transonic
THE MEASURE OF BETTER RESULTS.

Care Guidelines for Nanoprobes

A Transonic® Precision Nanoprobe is a delicate instrument for precise blood flow measurements in microsurgical research applications. Please handle this instrument with care to enjoy full use and trouble free maintenance over the lifetime of the product.

These Probes are warranted for 3 months.

HANDLING THE PROBES DURING USE

Never apply pressure or force on the reflector or the reflector hook.

- Handle the chronic style Nanoprobes by the cable just in back of the Probe head, or by gently grasping the Probe body. Never apply pressure or force on the reflector or the reflector hook, which is the most delicate part of the structure.
- Acute use Nanoprobes have a stainless steel handle along the cable to easily maneuver the Probe head on the vessel and maintain position of the Probe with a micromanipulator. As with chronic Probes that have no handle, do not apply pressure or force to the Probe head or reflector.
- We suggest using fine forceps or tweezers to lift the vessel into the Probe lumen, rather than using the reflector hook to scoop the vessel into the Probe lumen.
- Keep sharp objects away from the reflector face and Probe body (scalpels can chip the reflector surface and cut the epoxy Probe body).

CLEANING

Probes can be cleaned of adhering tissue by soaking in a solution of MadaCide-1 by Mada Inc. (www.madamedical.com) or Tergazyme® by Alconox, Inc. (www.alconox.com)

Probes can also be safely rinsed with 70% isopropyl alcohol.

Do not place the Probe in contact with acetone - this will dissolve the epoxies used in the Probe.

STERILIZATION

Probes can be sterilized by:

1. Ethylene Oxide gas sterilization
2. Soaking in liquid sterilizing solutions (such as 2% glutaraldehyde), then rinsing thoroughly with sterile saline.

Probes cannot be steam-sterilized, since steam-sterilization temperatures will degrade the piezoelectric crystals embedded inside the Probe.

For ethylene oxide sterilization, follow the standard sterilization cycle time for your EO system.

ETHYLENE OXIDE STERILIZATION	
PARAMETER	**VALUE**
Chamber Temperature	119° - 131°F
Chamber Humidity	45 - 75% RH
Load Temperature	104° - 141°F
Load Humidity	20 - 90% RH
Gas Mix	10% EO, 90% HCFC
Pressure	24.2 - 27.2 PSIA
Heated Aeration Time	12 - 48 Hours
Ambient Aeration Time	47 hours minimum

FLOWPROBE STORAGE

Nanoprobes should be stored in dry, room-temperature locations in their original plastic boxes with padding to protect the Probe from impact damage.

Probes should not be exposed to rapid temperature changes (such as immediately going from cold water to body-temperature water).

transonic
THE MEASURE OF BETTER RESULTS.

Tethers & Swivels for Conscious Measurements

Transonic® Flowprobes allow continuous volume flow measurements in conscious rats and mice. Following surgical Probe placement, the mini-connector of the Flowprobe is tunneled subcutaneously and exteriorized to the mid-scapular region. To monitor blood flow, the Probe connector must be attached to an extension cable connected to the Transonic® Flowmeter. The "extension cable tether" used for rats has a 9 inch metal spring covering the wire so that the animal does not damage the lead during periodic measurements. Mouse extension cables are not covered since the spring would be too heavy.

For long term continuous measurement, the use of an electrical swivel to transfer the Probe signals will allow free movement of the animal without twisting or damaging the cables. A Transonic® Flowprobe requires 4 electrical channels on the swivel plus ground to transmit and receive signals from the Probe.

To use a swivel device, the swivel must be spliced into the extension cable that attaches to the Flowmeter. If using a swivel from Dragonfly Inc. for mice, compatible cables with connectors that match the Dragonfly swivel may be purchased from Transonic®. For rats, the extension cable is cut and the wire ends are soldered to the swivel input and output contacts. The following procedure ensures that proper signal transmission to and from the Probe is maintained. (See "Research Equipment Sources" on page 12 for list of swivel providers)

General Steps to Attach a Swivel

1. Cut the spring and wire end of the 4-pin extension cable to the appropriate length for the cage.
2. Strip 1-2 inches of the wire to attach to the input end of the swivel.
3. Use rosin core electrical grade solder to attach the wires to the swivel contacts. Note: solder connections must be done carefully. A sloppy connection can add offset or noise to the measurement.
4. The Transonic® cable contains Kevlar threads which may be tied to the swivel end to provide mechanical strain relief.
5. Match the colored wire leads of the 10-pin Flowmeter portion of the extension cable to the corresponding contacts of the end of the swivel and solder the connections.
6. Insert the Probe calibration key in the Flowmeter when taking measurements.

Configurations for Rats

Three cable configurations are offered to attach a swivel for monitoring in rats. They differ by:

1. Where the attachment of the Probe is made
2. The type of spring and button that are used
3. The cable length requirements of the implanted Probe

RAT SWIVELS				
CONFIGURATION*	**CUSTOMER MODIFICATION**	**RAT ATTACHMENT TO SWIVEL**	**PROBE CABLE LENGTH**	**PROBE CONNECTOR**
A	Cut probe cable	Permanent for long-term monitoring	60 - 100 cm maximum length	CM4B removed - soldered to swivel
B	Cut extension cable	Spring tether attached to rat	60 cm or length up spring + animal	CA4 with spring silicone cuff
C	Cut extension cable	Rat can be separated from swivel for periodic measurements	9 - 20 cm from vessel site to scapula	CM4S with rigid cuff

*See next page for Configuration descriptions.

transonic
THE MEASURE OF BETTER RESULTS.

Tethers & Swivels for Conscious Measurements Cont.

A. Instech PO135 Spring Tether & Instech Polysulfone Skin Button

THE ANIMAL SUBJECT IS PERMANENTLY CONNECTED TO SWIVEL.

The Probe is supplied with a long cable. The connector is cut off with a small wire lead and the cable is threaded through the skin button and spring tether and soldered directly to the swivel input contacts. If cannulation tubing is required for drug infusion or pressure measurement, it is installed in the spring tether during this procedure. The removed Probe connector is soldered to the output contacts of the swivel to connect to the Flowmeter extension cable.

B. Transonic® MCS11 Spring Tether & Silicone CA4 Cuff Skin Button (AAPC103)

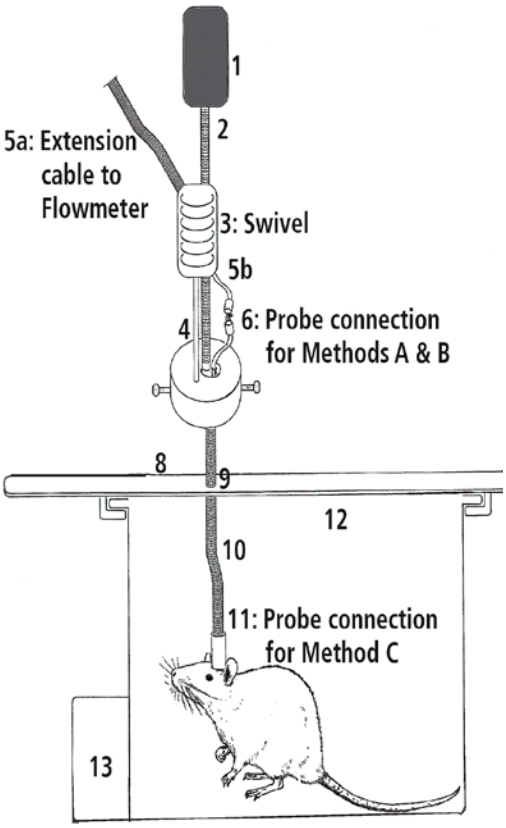
PROBE WITH TETHER ATTACHMENT CONNECT AT THE SWIVEL INPUT.

The Probe is supplied with a long cable and 4-pin micro connector that is passed through the silicone cuff and spring tether. The Flowmeter extension cable is cut and its 4-pin connector section is soldered to the swivel input contacts. The 10-pin section is soldered to the swivel output contacts to bring signals to the Flowmeter. The size and number of cannulation tubes may be limited by the diameter of the Transonic spring. Note: the Probe cable is left intact so that it may be easily tested on the Flowmeter before and after use. Probe cables should be ordered with long enough cable to exit the mid-scapular area, pass through the 18" spring tether and connect to the swivel attachment with enough ease in the cable so that it is not pulling on the exit wound. The silicone Cuffs are not intended for very long term studies and may need replacement after 3-4 weeks.

C. Attachment to Swivel at Mid-scapular Skin Button-Transonic® Rigid Cuff (AAPC104).

ANIMAL CAN BE DISCONNECTED FROM SWIVEL & TETHER.

The Flowprobe is ordered with a short cable that will terminate at the midscapular wound exit. A Transonic® rigid cuff is applied over the connector to secure the connector in place and convert it to a skin button. A standard 4-pin extension cable with spring cover is cut as above, and soldered to the swivel input and output contacts. The spring covered portion of the extension cable forms the tether and plugs directly into the skin button on the rat when measurements are required. This method allows the animal to be removed from the swivel and tether and returned to its home cage after periodic monitoring. Since the tether construction is pre-sealed on the extension cable, alternate methods for installing cannulas for pressure measurement or drug delivery must be made.



1. Instech hydraulic swivel
2. Spring segment for fluid cannula through center of Airflyte swivel
3. Airflyte electronic swivel for Flowprobe
4. Post to swivel
5. Transonic® CA4 extension cable
   a. Flowmeter end is cut and soldered to swivel output
   b. Probe connector end soldered to swivel input
6. Transonic® CA4 mini Flowprobe connector
7. Machined brass stabilization disk: 1" diameter with set screw for post/spring
8. Shelf
9. Hole in shelf
10. Transonic® #MCS111 (fits CA4 connector) -or- Instech PS135 spring tether (May require splicing of Flowprobe cable for threading with 2 cannula through tether)
11. Transonic® CA4 cuff (#AAPC103) inserted into spring receptacle - or- Polysulfone button (Instech)
12. Metabolic cage
13. Food compartment

transonic
THE MEASURE OF BETTER RESULTS.

Tethers & Swivels for Conscious Measurements Cont.

Swivels for Mice

The swivel movement requirements for mice are much greater than the apparatus for rats. A 20 gram mouse requires a swivel that will spin with no rotational torque and a cable that is nearly weightless. Transonic® has collaborated with Dragonfly Inc. to provide compatible input and output cables to their swivels (models SL-8X-6 and SL-8X-10) which provide noiseless signals and do not hamper the activity of the mouse.

Mouse Nanoprobes use very fine stranded wire in the cable and should be handled with more care than the Flowprobes for rats. Nanoprobe wires should not be cut or covered with a spring shield or significant offset or damage can result. By providing the cables that are compatible with the Dragonfly swivel, we ensure that signal integrity is maintained. Check model numbers with Transonic® before ordering to ensure compatibility.

Fig. 1: Miniature Slip-ring Swivel from Dragonfly Inc.

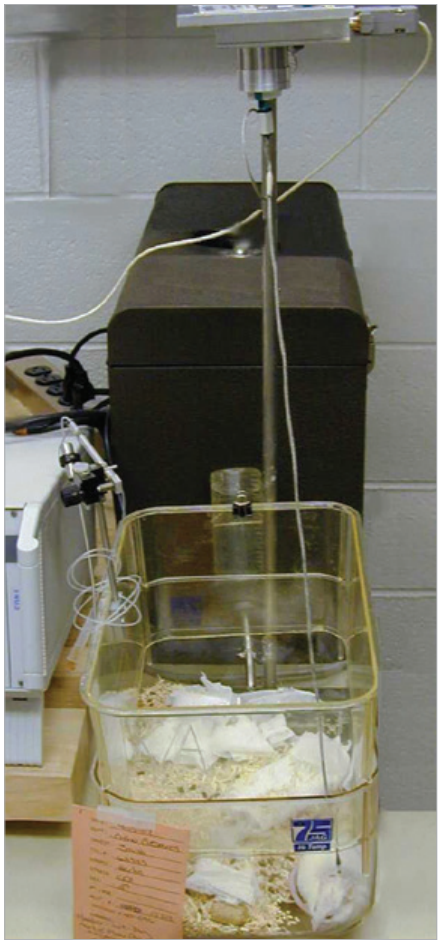


Fig. 2: Swivel Set-up

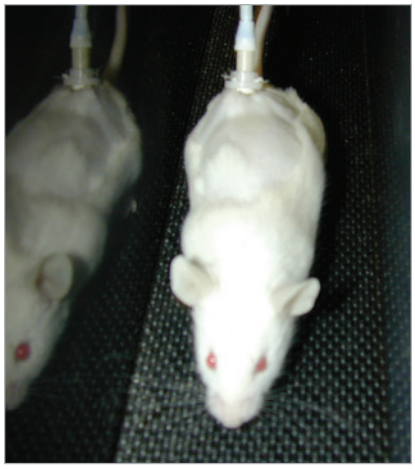


Fig. 3: Mouse running on treadmill; free movement enabled by electrical swivel interface to Flowmeter cables.

Precision Inline Flowsensors for Isolated Organ & Langendorff Preps

In vitro flow measurements for isolated perfused organ studies in rats and mice require precision instrumentation technology to record values that are typically very low and have different requirements than in vivo applications in an intact animal model.

- Transonic® PXN Inline Flowsensors measure absolute volume flow in laboratory tubing and in vitro perfusion apparatus.
- These Flowsensors can be used with non-particulate solutions such as saline, electrolyte and buffer solutions, in addition to blood.
- The Flowsensors are scaled for low or pulsatile flow applications with high sensitivity and low noise.
- Zero flow baseline measurements are easily achieved and low offsets can be nulled.

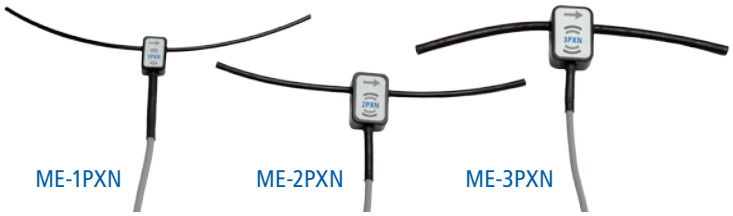
Transonic® Inline Flowsensors became the method of choice for isolated perfused organ studies in the late 1980's, replacing previous electromagnetic technology. They provide high resolution measurements of dynamic pulsatile flow and even low flow conditions. A hallmark of ultrasonic transit time technology is a stable baseline and minimal zero offset that is easily nulled and does not drift under stable conditions during the course of an experiment. The Flowsensors can be factory calibrated for the specific fluid perfusate that will be used, including Krebbs buffer, saline, blood and water.

The PXN-Series four transducer design for Transonic® 400-Series Flowmeters offers precision accuracy and ease of use. Flow range sensitivity is scaled to the sensor size; the 1PXN can measure flows < 1 ml/min to 25 ml/min (low flow scale) for mouse heart preps; the 2PXN and 3PXN are scaled higher for rat Langendorff and working heart preps. Miniature sensor sizes 1PXN (1.2 mm ID) – 3PXN (2.4 mm ID) are fabricated around flexible Pebax® tubing which may be cut to length for insertion into small tubing circuits or perfusion apparatus. These sensors are pre-calibrated for up to four user-defined fluid / temperature / flow range combinations.

Note: Inline Flowsensors are available in sizes from 1.2 mm inner diameter to 25.4 mm inner diameter. The sizes listed here are appropriate to the rodent model. For a complete listing of Inline Sensor sizes, please see our website: www.transonic.com.

SENSOR SIZE	BIDIRECTIONAL FLOW OUTPUTS				SYSTEM ACCURACY SPECIFICATIONS			ULTRASOUND FREQUENCY
	RESOLUTION	LOW FLOW (¼ SCALE)	STANDARD FLOW SCALE	MAX FLOW (STD SCALE)	MAX ZERO OFFSET	ABSOLUTE ACCURACY	LINEARITY	
	at 10 Hz in ml/min	1V output in ml/min	1V output in ml/min	5V output in L/min	ml/min	% of reading	%	MHz
1PXN	± 0.02	5	20	100	± 0.4	± 8	± 2	9.6
2PXN	± 0.02	10	40	200	± 0.6	± 4	± 2	9.6
3PXN	± 0.05	25	100	500	± 1	± 4	± 2	7.2

SENSOR SIZE	TUBING SPECIFICATIONS				PHYSICAL SPECIFICATIONS			
	TUBING ID		BARB OD		TOTAL LENGTH W/ TUBE ENDS		CASE LENGTH W/O TUBE ENDS	
	in	mm	in	mm	in	mm	in	mm
1PXN	3/64	1.2	Flexible tubing		3.9	100	0.3	8
2PXN	1/16	1.6			3.9	100	0.5	12
3PXN	3/32	2.4			3.9	100	0.6	14



transonic
THE MEASURE OF BETTER RESULTS.

ME-PXN Inline Sensors Care Guidelines

Transonic® PXN Inline Flowsensors are designed for laboratory use. The smooth cylindrical flow channel will not trap air bubbles or particulate material and can be easily flushed to keep it clean and free from a build up of material deposits on the interior surfaces.

Sizes 4PXN and larger are made with a rigid Ultem plastic tube with barbed ends to mate with flexible laboratory tubing. A variety of clamps are commercially available for high pressure applications.

Sizes 1PXN – 3PXN are manufactured with a flexible Pebax® tubing channel that optimizes the ultrasound signal transmission for the highest accuracy and sensitivity for low flow applications. Pebax® is a polyether block amide plastic with strong physical and mechanical properties. The tubing ends are supplied approximately 45 mm long, but may be cut to a preferred length without compromising the integrity of the sensor. Plastic connectors are available for connecting the sensor or the Pebax® tubing ends may be expanded to fit over the system tubing. (Pebax® is a registered trademark of ARKEMA)

Cleaning and Sterilization

Clean the outside surface and internal chamber of ME-PXN Inline Sensors with mild soap or detergent and warm water (55°C; 130°F). Remove any foreign material with a soft bristled brush. Avoid scratching the inner channel surface. A syringe with a plastic luer lock adapter, a pipe cleaner or small gauge covered wire may be used to mechanically clean the internal channel of the smaller Inline Sensors. The surfaces may be blown dry with compressed air to promote drying. Do not use alcohol or hydrogen peroxide on the internal Pebax® chamber or tubing ends as this will damage the plastic and the Sensor. The electronic connector should be washed only when necessary; wipe with 90% ethanol or propanol, but do not immerse the connector. ME-PXN Sensors may be sterilized by cold ethylene oxide gas (< 60°C; 140°F.) Avoid subjecting Sensor housing to temperatures higher than 60°C. ME-PXN Sensors should not be boiled, autoclaved or sterilized by cold liquid sterilization.

Sensor Storage

PXN Inline Sensors should be stored dry in their plastic shipping cases.

Mounting and Use

PXN Inline Flowsensors should be mounted into the tubing circuit so that they are supported on both ends. They should not be hung or supported by the Flowsensor cable. The arrow indicates direction of positive flow. Use the invert feature of the TS410 if the Sensor cannot be mounted in the positive position.

Larger sizes (4PXN and larger) have barbed ends to grip flexible laboratory tubing. The Ultem tubing edge is thin to provide a streamlined transition with circuit tubing. Protect these from sharp impact damage or dropping. Hard (high durometer) or thick walled tubes may slide onto the barbed ends more easily if the flexible tubing end is warmed prior to insertion. Nylon hose clamps are available to secure the junction for high pressure applications.

Smaller 1PXN - 3PXN Sensors are spliced into the flow circuit with short lengths of rigid plastic or metal tubing. Do not pull the flexible Pebax® tubing from the sensor housing: damage may result. The Pebax® tubing may be cut to a shorter desired length using a sharp blade. The ends may also be widened to mate with larger diameter tubing.

Pebax® Tubing

Pebax® tubing becomes pliable at higher temperatures. To achieve larger diameter tubing ends to fit tightly over custom apparati, the ends may be expanded by heating them over either a single gradually tapered rod or successively larger diameter rods. Transonic® recommends experimenting with this process on a sample of the Pebax® tube cut from one of the tubing ends or a sample obtained from Transonic® before altering the Sensor ends. Transonic® cannot be held responsible for damage made to the Sensor resulting from modification of the Pebax® tubing.

transonic
THE MEASURE OF BETTER RESULTS.

ME-PXN Inline Sensors Care Guidelines Cont.

Instructions for Expanding Pebax® Tubing Ends

1. Mount the end of the tubing (approximately 5 mm) onto a metal rod that is slightly larger than the inner diameter of the Pebax® tube (or a rod that tapers down to the tubing ID). The rod should not have sharp edges or come to a point. Dip the end of the rod in soapy water, and grip the Pebax® tubing with a paper towel or cloth to make insertion easier. Expanding the Pebax® tubing within 2 cm of the sensor housing is not recommended.
2. Vertically submerge the rod, but not the Pebax®tubing in near-boiling water (about 95°C) for 30 seconds. The metal rod will transfer the heat to the tubing end.
3. Keeping the assembly vertical, submerge the rod and Pebax® tubing (but not the Sensor housing) into cold water for 30 seconds. Grip the Pebax® tubing with a paper towel and carefully remove the rod.
4. Repeat the process with successively larger diameter rods until the desired diameter is achieved. It is possible to expand the 1.2 mm diameter 1PXN tubing to 2.0 mm.

Calibration Guidelines

ME-PXN Flowsensors are precalibrated at the factory for use with customer specified fluid and temperature. This calibration is performed with equipment that has been calibrated traceable to the standards of National Institute of Standards and Technology and to Transonic Systems Inc.® equipment performance standards. A Calibration Certificate, valid for 1 year, will be supplied with each Flowsensor upon purchase. Up to 4 fluid/temperature combinations may be pre-programmed into the EPROM. ME-PXN Flowsensors may be recalibrated by the user for other fluid/temperature conditions using the gain adjustment program in the TS410 Module.

Please note, the ultrasound signal amplitude is also normalized for the fluid/temperature use specified. Using the Sensor at a different temperature or with fluid other than specified may show a significant reduction in the normalized received signal of the sensor. Ultrasonic transmission is affected by the density of the fluid. Accurate measurements can be made even with a low received signal indication if a careful calibration is performed to correct the flow gain of the Flowsensor.

Keys to Accurate Perivascular Flow Measurements

Importance of Acoustic Coupling for Accuracy

Highest accuracy with ultrasonic transit-time Flowprobes is achieved when the ultrasound signal is transmitted under uniform acoustic conditions. This occurs when the acoustic properties of the coupling media and tissue are stable and most closely match the acoustic properties of the liquid being measured. Since volume flow measurement with Transonic® Flowprobes is derived from a phase shift (the difference in upstream and downstream transit times) and is impacted by changes in the acoustical velocity of the ultrasonic beam, discrete sources of error from acoustical mismatch can be eliminated by observing the following guidelines.

AIR

Air attenuates the Probe's ultrasound signal and effectively blocks ultrasound transmission. With large air pockets in the path of the ultrasound beam, the Flowprobe receives little or no transmitted signal and accurate flow measurements are not possible. Even small air bubbles can compromise measurement accuracy. Therefore, all spaces between the vessel and Probe must be filled with a suitable coupling agent (Fig. 1).

COUPLANT

The best acoustic couplant is Surgilube (E Fougera & Co.) because it matches the acoustic properties of blood. Media with lower acoustical velocity and impedance than blood are poor coupling agents for blood flow measurement with current ultrasonic transit time Flowprobes. These agents include saline, water, and NALCO 1181 mixed with saline. Aquasonic 100, an acoustic coupling agent used for sonography proved to be only on the borderline of acceptability for use with transit time Probes. Acoustically mismatched media cause reflections of the ultrasound at the vessel boundary, can substantially change the acoustical beam direction within the Probe, and impose uneven changes in the ultrasonic transit time. Measurements may be unstable and unpredictable in both positive and negative directions.

Perivascular Flowprobes completely filled with acoustic couplant.

FAT

Fatty tissue also has a low acoustic velocity and affects the ultrasonic beam similarly. A pad of fat on the vessel wall in the acoustic pathway of the ultrasonic beam can act like a lens, reflecting or defocusing the ultrasound and altering the transit time.

TEMPERATURE

Temperature also effects the velocity of ultrasound and should be controlled for the most accurate measurements. Acoustical velocity increases with temperature increase. Transitions of the ultrasound beam from room temperature coupling agent to body temperature vessel wall and blood will alter the transit time and may exacerbate errors from other sources.

SUMMARY

Subtle phase shifts in the ultrasonic beam may be caused by inappropriate acoustic conditions during the experiment and will affect the accuracy of the measurement. Acoustically tested and approved coupling agents should be used with Transonic® Probes. Fatty tissue should be carefully cleaned from the vessel where the Probe is placed. Controlling temperature in the acute experiment makes excellent physiological sense, in addition to being good acoustic practice. Perivascular Flowprobes are calibrated for measurements of blood at 37°C and will give the most accurate readings if used within a ± 2 - 3 degree range. Gels may be warmed on a heating plate and the Probe itself should be allowed to equilibrate to this temperature for about an hour prior to use.

transonic
THE MEASURE OF BETTER RESULTS.

Keys to Accurate Perivascular Flow Measurements Cont.

Optimizing Conditions

Accurate flow measurements with ultrasonic transit time technology depend on careful attention to several variables. These include:

CHOICE OF A PERIVASCULAR PROBE

Although Transonic® Flowprobes are designed for a non-constrictive fit on the vessel, the vessel/Probe fit can influence accuracy significantly. For acute applications, the vessel must fill at least 75% of the Flowprobe lumen to meet published accuracy specifications. A close or snug fit will result in the least measurement variability. A close fit lessens the amount of acoustic gel needed and minimizes its effect on the measurement.

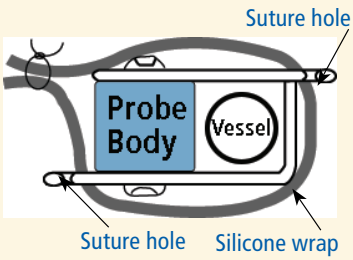
- Choose a range of Flowprobe sizes to cover variability in vessel diameter between subjects so that the 75% vessel fit rule is followed.
- Use Nanoprobes for a close fit on small vessels (< 700 microns) to maintain acoustic coupling more easily.
- Certain Flowprobes have been designed with increased sensitivity to minimize the effects of acoustic mismatch. These include V-Series Flowprobes for small vessels (<700 micron diameter). V-Series Probes are larger bodied and may be used instead of Nanoprobes as vessel length and surgical space allows being careful to follow vessel placement guidelines for V-Series Probes.

ADVANTAGE OF CHRONIC IMPLANTS

Many of the sources of error listed here are associated with acute use of ultrasonic Flowprobes and can be effectively eliminated when the Probes are implanted for long term measurements and chronic protocols. No coupling gel is required unless measurements are taken during the intraoperative procedure. Within 3 - 5 days during an animal's surgical recovery, the air spaces are filled with fibrous connective tissue. This tissue is a good acoustic conductant and also serves to center the vessel in the most sensitive position of the Probe. As in acute applications, the vessel should be stripped of fatty tissue prior to Flowprobe implantation and preventative measures should be taken to keep fat from infiltrating into the acoustic pathway over time. In species or vessel sites predisposed to fatty tissue deposits, a thin sheet of silicone wrapped around the outside of the Probe and sutured to adjacent tissues at the time of implant will keep the Probe fat free and also aid in stabilization (see sidebar on second page).

SILICONE WRAP

Precut silicone sheeting is applied during Probe implantation, after the Probe's sliding cover plate is closed around the vessel and before the Probe is sutured in place.



Wrap the silicone around the Probe so that both suturing ends of the Probe bracket assembly stick through the small rectangular cutouts in the sheet. The ends of the sheeting are then sutured together to hold the wrap around the Probe (Fig. 2).

The Probe may then be secured in place using its regular suturing points (the two suture holes in the Probe bracket assembly, and a suture around the Probe cable. Alternately, one may suture the silicone wrap to surrounding tissues, or to the artery wall, if appropriate.

In a chronic experimental design, the cardiovascular system will also be freed from intraoperative stresses. Conscious measurements may be made without cardiovascular influences from anesthesia. Under these stable acoustic and physiologic conditions, our customers have pushed the measurement capabilities of Transonic® Flowprobes to record low flow states in difficult applications such as bile flow in the cystic and common bile ducts in a dog model, and esophageal (amniotic fluid) flow in fetal lamb swallowing.

SCIENTIFIC PROTOCOL

While the ease of use of Transonic® precalibrated Flowprobes have earned plug and play status, the rigors of scientific protocol should not be ignored. Transonic®specifies its Probes for ± 10%-15% absolute accuracy (see specification tables for individual Probe series). Careful attention to the above considerations will ensure that measurements reliably meet these standards. Absolute accuracy may be further enhanced by in situ calibration of the Flowprobes to validate the measurement under their specific conditions of use.

transonic
THE MEASURE OF BETTER RESULTS.

Implantation of a Skin Button Connector

Preparing a Transonic® CA4 Connector for Installation into a "Rigid Cuff" to Convert into a Skin Button

In the mouse, an implanted Flowprobe maintains the best position if the cable is not disturbed by subcutaneous preparation. To do this, cut and close the skin over the Flowprobe's cable to the Probe connector at the midscapular region, leaving the Probe's CA4 connector exposed (Figs. 1, 2), instead of tunneling the connector subcutaneously.

Close the skin over the incision with 5-0 Vicryl sutures. Use a 3/4" square of Mersilene Mesh under the skin at the connector (Fig. 2) and suture the skin closed around cable (Fig. 1). This helps to fortify the skin and keep sutures from pulling out of the skin. Finally, install a skin button cuff (Fig. 3) over the CA4 connector suturing through the skin and the mesh to improve long term stability of the implant.

The wound is cleaned and the animal is allowed to recover before measurements are made. Generally, it takes 3 - 5 days to achieve a stable signal as fibrotic tissue helps to encapsulate the Probe, though the flow signal may be available as soon as one day post surgery.

1. Make a subcutaneous pouch to hold the Mersiline mesh (Ethicon). Placing mesh under the skin strengthens the skin so that the button can be placed long-term.
2. Place the mesh in the skin incision and try to get it to lay flat without folding back on itself (Fig. 2). Use something flat like the back side of forceps to get the mesh to lie down along the muscles. You may lift up the skin to get a view of the corners of the mesh. Put a drop of Nexaband onto the corners as a temporary anchor.

Fig. 3: Rigid cuff for CA4S Connector.

ACKNOWLEDGEMENT

Transonic Systems Inc.® gratefully acknowledges the assistance of the collaborator of T.L. Smith, Ph.D. and M.F. Callahan, Ph.D. in the development of this protocol and sharing of data.

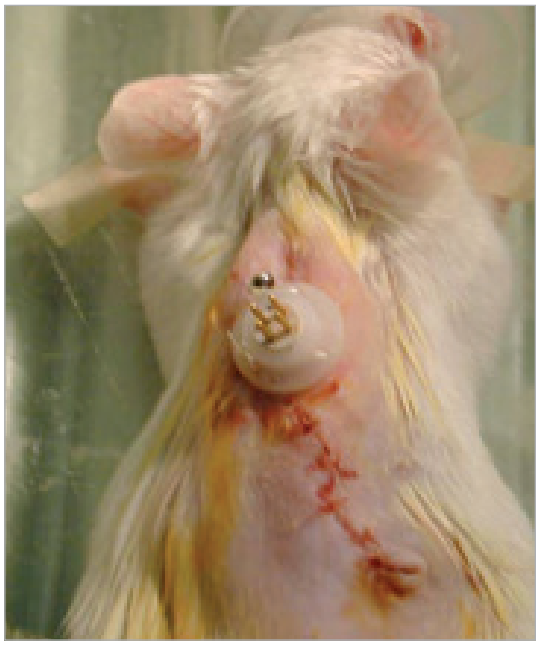


Fig. 1: The mouse is shown with the skin button positioned after surgery.

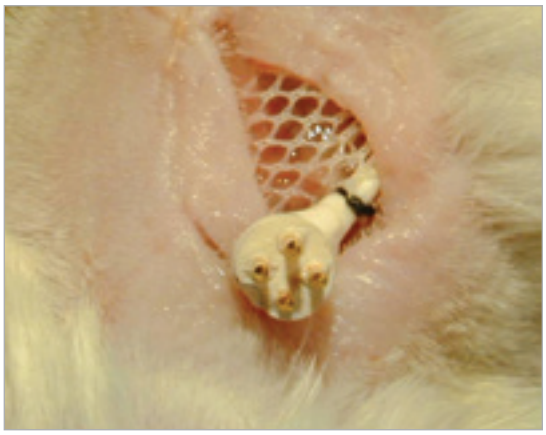


Fig. 2: Mersilene Mesh shown under skin in area prepared for button placement.

transonic
THE MEASURE OF BETTER RESULTS.

Implantation of a Skin Button Connector Cont.

3. To place sutures to hold the skin button in place, pass a needle (24G or larger in a rat) through the skin (Fig. 4), into the mesh, perhaps into a small bit of muscle, back through mesh and then back up through the skin to exit near where one of the sets of button holes would be on the skin (Fig. 5). Do this so that the skin entrance and exit holes are a little wider than the holes in the button. In doing this, you should look into the central skin incision to see that the needle doesn't run through your cables or catheters.
4. Pass a piece of vetafil (or similar non-absorbable suture) through the needle. Remove the needle so that you have the vetafil running down under the skin, through the Mersilene mesh and muscle and back up through the mesh and skin (Fig. 6).
5. Clamp the two loose ends of the vetafil with hemostats or Schwartz serrefines (that will hold these sutures together so you can approximate the other suture points (Fig. 7).
6. Repeat this procedure 3 more times for the other button holes (Fig. 8).
7. To close the incision with a purse-string, thread the four vetafil sutures through the button holes and tie the button down (Figs. 9, 10). Don't tie it so tight that it is tugging on the skin or it may cut through the skin and muscle. Leave a little space for the button to move.

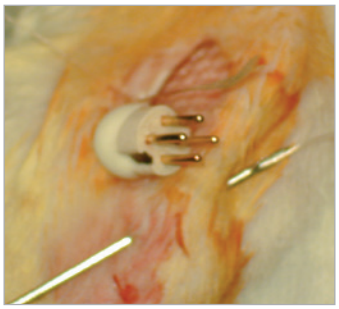


Fig. 4: Passing a needle through the skin.

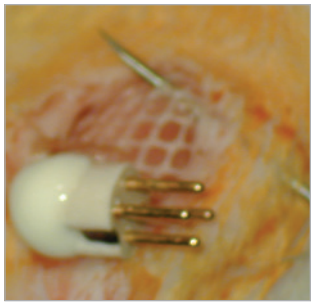


Fig. 5: The needle should pass through skin, mesh and muscle.

Fig. 6: Remove the needle leaving the suture in place.

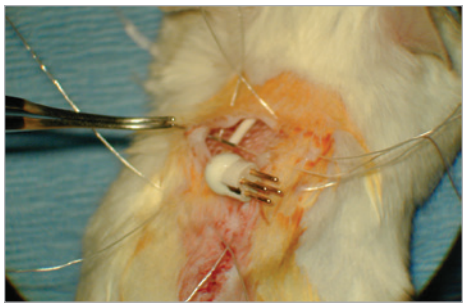


Fig. 7: Sutures placed for skin button.

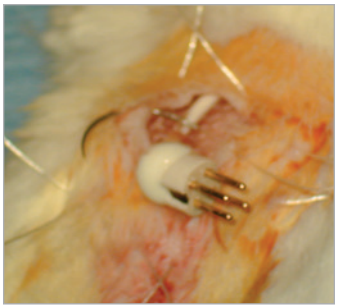


Fig. 8: Multiple sutures to hold the skin button in place.

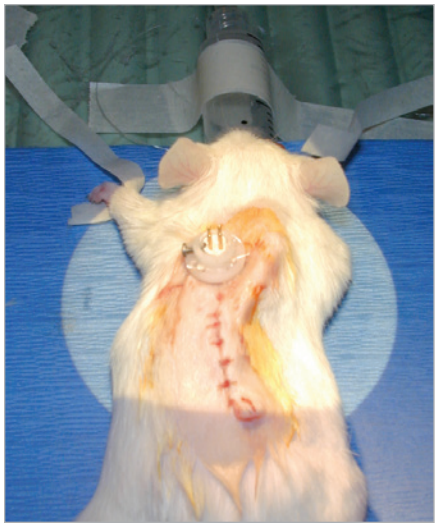


Fig. 9: Skin button sutured in place.

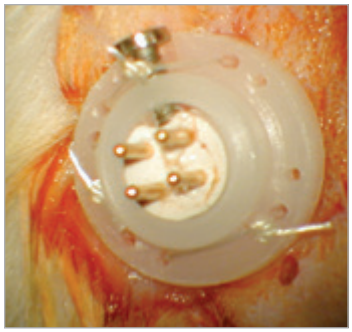


Fig. 10: Close up of skin button sutured in place.

transonic
THE MEASURE OF BETTER RESULTS.

Keys to Successful Cardiac Output Measurements

Common applications like the measurement of cardiac output can be challenging for the novice. Flow values that deviate from expectation may be difficult to understand and may arise from a combination of common errors. The following guidelines outline common pitfalls that affect flow readings and may present as "low" flow or "variable" flow measurement.

Target Vessel Position:

ASCENDING AORTA VS. PULMONARY ARTERY VS. DESCENDING AORTA

Cardiac Output (CO) is the total blood flow output of the heart. It can be measured in the Ascending Aorta or the Pulmonary Artery. In small animals (rats & mice) the pulmonary artery is much less accessible for Flowprobe placement and is rarely used unless the investigation requires assessment of blood flow distribution to the lungs.

Cardiac Output in rats and mice is directly measured with a Transonic® Flowprobe placed on the ascending aorta, just before the aortic arch. This Probe position most completely captures the total outflow of the heart on each beat, less the blood flow that is diverted into the coronary circulation (about 10%).

Estimates of CO can be measured more distal on the lower thoracic aorta or descending aorta. However, the measurement will not include flow to the head in the carotid branches or flow in the brachial arteries and forelimbs, and so would be an underestimation of cardiac output. Placement of a Flowprobe in this position is less technically challenging than on the ascending aorta, and is sometimes used to detect relative changes in CO in experiments where entering the thoracic cavity would be too invasive and not pertinent to the study design.

Study Design:

CHRONIC IMPLANTATION

Although chronic implantation protocols present their own challenges, measurements with a previously implanted Flowprobe can be made under much more stable and physiological conditions than in an open chest experiment. It generally takes 3 – 5 days after surgery for fibrotic tissue to encapsulate the Flowprobe and ensure good signal quality. This period is also necessary for the animal to recover from surgery and affects of anesthetic agents. With the chest cavity closed and negative pressure restored, hemodynamics can be monitored for normal baseline conditions. Measurements may be recorded in the conscious animal, or under less potent anesthetics for dose/response studies. Note that cardiac output in the conscious animal will vary during the time of day in circadian cycle and activity level.

ACUTE OPEN CHEST DESCENDING AORTA

Measurements of cardiac output made in an acute, anesthetized, open chest protocol are lower and less stable than measurements made in an animal with an implanted Flowprobe. In the acute experiment, measurements may be technically compromised by Probe position and acoustic coupling to achieve good signal quality, but also by the physiological condition of the animal. The following parameters should be considered to optimize results.

ANESTHETIC AGENT MAY DEPRESS CARDIAC OUTPUT

Common anesthetics used for small animal surgeries include pentobarbitol, urethane, halothane, isoflurane and ketamine/xylazine. These agents can have a significant affect on cardiac output and the effect can vary between species. These also depend on whether the measurements are to be made in acute open chest application or after recovery from implantation in the conscious animal. Pentobarbitol is known to depress cardiac output in rats, but is often used for acute experiments and CO values are lower than expected. Ketamine/xylazine is a useful anesthetic for application in rats, but has severe and lingering effects in mice. Isoflurane has fewer systemic hemodynamic side effects in the mouse and seems to preserve cardiac function. Know the effects of the anesthetic agent on the study subject.

Keys to Successful Cardiac Output Measurements Cont.

MAINTAIN BODY TEMPERATURE

A rat or a mouse loses core body temperature quickly when a body cavity is exposed during surgery. Measurements of cardiac output will drop considerably if core temperature is not maintained. We have observed flows that are 75% lower than typical reported values in open chest preps where the temperature has deviated 9° lower than normal (example: PA flow measured acutely in a rat: 16 ml/min @ 28°C; 40 ml/min @ 32°C; 60 ml/min @ 37°C). These dramatic affects are also reported in peripheral vascular flow measurements.

Temperature should be monitored and normal body temperature maintained during the experiment. Monitoring is easily achieved with a rectal temperature sensor. Heating pads or heated surgical platforms are available or can be easily constructed to provide adequate temperature control. A heating lamp light source can also be used. In both cases, be sure not to over heat the subject. Body temperature may also be conserved by covering the exposed area with gauze.

VENTILATION & SURGICAL INSTRUMENTS

Working in a small animal model requires surgical instruments that are scaled down to smaller vessels than those used for large animal surgery. An excellent selection of microsurgical vessel dilators, forceps, scissors and other accessories are recommended and available from Fine Science Tools. See the recommended listing “Special Surgical Equipment Needs” on page 10.

Animals that undergo open chest surgery must be ventilated mechanically with a respirator that will deliver the appropriate small stoke volumes and ventilation rates that are required by small animals. There are excellent products available for this purpose from Hugo Sachs Electronik of Harvard Apparatus.

CARDIAC OUTPUT FLOWPROBE RECOMMENDATIONS		
ANIMAL	**WEIGHT**	**PROBE**
Rat	< 250 g	2PS (2 mm)
	250 - 350 g	2.5PS (2.5 mm)
	400 - 500 g	3PS (3 mm)
Mouse	20 - 50 g	1.5PSL (1.5 mm)

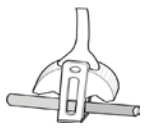
FLOWPROBE SIZE SELECTION FOR THE ASCENDING AORTA

It is important to choose the proper size Flowprobe for the animal species and strain that is used. Although Transonic® Flowprobes do fit a range of vessel sizes, the accuracy of the Flowprobe will be maximized in acute application if the Flowprobe fits the vessel more closely and the amount of gel required for acoustic signal coupling is therefore minimized. Transonic® surgical protocols recommend Flowprobe size by weight of the subject (see table).

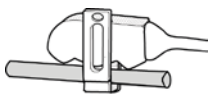
Use of a larger Probe than necessary has two potential problems. First, a larger than necessary Probe may be too large for the anatomical space within the chest cavity and the space along the vessel. Secondly, the Flowprobe ultrasonic field is not 100% uniform. PS-Series Flowprobes tend to have greater sensitivity in the center of the Probe and less sensitivity on the edges of the Probe. We specify that the vessel should fill 75 – 95% of the Probe lumen to meet our accuracy specification. A vessel that is smaller than specified for a given Probe size will exhibit variable reading depending on the position of the vessel within the Probe.

PROBE CONFIGURATION & SURGICAL APPROACH:

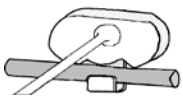
Generally, the orientation of the Flowprobe cable with respect to the Probe head (“back”, “side” or “lateral”) is not as critical for acute open chest protocols as it is for chronic implantation where the cable must be tunneled subcutaneously to the midscapular area. However, the cable orientation influences how the Probe is positioned on the vessel.



B- Back: perpendicular to vessel



S- Side: parallel to vessel



L- Lateral: for thoracotomy

transonic
THE MEASURE OF BETTER RESULTS.

Keys to Successful Cardiac Output Measurements Cont.

ASCENDING RIGHT INTERCOSTAL THORACOTOMY:

Probe with lateral or side cable. This approach is less invasive but requires more careful dissection of the aorta because the vessel is almost hidden from view. See"Rat Ascending Aorta: Chronic Blood Flow Measurement (Right Thoracotomy-Side Exit Probe)" on page 48 for details.

MEDIAN STERNOTOMY:

Probe with back cable. This approach is often preferred for acute measurements because the heart and aortic arch are easily visible. See "Rat Ascending Aorta: Chronic Blood Flow Measurement (Sternal Approach)" on page 51.

PROBE POSITION ON THE VESSEL

The aortic arch presents a unique challenge to transit time flow measurement technology. Although Transonic® Flowprobes are largely insensitive to vessel alignment within the Probe, incorrect placement with respect to the aortic arch can have a large affect on the measurement values, causing an underestimation of flow by as much as 40%. Correct Probe position is essential to overcome this limitation: the plane of the Flowprobe ultrasonic path must be oriented perpendicular to the plane defined by the curvature of the arch for accurate flow measurement. Placing the Flowprobe so that the ultrasonic wave is in the same plane as the curvature of the aortic arch is incorrect and may cause readings that are lower than expected values. In practice, the Flowprobe will exhibit variable readings as the Probe is rotated on the ascending aorta giving the lowest reading on the curve and the highest reading when the Probe is placed correctly with respect to the arch.

ACOUSTIC SIGNAL QUALITY & COUPLING GEL

An air bubble will block the ultrasonic signal and can cause variable or noisy readings. Acoustic signal coupling gel is supplied with the Flowmeter and should be used to displace any air that is trapped between the vessel and the Flowprobe. The type of gel that is used can also have an effect on the Flowprobe reading. Some ultrasonic gels affect the sensitivity of the reading and can contribute to a lowered measurement value. Transonic® recommends Surgilube lubricating gel as an acoustic couplant because the acoustic properties of this agent match the acoustic properties of blood and will produce the least affect on the flow measurement. See Technical Note RL-9-tn for information about acceptable coupling gels.

CARDIAC OUTPUT REPORTED VALUES		
ANIMAL	**WEIGHT**	**CO VALUE**
Rat (Sprague Dawley)	350 g	100 ± 8 ml/min
	350 - 430 g	85-103 ± 7 ml/min
Mouse	30 g	20 ± 4 ml/min resting
	30 g	26 ± 6 ml/min stimulated

REFERENCES

Torres LN, Torres Filho IP, Barbee RW, Tiba MH, Ward KR, Pittman RN, "Continuous peripheral resistance measurement during hemorrhagic hypotension," Am J Physiol Heart Circ Physi 2004; 287(5): H2341-5.

Woo YJ, Panlilio CM, Cheng RK, Liao GP, Atluri P, Hsu VM, Cohen JE, Chaudhry HW, "Therapeutic delivery of cyclin A2 induces myocardial regeneration and enhances cardiac function in ischemic heart failure," Circulation 2006; 4;114(1 Suppl):I206-13.

Krieger EM, Brum PC, Negrão CE, "State-of-the- Art lecture: influence of exercise training on neurogenic control of blood pressure in spontaneously hypertensive rats," Hypertension 1999; 34(4 Pt 2): 720-3.

Brands MW, Fitzgerald SM, Hewitt WH, Hailman AE, "Decreased Cardiac Output at the Onset of Diabetes: Renal Mechanisms and Peripheral Vasoconstriction," AJP 2000; 278(5): E917-924.

McKeown KP, Shoukas AA, "Chronic Isolation of Carotid Sinus Baroreceptor Region in Conscious Normo-tensive and Hypertensive Rats," AJP 1998; 275(1): H3220- H329.

Janssen BJ, De Celle T, Debets JJ, Brouns AE, Callahan MF, Smith TL "Effects of Anesthetics on Systemic Hemodynamics in Mice," Am J Physiol Heart Circ Physio. 2004 Oct; 287(4):H1618-24.

Potts JT et al, "Reduction in Arterial Compliance Alters Carotid Baroreflex Control of Cardiac Output in a Model of Hypertension," AJP 1998; 274(4 Pt 2): H1121-31.

Janssen B et al, "Chronic Measurement of Cardiac Output in Conscious Mice," AJP 2002; 282(3): R928-35.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Ascending Aorta: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Ascending Aorta
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Acute
Vessel Diameter:	1.2 - 1.3 mm
Length:	2.5 - 3.0 mm

PROBE

Size:	1.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-1.5PSL

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

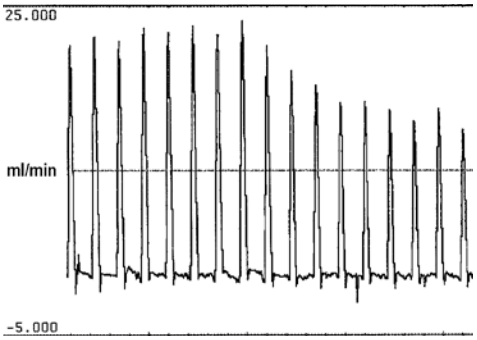


Fig. 1: Acute cardiac output measurement on ascending aorta in an anesthetized mouse.

Surgical Approach

Acute cardiac output measurements may be obtained in mice by conventional surgical techniques and ascending aorta blood flow measurements.

Fig. 2: 1.5PSL Probe for mouse ascending aorta blood flow.

PREPARATION

Anesthetize the mouse with a mixture of ketamine and xylazine as mentioned in Mouse Anesthetic Guidelines (RL-67-tn). After anesthesia is attained, shave the mouse along the sternum. Place the mouse on a respirator equipped for approximately 120 breaths per minute at a very small tidal volume which is adequate to move the chest an appropriate amount for respiration. An endotracheal tube is not really necessary. Expose the trachea through a mid-line incision over the trachea. Place a 3-0 silk ligature around the trachea. Using a scalpel blade, make an incision between cartilage rings below the larynx. Pass a small polyethylene tracheal tube directly into the trachea. Connect it via silastic tubing to a rodent respirator.

Fig. 3: Goldstein Lacrimal sac retractor modified (bottom) for rib retraction in a mouse.

STERNOTOMY

Once the animal is placed on a respirator providing positive pressure respiration, perform a median sternotomy by using scissors to cut the skin overlying the sternum. Bisect the sternum longitudinally using scissors, beginning at the manubrium and extending towards the xiphoid. Stop the incision prior to reaching the xiphoid so that the bifurcation point of the internal mammary arteries is not bisected. Try and stay as close as possible to the mid-line of the sternum to avoid cutting the internal mammary arteries.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Ascending Aorta: Acute Blood Flow Measurement Cont.

Surgical Approach cont.

Once the sternum has been incised longitudinally, place a modified Goldstein lacrimal sac retractor (Fig. 3) within the incision site and retract the ribs laterally. This exposes the heart and the thymus gland of the mouse. Retract the thymus gland rostrally towards the sternum to expose the ascending aorta and pulmonary artery.

PROBE PLACEMENT

Dissect the ascending aorta free of the pulmonary artery. Position a Transonic® 1.5PSL Probe perpendicular to the axis of the ascending aorta and pressed close to the origin of the aorta at the heart. This allows a long straight section of blood vessel to be illuminated with ultrasound without sampling the bend of the aortic arch. Acoustical coupling gel (Surgilube gel acoustically matches blood for the most accurate measurements and is available from Transonic®) should to be placed around the aorta, completely filling the lumen of the transit-time Flowprobe. This allows acoustical coupling of the flow Probe with the aorta itself. The Probes have been calibrated using this gel.

Verify zero flow and accurate positioning on the ascending aorta with a flow waveform. During diastole the flow waveform should parallel the electrical zero tracing on a recording oscillograph.

REFERENCES

Janssen, BJA et al, "Effects of anesthetics on systemic hemodynamics in mice," Am J Physiol Heart Circ Physiol 2004; 287: 1618-1624.

Gao, XM et al. "Cardiac output in mice overexpressing beta-2-adrenoceptors or with myocardial infarct," Clin Exp Pharmacol Physiol 2001; 28(5-6): 364-70.

Varghese, P et al, "β3-adrenoceptor deficiency blocks nitric oxide–dependent inhibition of myocardial contractility," J Clin Invest 2002; 106(5): 697-703.

Mouse Ascending Aorta: Chronic Blood Flow Measurement

APPLICATION BASICS

Site:	Ascending Aorta
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Chronic
Vessel Diameter:	1.2 - 1.3 mm
Length:	2.5 - 3.0 mm

PROBE

Size:	1.5 mm
Reflector:	J
Connector:	4-pin
Cable Length:	60 cm
Catalog #:	MC-1.5PSL-JN-WC60-CA4S-GA

FLOWMETER

TS420 Perivascular Module

Implantation Techniques

Continuous beat-to-beat measurements of mouse cardiac output (minus coronary flow) can be made directly with the 1.5PSL Flowprobe positioned on the ascending aorta. Surgeries on this scale are performed with the aid of a surgical microscope. The Flowmeter displays mean flow; recorded waveform data are used in calculations of stroke volume, peak flow, aortic flow acceleration dF/dt, aortic input impedance, systemic vascular resistance and heart rate. Flow measurements with pressure data are used to determine pressure volume relations in cardiac function and can now be applied in transgenic mouse models.

PROBE PLACEMENT

Under anesthesia and after the mouse has been properly ventilated on a respirator, a right thoracotomy is performed in the third intercostal space to expose the lungs in the thoracic cavity. The right lung is packed clear of the surgical field with a small piece of surgical sponge. The ascending aorta lies directly under the thymus gland. Carefully dissect the ascending aorta with blunt dissection using microsurgical vessel dilators to free the vessel from connective tissue. The vessel can be manipulated most easily by grasping the small fat pad at the base of the pulmonary artery. After the aorta has been isolated from the pulmonary artery, two pieces of surgical silk are passed under the vessel to aid in placing the vessel within the lumen of the Probe. The Flow Probe is introduced into the thoracic cavity and the vessel lifted into the Probe lumen so that the J-shaped reflector encircles the aorta.

Flow Ranges Observed

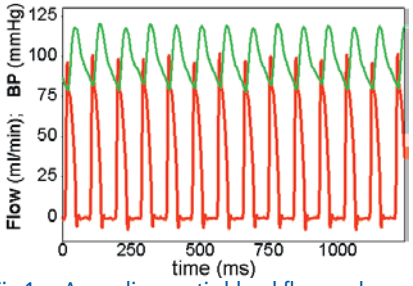


Fig 1: Ascending aortic blood flow and pressure in a conscious mouse 7 days after implantation.
Courtesy, B. Janssen, Univ. of Maastricht

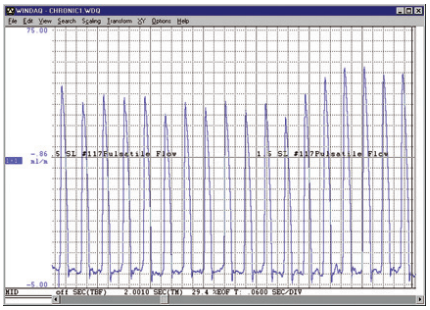


Fig 2: Chronic CO in the Mouse: Transonic 1.5PSL Probe on Ascending Aorta Flow 14.2 ml/m; Heart Rate 510 bpm; SV = 28 µl.

Fig 3: 1.5PSL Probes are designed specifically for chronic implantation on the ascending aorta of the mouse. The transducer housing is less than 5 mm and is easily accommodated in the mouse thoracic cavity. The non-constrictive fit does not interfere with the ascending aorta or pulmonary artery. For connector exteriorization, the Probe's flexible cable is tunneled under the skin.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Ascending Aorta: Chronic Blood Flow Measurement Cont.

Implantation Techniques cont.

After confirmation of vessel placement, the sutures can be removed and the 1.5PSL Flowprobe is rotated so the Probe cable exits laterally to the right. The thoracotomy can be closed over the Probe to establish negative pressure in the thoracic cavity and improve venous return and cardiac output.

For acute measurements, an acoustic coupling gel injected into the Probe lumen is used to transmit the ultrasound signal. Gel is not required for chronic applications; fibrous tissue encapsulation of the Flow Probe will provide good signal transmission after the animal recovers (3-5 days) and is ready for measurement and experimentation.

Surgical Implantation of 1.5PSL Flowprobe on the Ascending Aorta

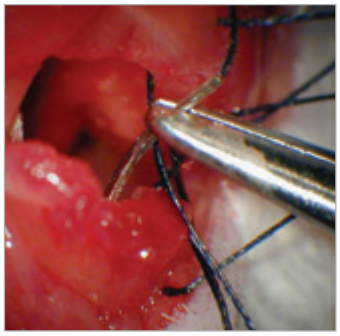


Fig 4: After the mouse has been anesthetized and properly ventilated, a right thoracotomy is performed in the 3rd intercostal space. Closure sutures are placed in the muscle layers at the beginning of the procedure.

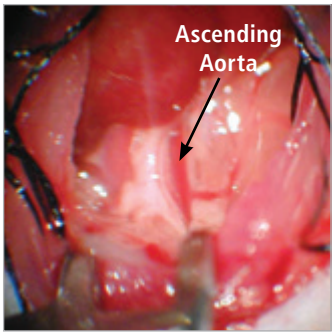


Fig 5: A modified rib retractor is inserted to visualize the heart and ascending aorta. The ascending aorta lies directly under the thymus gland.

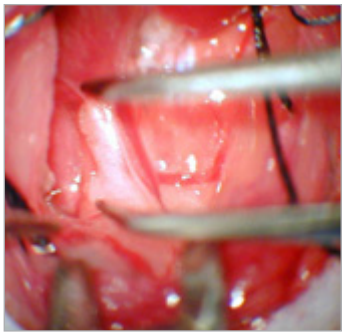


Fig 6: Careful dissection along the ascending aorta is performed by blunt dissection using microsurgical vessel dilators to free the vessel from connective tissue.

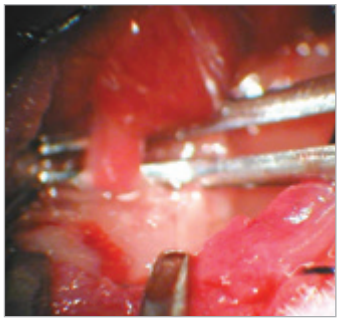


Fig 7: The aorta has been isolated from the pulmonary artery. Gently lift under the aorta to pass surgical silk under the vessel to aid in placing the vessel in the lumen of the Probe.

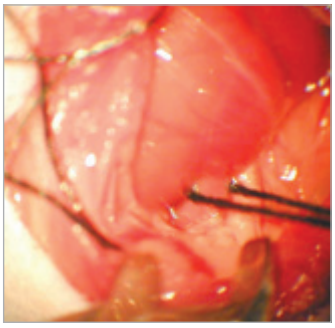


Fig 8: Note the surgical silk around the ascending aorta at the base of the heart.

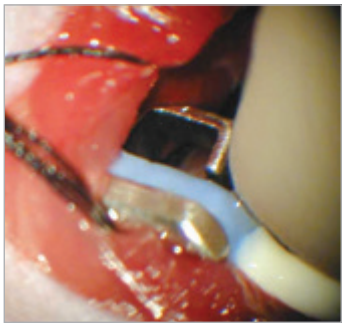


Fig 9: The retractors are removed and the 1.5PSL Flowprobe is introduced into the thoracic cavity.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Ascending Aorta: Chronic Blood Flow Measurement Cont.

Surgical Implantation of 1.5PSL Flow Probe on the Ascending Aorta cont.

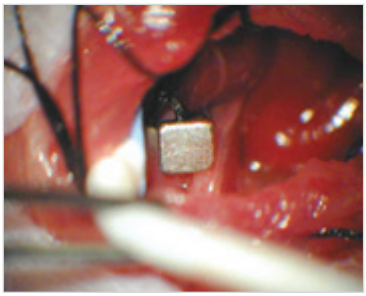


Fig 10: The aorta is gently lifted into the opening of the flow Probe. Confirm that the vessel is within the Probe by gently rotating the Probe. Remove the surgical silk from around the aorta to prepare for closure.

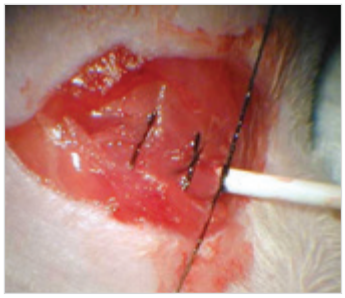


Fig 11: The cable is rotated laterally to the right and the thoracotomy closed over the Probe. Reapproximate the muscles. A suture is passed around the Flowprobe cable to stabilize the Probe in position.

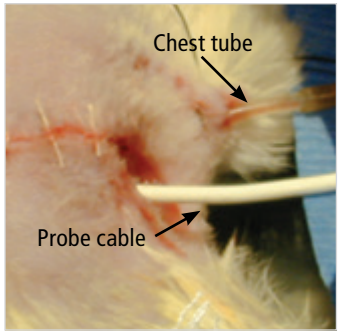
Measurement of Ascending Aorta Blood Flow in the Mouse Protocol©

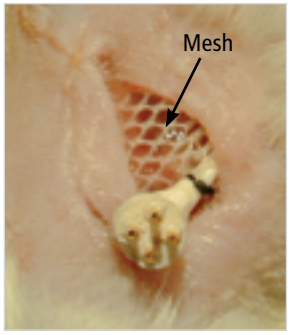
Courtesy of TL Smith, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC. Wake Forest University School of Medicine, Department of Orthopaedic Surgery; used with permission.

Produced by: Margo Sosa, Senior Product Manager, Transonic Systems, Inc., 34 Dutch Mill Rd., Ithaca, NY 14850

For the complete PowerPoint application presentation see: www.transonic.com

Final Closure Procedures





Figs 12, 13: A chest tube attached to a negative pressure system can be monitored for bubbles indicating pneumo-thorax. Close the skin incision. For chronic monitoring, the Probe connector is passed under the skin to the midscapular area by creating a subcutaneous tunnel using a pair of straight hemostats and gently grasping the CA4S connector. Dacron mesh is placed under the skin at the connector and the incision closed. A button cuff is installed over the connector and sutured in place through the dacron mesh.

Fig 14: The mouse can be monitored for cardiac output after recovery in 3 - 5 days. During this time, the flow Probe will encapsulate in fibrous tissue to provide good signal transmission.

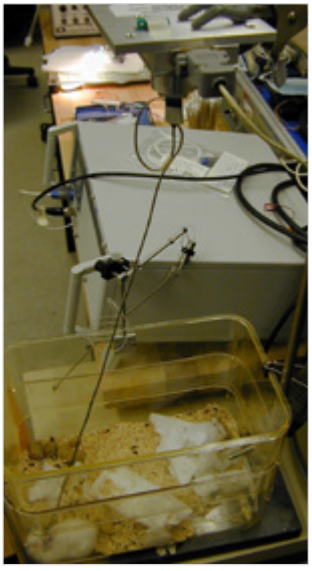


Fig 15: Mouse attached to Dragonfly swivel for flow measurement recording.

Mouse Ascending Aorta: Chronic Blood Flow Measurement Cont.

Continuous Conscious Cardiac Output in Mice

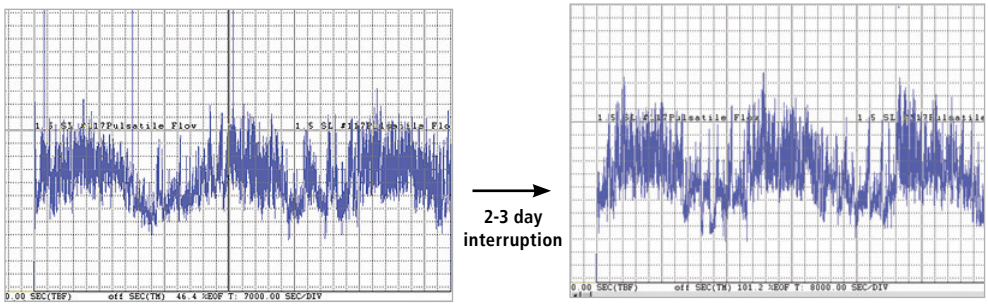


Fig 16: Recording over ~ 8 days with one 2-3 day interruption in recording; 0.1 Hz; 10 samples/sec. Note circadian cycles in flow.

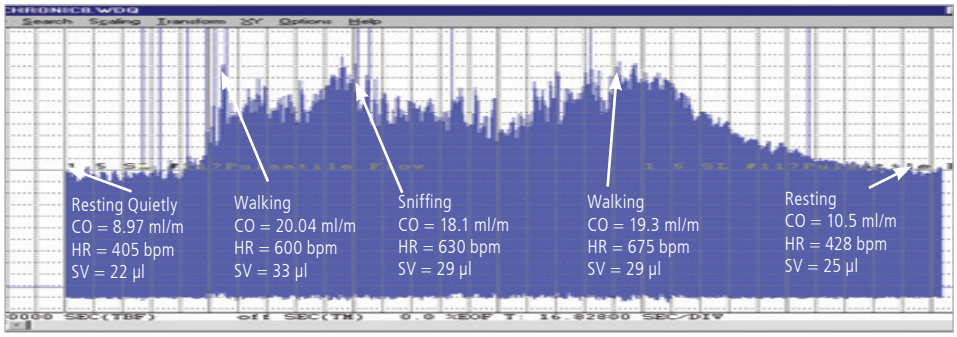


Fig 17: 8 Minute Recording Of Cardiac Output: Dramatic variability demonstrates dynamic range of mouse CO related to activity which cannot be recognized under acute anesthetized protocols.

REFERENCES

Gross V, Luft FC, "Systemic Hemodyna-mics in non-anesthe-tized L-NAME- and DOCA-salt-treated Mice," Obst J Hypertens 2004 ;22(10): 1889-94.

Janssen BJ et al, "Effects of Anesthetics on Systemic Hemodynamics in Mice," Am J Physiol Heart Circ Physio 2004; 287(4): H1618-24.

Janssen BJ et al, "Chronic Measurement of Cardiac Output in Conscious Mice," AJP 2002; 282(3) R928-35.

Lorenz JN, "A Practical Guide to Evaluating Cardiovascular, Renal, and Pulmonary Function in Mice," AJP 2002; 282(6): R1565-582.

Tournoux F et al, "Validation of Noninva-sive Measurement of Cardiac Output in Mice Using Echocardiography," J Am Soc Echocardiogr. 2011.

Janssen BJ, Smits, JF, "Autonomic Control of Blood Pressure in Mice: Basic Physiology and Effects of Genetic Modification," AJP 2002; 282(6): R1545-64.

ACKNOWLEDGEMENT

Transonic® gratefully acknowledges the assistance of collaborators T.L. Smith and M.F. Callahan, Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, in the development of this protocol and gracious sharing of data.

Surgical Protocol (Video)
T.L. Smith, Ph.D,

Detailed procedural video showing step-by-step surgery for implantation of the Transonic® 1.5PSL Flowprobe on the mouse ascending aorta.

www.transonic.com

transonic
THE MEASURE OF BETTER RESULTS.

Rat Ascending Aorta: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Ascending Aorta
Species:	Rat
Body Weight:	230 grams
Duration:	Acute
Vessel Diameter:	2 mm

PROBE

Size:	2 or 2.5 mm
Reflector:	JS
Connector:	CRA10: 10-pin
Cable Length:	100 cm
Catalog #:	MA-2PSB or MA-2.5PSB

FLOWMETER

TS420 Perivascular Module

2PSB

Flow Ranges Observed

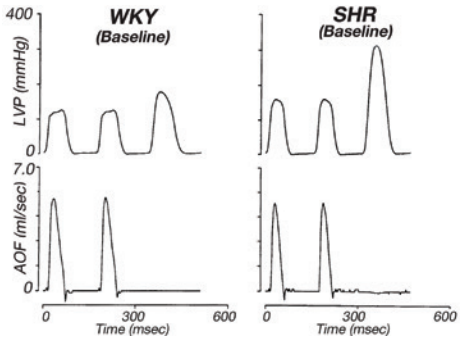


Fig. 1: A typical example of left ventricular pressure (LVP) and aortic flow (AOF) data from in-situ experiment. Base-line data are depicted for 2 steady-state contractions followed by an isovolumetric contraction obtained by occluding ascending aorta in diastole. Left and right panels correspond to data for Wistar-Kyoto (WKY) and spontaneous hypertensive rat (SHR), respectively.

Note: Instantaneous flow in a ketamine anesthetized rat peaked at over 300 ml/min. Cardiac index was 166 ml/kg/min. These values are substantially higher than those in rats anesthetized with pentobarbital.

Application

Measurement of cardiac output has many applications. One researcher studies vasoconstrictors by combining CO with pressure and flow measurements in peripheral vessels. Another studies the effect of altering isomyosin composition on left ventricular resistance. This application requires high speed (1000 Hz) data acquisition and sophisticated digital signal processing.

Comparative Anatomy

The anatomy of the rat may be initially disorienting to the surgeon familiar with larger animals. The normal rat has a persistent left anterior vena cava and an prominent aortic arch that is slightly rotated to the right. The following mental exercise may aid orientation; visualize grafting an additional vessel to the caudal vena cava of the canine heart in Fig 2. Mentally pull the vessel cranially, then merge and move the common carotid arteries from brachiocephalic trunk to the aorta. The result is the remarkably rat-like presentation shown in Fig 3.

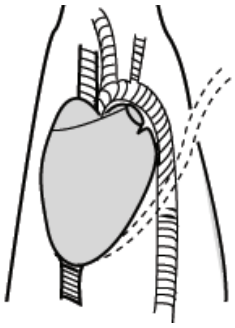


Fig. 2: Canine heart

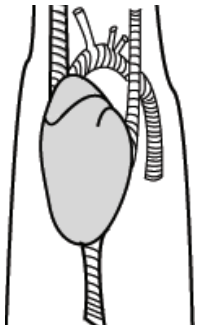


Fig. 3: Canine heart modified to look like a rat heart

Surgical Protocol

Anesthetize the rat with ketamine hydrochloride (70 mg/kg IP) and mechanically ventilate. Perform a median sternotomy and open the pericardium taking care to avoid the vagus nerve. Accuracy is greatest when the Flowprobe fits the vessel very closely and a minimum of acoustic gel is needed.

transonic
THE MEASURE OF BETTER RESULTS.

Rat Ascending Aorta: Acute Blood Flow Measurement Cont.

Surgical Protocol cont.

Place the bracket around the ascending aorta just above the coronary arteries. If you are using a Probe with a slide, close the slide. Position the Flowprobe as shown in Fig 4. In this location, the Flowprobe is perpendicular to the curvature of the arch. Incorrect placement is shown in Fig 5. In Fig 5, the sound beam of the Flowprobe is parallel to the arch and flow may be significantly underestimated.

We recommend Surgilube gel as a couplant because its acoustic velocity is within 30 m/sec of blood. Most ultrasonic coupling gels have an acoustical velocity different than blood and tend to lower sensitivity. To apply gel, remove the plunger of a 30 cc syringe and load the syringe with sterile lubricating gel, taking care to prevent the formation of air bubbles. Place a flexible catheter on the tip of the syringe. Insert the flexible catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The lubricating gel must replace all air space to be effective as an acoustical couplant. Select test mode on the Meter to verify that signal amplitude is close to 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of gel or an air bubble.

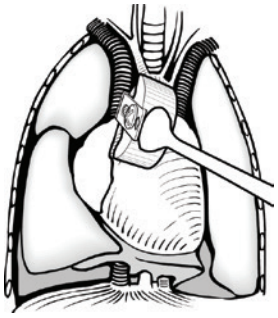


Fig. 4: Correct Flowprobe position

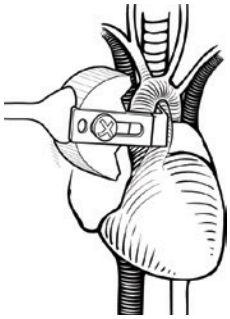


Fig. 5: Inorrect Flowprobe position

REFERENCES

Shroff SG et al, "Isomyosin and Systolic Mechanical Properties of the Intact Hypertrophied Left Ventricle," Circ 1987; 76(4): IV-334.

Shroff SG, Motz W, "Left Ventricular Systolic Resistance in Rats with Hypertension and Hypertrophy," AJP 1989; 257: H386-394.

Shroff SG et al, "Relation Between Left Ventricular Systolic Resistance and Contractile Rate Processes," AJP 1989; 257.

Hoffman A et al, "Endothelin Induces An Initial Increase in Cardiac Output Associated with Selective Vasodilation in Rats," Life Sciences 1989; 45(3): 249-255.

For additional references, visit www.transonic.com

Rat Ascending Aorta: Chronic Blood Flow Measurement (Right Thoracotomy-Side Exit Probe)

APPLICATION BASICS

Site:	Ascending Aorta
Species:	Rat
Body Weight:	270 grams
Duration:	Chronic
Vessel Diameter:	2.5 mm

PROBE

Size:	2.5 mm
Reflector:	JN
Connector:	4-pin
Cable Length:	10 cm
Catalog #:	MC-2.5PSL-JN-WC10-CM4S-GC

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

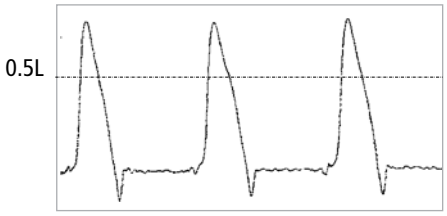


Fig. 1: Instantaneous flow in a conscious 300 gram rat peaked at 550 ml/min. Mean flow was 90 ml/min.

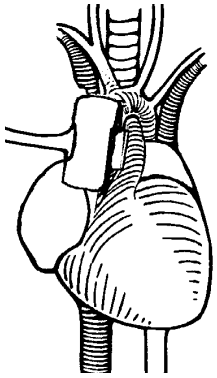


Fig. 2: Lateral cable Probe

Right Thoracotomy

Anesthetize the rat with sodium pentobarbital (60 mg/kg/IP) and ventilate mechanically. Place the rat in left lateral recumbency and make an incision in the right 3rd intercostal space, being careful to avoid the internal mammary artery. Retract the ribs and the right lobe of the lung to expose the aortic arch. Carefully isolate the ascending aorta from the pulmonary artery using blunt dissection. Pass two pieces of 5-0 silk around the aorta to aid in placing the vessel within the lumen of the Probe. Insert the Probe into the thoracic cavity and gently compress the aorta so that the J-reflector bracket can be passed around the vessel.

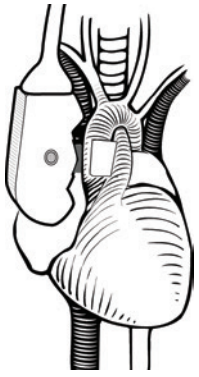


Fig. 3: Side cable Probe

Choice of Flowprobe

2.5PSL Lateral Cable Probe (Fig. 2): The 2.5PSL lateral Probe configuration allows the cable to be anchored in place at the thoracotomy incision site without a separate puncture to properly orient a "side" cable orientation Probe. Position the Probe so that the smooth back of the Probe opposite the reflector is positioned against the sternum. The cable is passed through the thoracotomy and closed routinely.

2.5PSS Side Cable Probe (Fig. 3): This side cable orientation requires a second puncture to pass a trocar through the pectoral muscles and the 2nd intercostal space to correctly orient the cable to keep the Probe from twisting on the vessel. Pass the CM4S or CA4S connector and cable through the trocar to exteriorize it. Close the thoracotomy site routinely.

Rat Ascending Aorta: Chronic Blood Flow Measurement (Right Thoracotomy-Side Exit Probe) Cont.

Exteriorizing the Connector

Create a subcutaneous tunnel with straight hemostats between the forelimbs and up to the neck. Place the animal in ventral recumbency and make a 1.5 cm mid-scapular incision. Route the connector and cable to exit between the shoulder blades. Place a patch of Dacron mesh under the skin to reinforce skin at the mid-scapular exit site. Close the skin incision and place the CM4S or CA4S connector in the cuff. Flexible silicone and rigid delrin cuffs are available for both connector types. Suture the cuff to the skin through Dacron mesh placed subdermally to reinforce sutures.

ACKNOWLEDGEMENT

Thomas L. Smith, Ph.D., Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

REFERENCES

Johnson RA, Lavesa M, Askari B, Abraham NG, Nasjletti A, "A Heme Oxygenase Product, Presumably Carbon Monoxide, Mediates a Vasodepressor Function in Rats," Hypertension 1995; 25:166-169. (531A)

Greene, E.C, Anatomy of the Rat, Hafner Publishing Co., New York, 1963.

Rat Surgical Video
Blood Flow Measurement in the Rat with "Implantation Techniques of the Transonic Flowprobe on the Rat Ascending Aorta," Smith, vp-10

PROBE RECOMMENDATIONS FOR ANIMAL WEIGHT & PROTOCOL				
WEIGHT (GRAMS)	**PROBE SIZE**	**PROBE MODEL**	**CABLE ORIENTATION**	**SURGICAL APPROACH**
< 250	2 mm	2PSB	Back	Medium sternotomy
		2.5PSL	Lateral	Right thoracotomy at 3rd intercostal
250 - 350	2.5 mm	2.5PSS	Side	Right thoracotomy with CA4S micro-connector exited at 2nd intercostal
400 - 500	3 mm	3PSL	Lateral	Right thoracotomy at 3rd intercostal
		3PSB	Back	Medium sternotomy

Rat Ascending Aorta: Chronic Blood Flow Measurement (Right Thoracotomy-Back Exit Probe)

APPLICATION BASICS

Site:	Ascending Aorta
Species:	Rat
Body Weight:	325 - 400 grams
Duration:	Chronic
Vessel Diameter:	2.8 mm

PROBE

Size:	3 mm
Reflector:	LS
Connector:	4-pin
Cable Length:	12 cm
Catalog #:	MC-3PSB-LS-WC12-CM4S-GC

FLOWMETER

TS420 Perivascular Module

3PSB

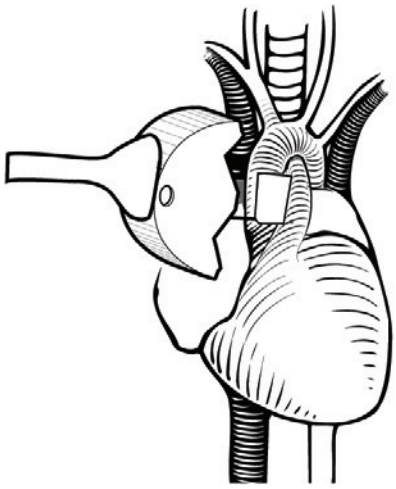


Fig. 1: PSB Probe on Ascending Aorta

Right Thoracotomy Using Back Exit Flowprobe

Proper stabilization and orientation of the Probe cable is essential to keep the Probe in position on the ascending aorta and to avoid the Probe twisting on the vessel during closure and exteriorization of the connector to the back.

Anesthetize the rat with sodium pentobarbital (50 mg/kg i.p.) and ventilate mechanically. Perform a right thoracotomy between the second and third ribs. Isolate the ascending aorta and clear of adipose and connective tissue. Position a 3 mm PS-Series back cable exit Probe around the aorta as close to the heart as possible. Pack the space between the body of the Probe and the aorta with surgical sponge (Mericel). Pass the cable from the Probe through the thoracotomy and tunnel it subcutaneously to the top of the skull.

Close the thoracotomy, suture the muscle layers and reduce the pneumothorax. Affix the Probe connector to the top of the skull with a skin button (AAPC104) and dental acrylic. Suture the wounds and treat with a topical antibiotic. Administer a suspension containing ampicillin (10 mg) subcutaneously.

ACKNOWLEDGEMENT

R. Allan Buchholz, Ph.D., Pfizer Central Research Div., Groton, CT and R.L. Dundore, Ph.D., Sterling Drug Co., Dept. of Pharmacology, Rensselaer, NY.

REFERENCES

Dundore RL, Habeeb PG, Pratt PF, Becker LT, Clas DF, Buchholz RA, "Differential Hemodynamic Responses to Selective Inhibitors of Cyclic Nucleotide Phosphodiesterases in Conscious Rats," J of Cardiovasc Pharm 1992; 19: 937-44.

Rat Ascending Aorta: Chronic Blood Flow Measurement (Sternal Approach)

APPLICATION BASICS

Site:	Ascending Aorta
Species:	Rat
Body Weight:	277 - 300 grams
Duration:	Chronic
Vessel Diameter:	2.5 mm

PROBE

Size:	2.5 mm
Reflector:	JS
Connector:	4-pin
Cable Length:	12 cm
Catalog #:	MC-2.5PSB-JS-WC12-CM4S-GC

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

90-100 ml/min in a 300 gram conscious rat.

2.5PSB

Application

This protocol was developed to evaluate systemic hemodynamic effects of selective inhibitors of cyclic nucleotide phosphodiesterase in the conscious rat model.

Sternotomy

A mediosternal approach can allow better visualization into the thoracic cavity for Probe placement on the aorta. Though this approach would seem more invasive, the procedure can be performed more quickly and animals may recover faster from a more expedient surgery. It also has allowed Probe placement in younger, smaller rats (150 grams).

Advantages of Sternal Approach

1. Least stressful to the rat for healing
2. Easy to visualize the aorta and heart
3. Fast
4. Low risk of damaging the lungs
5. Allows implants on young (150 g) rats

Anesthesia and Surgical Preparation

1. Anesthetize with pentobarbital (60 mg/kg). Administer 0.1 mg/kg atropine simultaneously. Once the rat is anesthetized, administer 2 mg gentamicin.
2. Intubate with PE-190.
3. Place rat in dorsal recumbency.
4. Scrub surgical area of rat with 1.0% betadine solution and place a surgical drape over the rat.

Rat Ascending Aorta: Chronic Blood Flow Measurement (Sternal Approach) Cont.

Flowprobe Implantation

1. Make a 3.5 cm. midline muscle and skin incision over the sternum using a #11 scalpel blade.
2. Attach tracheal tube to the respirator.
3. Lift the sternum with rat-toothed forceps. Using a #11 scalpel blade, make a puncture just lateral to the midline on the left side. Puncture should be mid-thoracic.
4. Insert one blade of a 5" straight scissor into the puncture and cut on the midline of the sternum 1.25 cm rostral and caudal. Caudal extent should not exceed 1 cm above the diaphragm. Keep pleural membrane on right side intact.
5. Retract with a microretractor to expose the thymus and heart. Use a retractor with short, blunt prongs to maintain an intact right pleura.
6. Divide the thymus into the left and right lobes to expose the aorta.
7. Gently separate the ascending aorta from the pulmonary artery with a pair of curved 4" micro-dissecting forceps.
8. Use these forceps to clear 3 mm along the aorta for subsequent placement of the J-reflector of the Flowprobe.
9. Place a 5" piece of 3-0 silk suture around the aorta.
10. Position the Probe with the open side of the J-reflector toward the aorta and the back side facing the pulmonary artery.
11. Using the suture, gently guide the aorta into the J-reflector, close the J-reflector, and remove the suture (Fig. 1).
12. Remove the retractor and position the cable to exit at approximately a 45 degree angle cranially such that the aorta is parallel with the J-reflector. The position of the heart and aorta within the chest are such that the Probe naturally assumes this position (Fig. 2).
13. Placing one suture on each side of the cable, suture cable in place by closing sternum rostrally.

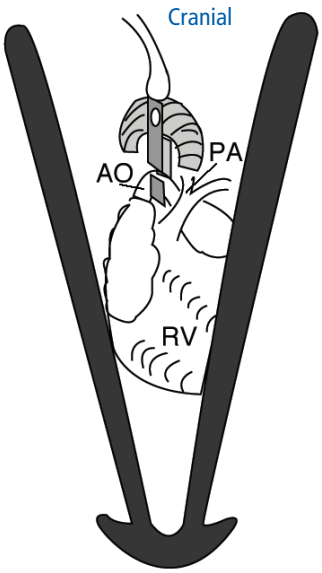


Fig. 1: Schematic of sternum separated to xyphoid.

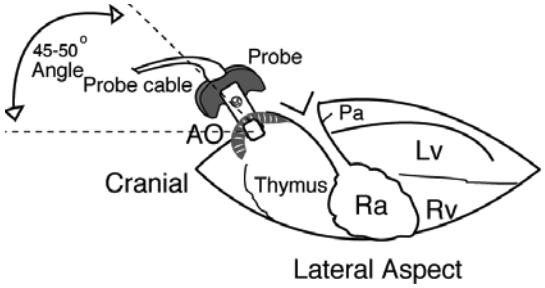


Fig. 2: Site and orientation of Probe on the ascending aorta.

Rat Ascending Aorta: Chronic Blood Flow Measurement (Sternal Approach) Cont.

Restoration of Negative Pleural Pressure

1. From the midline incision, the skin on the left side of the thorax is pulled away by blunt dissection to expose the pectoralis minor muscle.
2. Place a 3-0 silk purse string suture in the pectoralis minor muscle 1.5 cm lateral to midline.
3. Brace the inside of the ribcage with a pair of 4" straight dissecting forceps. Make a puncture wound between the ribs inside the purse string using a pair of 4" curved micro-dissecting forceps (Fig. 3).
4. Insert a 5 cm silastic tube (0.03in. x 0.065 in) 1.5 cm into the chest cavity, through the puncture wound, and tie loosely.
5. Complete the closure of the sternal incision and muscle in two layers.
6. Using a 3 cc syringe and 19-gauge needle, draw a negative pressure through the chest tube.
7. Check sternal incision for leaks.
8. Tighten purse string while removing the chest tube.
9. Retract skin flap over purse string.
10. Close skin caudally and leave enough open rostrally for tunneling the connector.

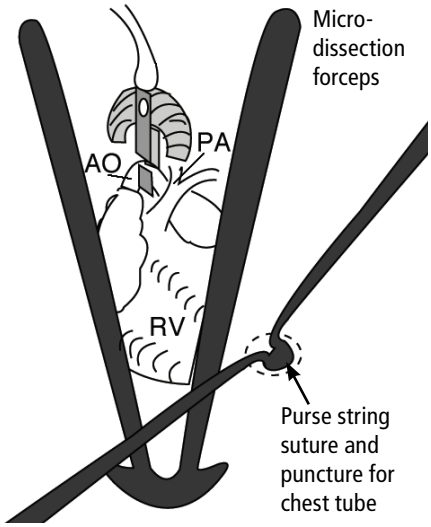


Fig. 3: Schematic showing site of suture and chest tube.

Connector Placement

1. Using a curved hemostat, tunnel from the base of the neck, above the left shoulder, to the cranial end of the incision.
2. Grasp the connector and pull through to base of the neck.
3. Close the sternal skin incision.
4. Place connector into plastic connector plate.
5. Suture connector plate into the neck muscles cranial to scapulae.

Weaning Off Respiration

1. Apply positive end-expiratory pressure by placing exhaust line of respirator in 2 cm of water for 1 minute.
2. Disconnect respirator from tracheal tube and check for spontaneous breathing. This may require up to 1 minute to occur.
3. Extubate when the rat exhibits a strong gag reflex.

ACKNOWLEDGEMENT

John W. Osborn, Ph.D., Departments of Animal Science and Physiology, AnSci/Vet Rm. 435, Univ. of Minnesota, St. Paul, MN

Ingegerd M. Keith, Ph.D., College of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive West, Madison, WI.

For additional references, visit:
www.transonic.com

Rat Abdominal Aorta: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Abdominal aorta
Species:	Rat
Body Weight:	270 grams
Duration:	Acute
Vessel Diameter:	1.2 mm

PROBE

Size:	1.5 mm
Reflector:	JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-1.5PRB

FLOWMETER

TS420 Perivascular Module

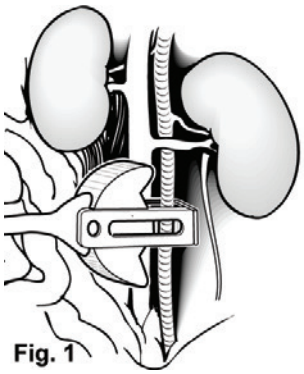


Fig. 1: Application of Flowprobe on the Abdominal Aorta.

Flow Ranges Observed

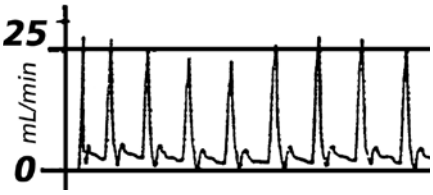


Fig. 2: Anesthetized rat, instantaneous abdominal flow: 2 to 25 ml/min.

Application

The measurement of blood flow on the abdominal aorta is commonly used by researchers for protocols that require relative cardiac output, vascular resistance or absolute flow to the hind limbs. The surgical approach to the abdominal aorta is less difficult than the corresponding approach to the ascending aorta and does not require mechanical ventilation.

Surgical Approach

Anesthetize the rat with ketamine/xylamine solution (0.09 ml/ 100 g body weight IM, thigh). The use of a heating pad or hot water bottle is recommended as hypothermia also reduces flow. In long procedures, fluid infusion (0.9% NaCl @ 1 ml/hr) through a femoral catheter is also recommended.

Place rat in dorsal recumbency and make a ventral midline abdominal skin incision. Extend the abdominal incision through the linea alba into the abdominal cavity. Deflect the intestines to the rat's right to expose the abdominal aorta and the left kidney. Carefully dissect free a 1 cm segment of the aorta just caudal to the kidneys. Remove adjacent fat for proper acoustical coupling. Place the Probe around the artery and close the slide. Manually position the Probe so that the artery is centered within the window and then tape down the Probe cable to help stabilize the Probe. If there is sufficient connective tissue, the Probe may also be sutured in position.

Remove the plunger of a 30 cc syringe and load the syringe with Surgilube gel, taking care to prevent the formation of air bubbles. Place a flexible catheter (or angiocatheter) on the tip of the syringe; the catheter may be inserted into the Probe's acoustic window adjacent to the vessel and the gel deposited as the syringe is withdrawn. A low signal or an acoustic error can usually be traced to an insufficient amount of lubricating gel or an air bubble.

ACKNOWLEDGEMENT

Flow trace courtesy of Dr. Wayne Schwark, Department of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY.

REFERENCES

Validation

D'Almeida MS, Gaudin C, Lebrec D, "Validation of 1- and 2- Transit Time Ultrasound Flow Probes on Mesenteric Artery and Aorta of Rats," AJP 1995 268(3) Pt. 2 H1368-1372.

Applications

Haque SM et al,"Quantification of Intestinal Blood Flow by Ultrasonic Transit Time Flowmetry in Fed and Endotoxaemic Rats," Eur J Surg 1996; 162(7): 561-565. (857A)

Cohn SM et al, "Diaspirin Cross-Linked Hemoglobin Resuscitation of Hemorrhage: Comparison of a Blood Substitute with Hyperteonic Saline and Isotonic Saline," J Trauma: Injury, Infection and Crit Care 1995; 39(2): 210-216. (790A)

Myers SI et al, "Endotocis Shock After Long-Term Resuscitation of Hermorrhage Reperfusion Injury Decreased Splanchnic Blood Flow and Eicoanoid Release," Annals Surg 1996; 224(2): 213-218.

For additional references, visit www.transonic.com

transonic
THE MEASURE OF BETTER RESULTS.

Rat & Mice: Coronary Blood Flow: Isolated Perfused Heart Preparation: Langendorff and Working Heart Models

APPLICATION BASICS				
MODEL	MOUSE		RAT	
	FLOW RANGE (ml/min)	INLINE FLOWSENSOR	FLOW RANGE (ml/min)	INLINE FLOWSENSOR
Langendorff	2 – 8	ME1PXN	7 - 30	ME2PXN
Working Heart (2 Inline Sensors)	8 – 15	ME2PXN	40 – 80	ME3PXN
Flowmeter	TS410 Tubing Module			
Calculated Cardiac Output = Coronary (Atrial Inflow) – Aortic Outflow				

Fig. 1: Inline Flowsensors: ME1PXN, ME2PXN and ME3PXN

Background

FLOWSENSOR CALIBRATION	
SOLUTION	Krebs blood or other Physiological Buffer Solution (sample may be required for factory calibration)
TEMPERATURE	37 C°
FLOW RATE	Isolated heart applications require calibration in a custom low flow range for the flow rates indicated above that are typical for isolated heart preps. Higher flow rates may be requested for working heart preparations.
Note: Isolated heart applications require calibration in a custom low flow range for the flow rates indicated above that are typical for isolated heart preps. Higher flow rates may be requested for working heart preparations.	

The first isolated perfused mammalian heart preparation was developed by Oscar Langendorff in the 1890's and continues to be a viable technique to study myocardial function today. Early discoveries in heart physiology originating from this technique include the roles of temperature, oxygen, calcium ions, the electrical activity during the cardiac cycle, the role of coronary circulation to deliver nutrient and oxygen rich blood to the heart and, importantly, that cardiac mechanical function is affected by changes in the coronary circulation.

Langendorff Method

This in vitro isolated organ technique allows the study of contractile force, heart rate, coronary resistance and other parameters of the heart under known physiological conditions without the neural and hormonal complications of an in vivo, whole animal experiment. In the Langendorff heart preparation, the heart is isolated from the animal, a cannula is inserted into the ascending aorta and the heart is perfused in a retrograde direction with blood or, more commonly, oxygenated nutrient rich crystalloid solution from a gravity fed reservoir. Pressure from the retrograde perfusion causes the aortic valve to close and forces the solution into the coronary circulation, draining via the coronary sinus into the right atrium. Contractions in the heart will continue in this state and various parameters can be measured.

There have been several advances in the apparatus and instrumentation to conduct these experiments, but the methodology remains fundamentally the same in the Langendorff preparation. The heart is perfused in a retrograde fashion in one of two modes: by constant pressure or constant flow.

Constant Pressure Mode

The perfusion of the heart is maintained at a constant pressure. Changes in resistance of the heart will result in fluctuations in the flow rate that are measured with a Transonic® Inline Flowsensor.

Rat & Mice: Coronary Blood Flow: Isolated Perfused Heart Preparation: Langendorff and Working Heart Models Cont.

Background Cont.

Constant Flow Mode

The perfusate is pushed through the heart at a constant flow rate. Changes in the resistance of the heart will result in fluctuations in pressure that can be monitored with a pressure transducer.

Additional parameters that can be measured in the isolated beating heart include:

- Left Ventricular Pressure via a balloon inside the ventricle equipped with a pressure transducer.
- Contractile Force via a force transducer attached to the apex of the heart.
- Electrical Activity is monitored via electrodes attached to the apex and atria.

Working Heart Method

The Langendorff isolated heart method was modified later by Neely et al to produce a model that would allow the study of the isolated heart under normal circulatory dynamics. In the working heart model, a second cannula is inserted into the pulmonary vein to perfuse the heart via the left atrium. The working heart pumps the fluid from the left ventricle out of the aorta under experimentally controlled preload (atrial pressure) and afterload (aortic resistance) conditions. This allows the measurement and calculation of additional parameters under the conditions of work. Flow in the atrial perfusion inflow cannula and aortic outflow cannula are measured with Transonic® Inline Flowsensors to calculate cardiac output (aortic flow plus coronary flow) and coronary flow (atrial inflow – aortic outflow). Coronary flow may be derived from the two Flowsensors or by collecting and weighing the coronary effluent. Other parameters that can be derived from the pressure and volume flow measurements are stroke volume, stroke work and vascular resistance.

Significance

Both the Langendorff isolated perfused heart and the working isolated heart methodologies have been used extensively in pharmacological and physiological studies to access ischemia and reperfusion injury with various pharmacological agents. This method remains popular because of the simplicity and ease of control, reproducibility and relatively low economic cost.

Common Donor Animals

Rats, transgenic mice, guinea pig and rabbit

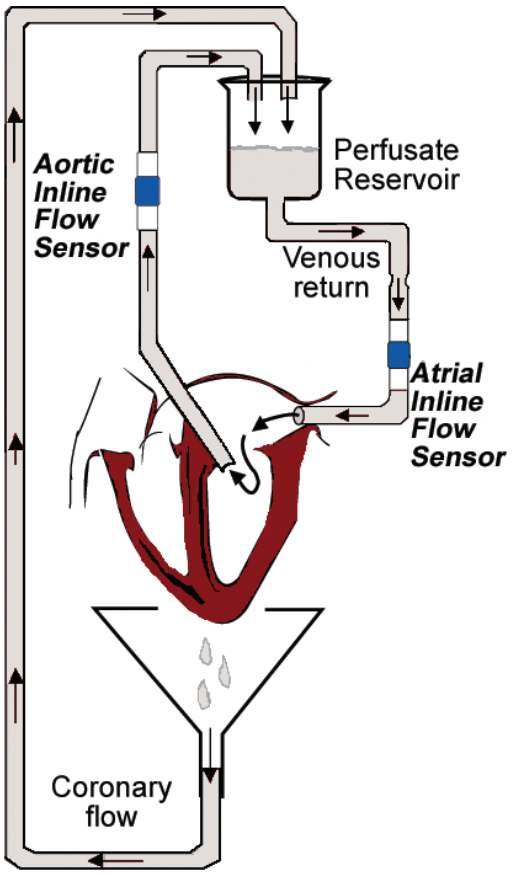


Fig. 2: Schematic of the working heart model of an isolated perfused heart preparation.

Langendorff Apparatus Sources:

Harvard Apparatus, Inc.

Hugo Sachs Electronik
(see Harvard Appartaus)

Rattus (Kent Scientific)

Radnoti Glass Technology, Inc.
Monrovia, CA www.radnoti.com

Rat & Mice: Coronary Blood Flow: Isolated Perfused Heart Preparation: Langendorff and Working Heart Models Cont.

Preparation

Anesthetize the rat with pentobarbital (60 mg/kg IP). To prevent coagulation, administer heparin (1000 IU/kg) intravenously in the right femoral vein (See Anesthetic Guidelines RL-67-tn for more information). A cannula is placed in the trachea for ventilation. Make a longitudinal skin and muscle incision opening the abdomen from the diaphragm to the throat. Cut the diaphragm free from the ribs. Open the thorax following the bone-cartilage border on the left and right sides parallel to the sternum from the diaphragm cranially to the first rib. Turn the complete anterior thoracic wall upwards over the head to expose the heart. Remove the pericardium. Separate the ascending aorta from connective tissue and the pulmonary artery using blunt dissection. Preplace a thread around the aorta.

Prepare for insertion of the aortic cannula: Prime the cannula to remove air bubbles and allow a small stream of perfusate during insertion. Clamp the vena cava above the diaphragm to minimize bleeding. Sprinkle the heart with cold physiological saline (4° C) so the heart slows down and stops beating. Incise the pulmonary artery to avoid distension of the right ventricle. Incise the aorta as far cranially as possible and insert the cannula, taking care that the position of the cannula is not too low to impede the aortic valves or the coronary ostia. Tighten the thread around the end of the cannula. Fully perfuse the heart. Completely isolate and remove the heart for transfer to the Langendorff apparatus.

REFERENCES

Döring HJ, Dehnert H, The Isolated Perfused Heart According to Langendorff, English edition, Biomess-technick-Verlag, West Germany, 1988.

Yasutake M et al, "Na+/H+ Exchange and Reperfusion Arrhythmias: Protection by Intracoronary Infusion of a Novel Inhibitor," AJP 1994; 267: H2430-40 (452A)

Galiñanes M et al, "Can Ischemic Preconditioning Ensure Optimal Myocardial Protection When Delivery of Cardioplegia Is Impaired?" Circulation 1995; 92 (Pt 2): 389-94. (580A)

Bouma P et al, "Nitric Oxide is An Important Determinant of Coronary Flow in the Isolated Blood Perfused Rat Heart," Basic Res Cardiol 1992; 87: 570-584. (327A)

Bouma P et al, "Coronary Arterial Inflow Impediment during Systole is Little Affected by Capacitive Effects," Am J Physiol 1993; 263: H715-21. (328A)

Zhang J et al, "Endothelin-1 Induces Direct Constriction of Hepatic Sinusoids," AJP 1994; 266(29): G624-G652. (462A)

Habazettl H et al, "Improvement in Functional Recovery of the Isolated Guinea Pig Heart after Hyperkalemic Reperfusion with Adenosine," J Thorac & Cardiovasc Surg, 1996; 111: 74-84. (599A)

Szczesna-Cordary D et al, "Myosin regulatory light chain E22K mutation results in decreased cardiac intracellular calcium and force transients," FASEB J 2007; 21(14): 3974-85.

Sambandam N et al, "Chronic activation of PPAR is detrimental to cardiac recovery after ischemia," AJP Heart Circ. Physiol. 2006; 290: H87-H95.

Skrzypiec-Springa M et al, "Isolated heart perfusion according to Langendorff—Still viable in the new millennium," J Pharmacol Toxicol Methods. 2007; 55(2):113-26.

For additional references, visit www.transonic.com

Rat & Mouse Carotid Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Carotid artery
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Acute
Vessel Diameter:	0.55 - 0.60 mm
Length:	2.5 - 3.0 mm

PROBE

Size:	0.5 mm or 0.7 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.5PSB, MA-0.7PSB, MA-0.5VB
FLOWMETER	TS420 Perivascular Module

APPLICATION BASICS

Site:	Carotid artery
Species:	Rat
Body Weight:	280 grams
Duration:	Acute
Vessel Diameter:	0.7 - 1.2 mm

PROBE

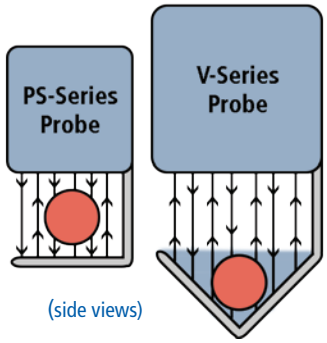
Size:	0.7 mm or 1 mm
Reflector:	JN or JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.7PSB, MA-0.7VS MA-1PRB w/handle
FLOWMETER	TS420 Perivascular Module

Application

The common carotid artery is an easily accessible vessel for measurements for relative cardiac output and cerebral blood flow. One advantage of the common carotid artery over the abdominal descending aorta is that the surgical approach for the common carotid artery does not require opening a major body cavity resulting in better thermoregulation and less physiological shock.

Transonic® Flowprobes are routinely used in rats and mice in standard study protocols for thrombosis formation and lysis because of their measurement precision. The recent explosion of genetic modeling techniques in mice for *in vivo* studies and the advanced technologies for bench marking results have become critical to understanding the pathophysiology of thrombosis formation and its treatment.

There are a handful of accepted methods to model arterial thrombosis in genetically altered mice. Two have been used routinely on the mouse carotid artery. The Ferric Chloride Model applies a 1 x 2 mm patch of ferric chloride-saturated paper directly on the adventitia of the isolated carotid artery. The Photochemical Model of Arterial Thrombosis uses circulating Rose Bengal that is activated by green laser illumination (545 nm wavelength) of the isolated carotid artery to cause oxygen-radical injury to the endothelium.



PS-Series Nanoprobe: The full height of the ultrasonic window has the same flow sensitivity, so that the vessel can be positioned anywhere within the Probe lumen.

V-Series: Only within the triangle (shaded) portion of the V will the Probe reach its full flow sensitivity

Fig. 1: Side-by-side comparison of a V-Series Flowprobe (on left) and Nanoprobe (on right).

Rat & Mouse Carotid Artery: Acute Blood Flow Measurement Cont.

Application cont.

For results that can be interpreted across studies, these methods rely on accurate "time to occlusion" blood flow measurement using Transonic® Flowmeters.

Transonic® Nanoprobes are the method of choice for carotid artery occlusive thrombosis studies which require precision measurement of zero blood flow to discern "time to occlusion". In the literature they are often mistaken for "Doppler Probes." They are not Doppler velocity Probes. Transonic® Nanoprobes are miniaturized ultrasonic transit-time Flowprobes that loosely cradle the target vessel in the mouse and directly measure the volume flow of blood in the vessel in ml/min. They are sized for mouse-size vessels and mouse-sized flow rates. Mouse carotid flow rates average approximately 0.24 to 0.7 ml/min in sham operated pre-occluded experimental animals depending on anesthetic and protocol that includes or does not include mechanical ventilation.

Introduction

The carotid artery is a long vessel that is free of branches and very easy to locate and isolate. In a mouse, the vessel is 0.5 to 0.6 mm diameter. Transonic® 0.5PSB and 0.7PSB Nanoprobes fit the vessel very closely, thereby minimizing the amount of acoustic gel required to achieve and maintain good signal. These small bodied Probes allow best visualization of the vessel and experimental site since they occupy little space along the vessel. Nanoprobes for acute use are fitted with handles, useful in stabilizing the position of the Probes for precision experiments such as measuring the time to occlusion in thrombosis studies.

In a rat, the vessel is minimally 0.7 mm diameter to 1.2 mm diameter, depending on the age of the animal and the treatment protocol (eg. ligation of the contralateral carotid artery) A 0.7PSB Nanoprobe will fit the vessel very closely and may be used in smaller animals, thereby limiting the amount of acoustic jelly that is required to achieve good signal. However, the Nanoprobe will be constrictive on many animals. For vessels larger than 0.7 mm diameter, use a 1 mm 1PRB Flowprobe fitted with a handle.

Many studies also reference high sensitivity 0.5VB mouse and 0.7VB rat Flowprobes for thrombosis applications. These may be used in this vessel location since the carotid is a long vessel and affords the space for the larger bodied Probe. If using the V-series, make certain that the vessel is fully within the sensitive "V" area of the Probe reflector and fill the remaining space with acoustic gel (Fig. 1). Only the "V" area of the Probe is sensitive to total flow. Flow outside this "V" position will be underestimated.

Flow Ranges Observed

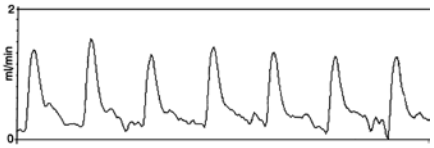


Fig. 2: Instantaneous flow in the common carotid artery ranged from 0 to 2 ml/min in a deeply anesthetized 200 gram rat.

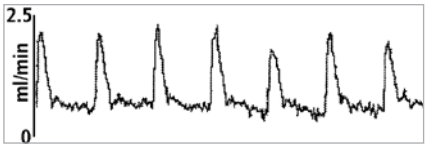


Fig. 3: Acute carotid arterial flow in a 31.2 gram mouse with a 0.7 mm V-Series Flowprobe.

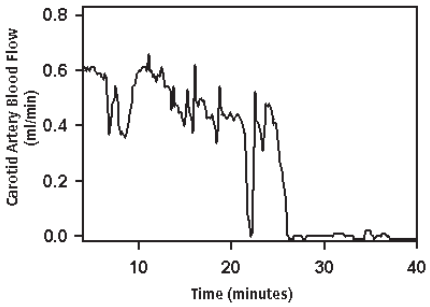


Fig. 4. Photochemical-induced injury set-up: Green laser light illumination on isolated mouse carotid artery proximal to Transonic® 0.5PSB Flowprobe. Sustained zero blood flow measured by the Flowprobe indicates carotid artery occlusion from thrombosis.

ACKNOWLEDGEMENT

Data and pictures courtesy of Katina Wilson and Steven Lentz, Dept. of Internal Medicine, Univ. of Iowa, Iowa City, IO.

Rat & Mouse Carotid Artery: Acute Blood Flow Measurement Cont.

Surgical Approach

Mice are anesthetized with sodium pentobarbital (70-90 mg/kg intraperitoneally) and secured in a supine position under a dissecting microscope. A heating pad is used to maintain body temperature at 36-37 °C. Supplemental anesthesia is administered as needed. A midline cervical incision is made and the trachea and right common carotid are dissected free, Mice are ventilated mechanically with room air and supplemental oxygen (80 breaths per minute, stroke volume 0.5 ml) using a Harvard rodent respirator modified with a 1.0 ml cylinder and piston assembly. Carotid artery blood flow is measured with a 0.5 PSB Flowprobe.

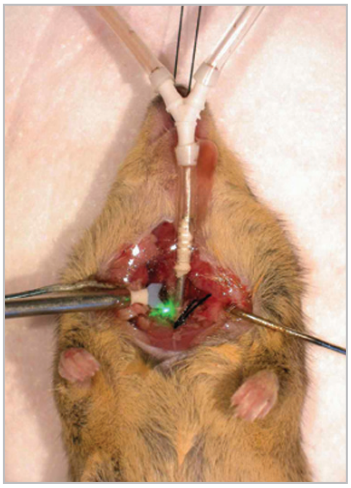


Fig. 5: Ventilated mouse undergoing photochemical thrombosis in the carotid artery.

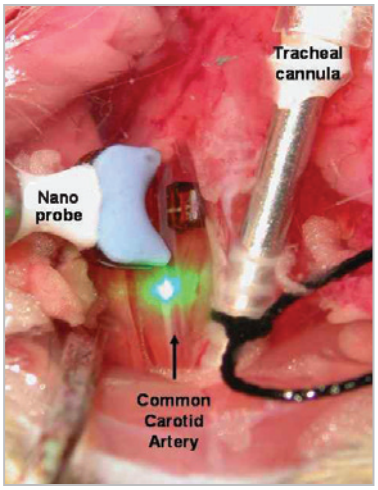


Fig. 6: Close-up of 0.5PSB Nanoprobe measuring carotid arterial blood flow in the mouse.

REFERENCES

Wilson KM et al, "Prothrombotic effects of hyperhomocysteinemia and hypercholesterolemia in ApoE-deficient mice," Arterioscler Thromb Vasc Biol 2007;27:233-40.

Wilson, KM et al, "Effect of mechanical ventilation on carotid artery thrombosis induced by photochemical injury in mice," J Thromb Haemost. 2003 Dec; 1(12): 2669-74. (2849A)

White TA et al, "Endothelial-derived tissue factor pathway inhibitor regulates arterial thrombosis but is not required for development or hemostasis," Blood 2010; 116(10): 1787-1794. (7997A)

Westrick RJ et al, "Murine models of vascular thrombosis (Eitzman series)," Arterioscler Thromb Vasc Biol 2007; 27(10): 2079-93.

Sturgeon SA et al, "Adaptation of the Folts and electrolytic methods of arterial thrombosis for the study of anti-thrombotic molecules in small animals," J Pharmacol Toxicol Methods 2006; 3(1): 20-9. (7082A))

Dörffler-Melly J et al, "Functional thrombomodulin deficiency causes enhanced thrombus growth in a murine model of carotid artery thrombosis," Basic Res Cardiol 2003; 98(6):347-52.

Eitzman, DT et al, "Plasminogen Activator Inhibotir-1 Deficienct Protects against Arteroscherosis Progression in the Mouse Carotid Artery," Blood 2000; 96(13): 4212-4215. (2569A)

Rat Carotid Artery: Chronic Blood Flow Measurement

APPLICATION BASICS

Site:	Carotid artery
Species:	Rat
Body Weight:	280 grams
Duration:	Chronic
Vessel Diameter:	0.7 - 1.2 mm

PROBE

Size:	1 mm
Reflector:	JS
Connector:	4-pin
Cable Length:	10 cm
Catalog #:	MC-1PRS-JS-WC10-CM4S-GC MC-1PRB-JS-WC10-CM4S-GC

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

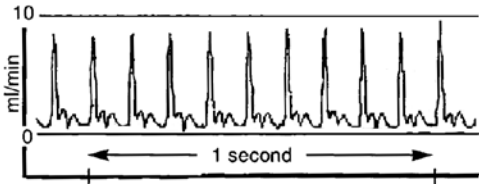
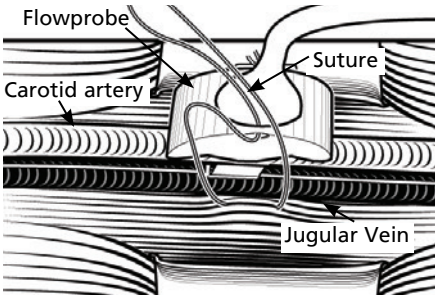


Fig. 1: Typical flow rate of a fully awake rat.

Note: We recommend that side cable configuration be used. It can be anchored in place with a suture around the cable and a loop through the slide cover.



Application

A study investigated the effects of hypergravic stress on cerebral blood flow (CBF). Rats were chronically instrumented with a Flowprobe on the right carotid artery and biparietal EEG electrodes. Four days post surgery the rats were exposed to 5-25 +Gz exposure (head to foot inertial stress). Each centrifuge exposure was 30 seconds in duration with 15 minutes of rest between +Gz exposures. A total of nine exposures were done on each rat at 2.5 +Gz increment.

Surgical Approach

Rats (250-350 g) were anesthetized using 3% halothane administered with medical grade oxygen via an Ohmeda vaporizer (BOC Health Care, England). The surgical sites (tracheal and scapular region) were shaven and aseptically prepared. A midline incision of the tracheal area was made. Blunt dissection exposed the common carotid with skin and muscles held open using bulldog clamps. Adherent tissues were retracted using 3-0 silk sutures anchored to a pair of hemostats. Approximately 2 cm of the common carotid artery was freed from connective tissues, the jugular vein, and vagus nerve by carefully passing a pair of serrated full curved forceps several times. The freed section of the artery was held in place using a halsted mosquito forcep (Roboz).

A Flowprobe (1PRB) was placed around the artery. Its signal was verified and monitored prior to closure. The metal slide of the Probe's reflector was closed using a small amount of cyanoacrylate glue. The artery and the acoustic portion of the Probe was soaked with 0.9% NaCl to avoid glue contamination. The overlying muscles were then approximated and sutured. The Probe's cable was anchored subcutaneously and then passed around the neck to exit at the back of the animal just above the scapular region. The connector side of the Probe was held in place using a silicone or delrin cuff sutured to the skin. Postoperatively, the animals recovered in individual cages and were given buprenex HCl analgesia (0.1mg/Kg), subcutaneously .

Rat Carotid Artery: Chronic Blood Flow Measurement Cont.

Flow Ranges Observed

The Flowprobe signal returned 48-72 hours post surgery. This signal lasted for 3-4 weeks depending on the fibrotic build-up surrounding the Probe's acoustic window. This recovery period is very advantageous because it eliminates the effect of surgery and anesthesia and allows us to obtain measurements on fully awake animals. Baseline flow in the common carotid artery of a fully awake animal is 7-10 ml/min (Fig. 1). Our protocol concentrates on cerebral blood flow and its changes under different conditions, which includes hypergravic stress, complete and incomplete ischemia. We developed a calibration procedure to verify the signal at the minimum (complete ischemia; 0 ml/min) and at the maximum (hyperemia 32 ml/min) blood flows (Fig. 2).

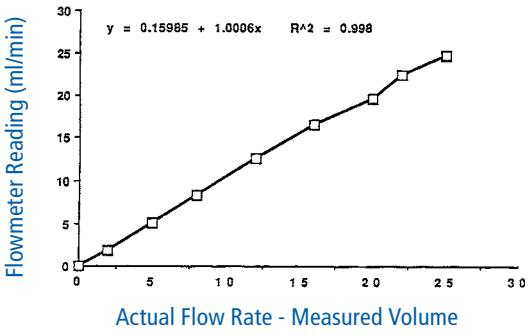


Fig. 2: Representative calibration curve of Flowmeter volume flow measurement against measured volume of flow.

ACKNOWLEDGEMENT

Dr. Paul Werchan, Brooks Air Force Base, TX, 78235; Roger M. Echon, CNS Mechanism of GLOC Laboratory, AL/CFTS Brooks Air Force Base, TX 78235

REFERENCES

Werchan PM, Echon RM, Barber JA, Galindo Jr S, Shahed JA, "Estimation of Rat Cerebral Blood Flow (CBF) during +Gz Centrifuge Exposures Leading to G-induced Loss of Consciousness (G-LOC)," Soc. of Neuroscience 1993; 19(Part 2): 1220 Abstract.

For additional references, visit www.transonic.com

Mouse Superior Mesenteric Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Superior Mesenteric artery
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Acute
Vessel Diameter:	0.60 mm
Length:	10 mm

PROBE

Size:	0.7 mm
Reflector:	JN
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.7PSB

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

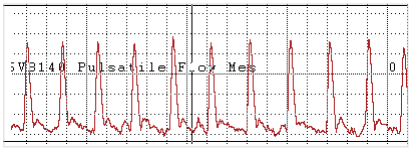


Fig. 1: Superior mesenteric arterial flow in the mouse.

Reported Flows: 0.9- 3.0 ml/ min in sham operated C57BL6/N male mice, body weight 25-33 g

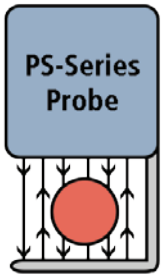


Fig. 2: Nanoprobe PS-Series: The full ultrasonic window has the same flow sensitivity, so that the vessel can be positioned anywhere within the Probe lumen.

Surgical Protocol

1. Administer anesthesia, clip from xiphoid process to lower abdomen.
2. Make a midline incision into abdomen (either one incision or first through skin then muscle).
3. Reflect small and large intestines laterally to the right.
   a. Major landmarks at this point include:
      - Vena cava and abdominal aorta on midline
      - Left kidney in more ventral position
      - Right kidney located more rostrally is usually obscured by intestine
      - Liver midline and superior usually partially obscures the celiac ganglia
   b. The coeliac ganglia is the large white body located in midline and slightly on the right in the area between the two kidneys. Its posterior border is perpendicular to the axis of the aorta. The ganglia contains a number of lymphatic vessels which are usually easy to visualize.
   c. The superior mesenteric artery is located within the ganglia or at its posterior border, running almost perpendicular to the aorta. Occasionally (especially in rats), it is located superiorally (under) the posterior border of the ganglia.
4. Carefully dissect the artery from the ganglia and lymphatic vessels. You should be able to clear at least a 10 mm section of the artery from the portion within the ganglia before encountering branches. Also, you can clear additional space back to the aorta. Occasionally there is a small branch near where the artery exits from the aorta.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Superior Mesenteric Artery: Acute Blood Flow Measurement Cont.

Surgical Protocol Cont.

5. Place the vessel gently within the Probe lumen and attach the handle of the Flowprobe to a micromanipulator to stabilize the Probe on the vessel. Fill the space between the vessel and Probe with Surgilube gel to aid in transmission of the ultrasound signal. Check that the "Signal Quality" indicator on the Flowmeter shows 4 – 5 lit bars; 3 or less bars may indicate an air bubble.
6. Measure flow.

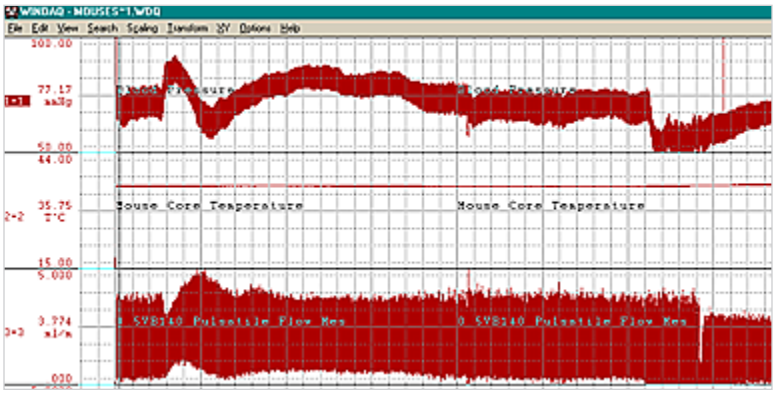


Fig. 3: Mouse Arterial Pressure (top) and Mesenteric Blood Flow measured with a 0.5PSB Nanoprobe showing dose response data.

REFERENCE

Albuszies G, et al, " Effect of increased cardiac output on hepatic and intestinal mcrocirculatory blood flow, oxygenation, and metabolism in hperdynamic murine septic shock," Crit Care Med. 33(10): 2332-2338, 2005.

Rat Mesenteric Branches: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Mesenteric branches jejenal & ileal arcades
Species:	Rat
Body Weight:	400 grams
Duration:	Acute
Vessel Diameter:	270 µm

PROBE

Size:	0.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.5PSB

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

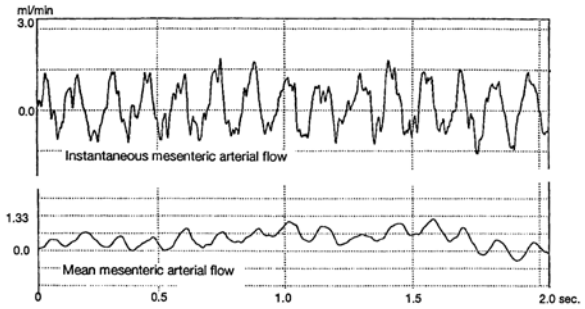


Fig. 1: Mean flow over 90 seconds was 0.494 ± 0.276 and 0.527 ± 0.233 ml/min, respectively. After treatment with isoprenaline and superfusion of the preparations with papaverine, mean flow increased to 0.666 ± 0.379 ml/min.

Application

The combination of this model and the new microcirculation transit-time Probe, allowed us to make the first measurements of true flows in the microcirculation. These were until now estimated from diameters and velocity measurements; this method lacked precision and prevented any appreciation of the instantaneous dynamic of flow. This allowed us to examine the instantaneous dynamic and to observe the cyclic variation of flow caused by the periodic contractions of the bowel.

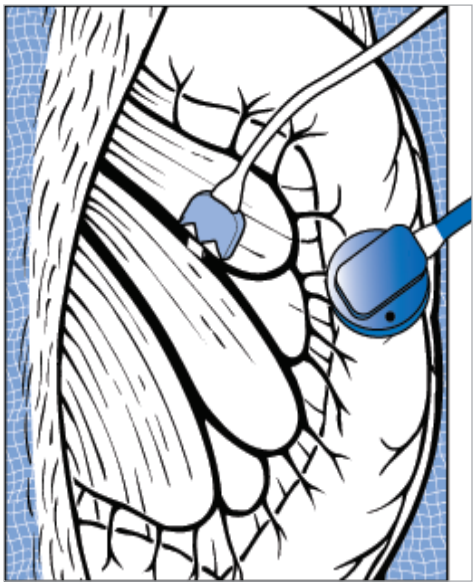


Fig. 2: Transit-time ultrasound Flowprobe on mesenteric (jejunal) branch (center); Laser Doppler Probe on mucosa on the right.

Surgical Approach

Note: This protocol is for mesenteric branches, jujunal and ileal arcades. The superior mesenteric artery (0.7 - 0.9 mm diameter) is larger than the branches and requires 1 mm Probes for full ultrasonic illumination.

Male Sprague-Dawley rats weighing approximately 400 g were anesthetized with 0.1 ml / 100 g pentobarital (6%) i.p. Anesthesia was maintained by additional i.p. injections of diluted pentobarital. The right jugular vein was cannulated with polyethylene tubing for the administration of drugs.

After a small abdominal incision, a section of the ileum was pulled out and spread over the transparent stage. Fat and connective tissues surrounding the mesenteric arteries (internal artery diameters were approximately 270 µm) were carefully removed under a dissecting microscope.

transonic
THE MEASURE OF BETTER RESULTS.

Rat Mesenteric Branches: Acute Blood Flow Measurement Cont.

Surgical Approach cont.

The preparation was mounted under a biological binocular microscope (Leitz) connected to a color video recording system. The surface of the mesenteric artery was covered with a Saran film. Warm Krebs' solution was superfused on the artery at a rate of 2 ml/min. The microscope magnification was 145 fold. The stainless steel handle of the Probe was connected to a micromanipulator in order to allow positioning of the Probe around the vessel near the objective. The vessel was positioned in the lumen of the Probe, making sure the reflector bracket was not tugging on the vessel wall to reduce the flow. Zero flow reading was obtained by transient clamping of the arteriole under investigation.

Basal flow measurements were done at a few minute intervals, the mean flows over 90 seconds were 0.494 ± 0.276 ml/min and 0.527 ± 0.233 ml/min respectively. The reproducibility seems excellent despite the elevated standard deviation due to the physiological periodic reduction of flow provoked by intestinal peristalsis.

After treatment of the animals with isoprenaline and superfusion of the preparations with papaverine, the mean measured flow increased to 0.666 ± 0.379 ml/min.

REFERENCES

Marque S et al, "APC Inhibits FMLP Induced Leukocytes Sticking in the Micro-circulation," FASEB J 1993; 7(3.1)1: A344.

Superior Mesenteric Blood Flow

Myers SI et al, "Oxygen Free Radicals Regulate Splanchnic Nitric Oxide Synthesis and Blood Flow," Cardiovascular Surgery 1995; 3(2): 207-10.

Wang J F et al, "The Roles of Nitric Oxide and Hydrogen Peroxide Production in Lipopolysaccharide-Induced Intestinal Damage," Shock 1995; 2(3): 185-191.

Turnage R H et al, "Neutrophil Regulation of Splanchnic Blood Flow after Hemorrhagic Shock," Ann Surg 1995; 222(1) 66-72. 575A)

Pofahl, W.F. et al, "Small Intestinal Microcirculatory Effects of Octreotide," J Surg Res 1994; 56:345-350. (397A)

Kost C K et al, "Vascular Reactivity to Angiotensin II is Selectively Enhanced in the Kidneys of Spontaneously Hypertensive Rats," J Pharmacol & Expe Therap 1994; 269(1) 82-8. (426A)

Jackson EK, Herzer WA, "Angiotensin II/Prostoglandin I2 Interactions in Spontaneously Hypertensive Rats," Hypertension 1993, 22: 688-98. (344A)

Turnage RH et al, "Splanchnic PG12 Release and "No Reflow" Following Intestinal Perfusion," J Surg Res 1995; 222(1): 66-72.

Rat Hepatic Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Hepatic artery
Species:	Rat
Body Weight:	430 grams
Duration:	Acute
Vessel Diameter:	0.25 - 0.4 mm

PROBE

Size:	0.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.5PSB

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

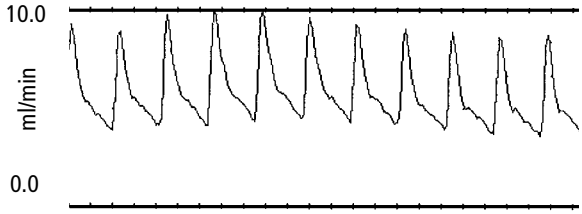


Fig. 1: Instantaneous flow in this anesthetized 330 g rat ranged from 5 to 10 ml/min. The pulse rate was approximately 380 bpm. This measurement was made directly on the hepatic artery and includes the flow components going to the gastroduodenal and right gastric arteries.

Application

In other species, measurement of hepatic arterial blood flow is often combined with portal vein flow for studies on nutrition, septicemia and toxicology. Since the net flux of a metabolite is the product of its arteriovenous or portovenous concentration difference and blood flow, the net hepatic uptake (or secretion) of any metabolite may be determined with three sampling catheters and two Flowprobes.

Hepatic arterial flow is also an important parameter in several models of liver transplant. In one study in pigs, hepatic arterial flow increased at the expense of portal vein flow following liver denervation. In humans, the routine intraoperative measurement of hepatic arterial flow in pediatric liver transplants has been advocated as a means of predicting early hepatic arterial thrombosis.

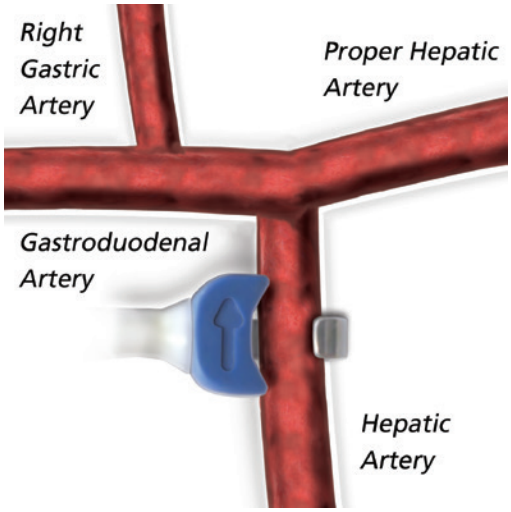


Fig. 2: Schematic of Flowprobe on a rat hepatic artery.

Surgical Approach

Anesthetize the rat with pentobarbital anesthesia (20 mg/kg IP). Note that pentobarbital anesthesia may significantly depress flow when compared to flow in the conscious animal (See Anesthetic Guidelines RL-67-tn for more information). The use of a heating pad or heat lamp is recommended as hypothermia also reduces flow. A 0.5 ml bolus of saline placed subcutaneously every half hour is also recommended.

Place the rat in dorsal recumbency and make a ventral midline abdominal skin incision. Extend the abdominal incision through the linea alba into the abdominal cavity. Retract the lobes of the liver cranially to locate the splanchnic vessels. Locate the pulsing hepatic artery where it branches from the short celiac artery.

Rat Hepatic Artery: Acute Blood Flow Measurement Cont.

Surgical Approach Cont.

The hepatic artery is easily dissected from adjacent tissue at this site as there is no immediately adjacent vein. However, at this site the hepatic artery does have flow components destined for the stomach and small intestine. For a more accurate assessment of hepatic flow, it may be necessary to trace and dissect the proper hepatic artery from the portal vein or ligate the right gastric and gastroduodenal branches. Clean off the fat on the vessel for proper acoustic coupling and place the Probe around the vessel making sure that the vessel is within the lumen of the Probe ultrasonic window. For extended measurements, a micromanipulator can be used to maintain proper position of the Flowprobe.

Fill the plunger of a 30 cc syringe and load the syringe with sterile Surgilube acoustic gel, taking care to prevent the formation of air bubbles. Place a flexible 20 gauge catheter on the tip of the syringe. Insert the catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The Surgilube gel acts as an acoustical couplant and must replace all air space. Check the signal bar indicator on the meter to verify that signal amplitude is close to 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of lubricating gel or to an air bubble.

REFERENCES

Nishida, T., Ueshima, S., Kazuo, H., Ito, T., Seiyama, A., Matsuda, H., "Vagus Nerve is Involved in Lack of Blood Reflow into Sinusoids after Rat Hepatic Ischemia", AJP 278(5): H1565-1570, 2000

Kazuo, H., Nishida, T., Seiyama, A., Ueshima, S., Hamada, E., Ito, T., Matsuda, H., "Recovery of blood flow and oxygen transport after temporary ischemia of rat liver", AJP 275(44): H243-249, 1998

Pannen BH, Bauer M, "Differential Regulation of Hepatic Arterial and Portal Venous Vascular Resistance by Nitric Oxide and Carbon Monoxide in Rats", Life Science 62(22): 2025-2033, 1998

Flynn WJ, Cryer HG, Garrison RN, "Pentoxifylline but not saralasin restores hepatic blood flow after resuscitation from hemorrhagic shock", J Surgical Research 50(6): 616-21, 1991

Turnage, R.H., Kadesky, K.M., Myers, S.I., Guice, K.S., Oldham, K.T, "Hepatic Hypoperfusion after Intestinal Reperfusion", Surgery 119(2): 151-60, 1996

Rat Portal Vein: Chronic Blood Flow Measurement

APPLICATION BASICS

Site:	Portal vein
Species:	Rat
Body Weight:	270 grams
Duration:	Chronic
Vessel Diameter:	1.5 mm

PROBE

Size:	2 mm
Reflector:	JS
Connector:	4-pin
Cable Length:	13 cm
Catalog #:	MC-2PSB-JS-WC13-CM4S-GC

FLOWMETER

TS420 Perivascular Module

2PSB

Flow Ranges Observed

Mean portal blood flow in a 270 gram rat is 20 ml per min. Flow may decrease markedly when the rat is sleeping or excited. A flow of 5 ml/min is not unusual.

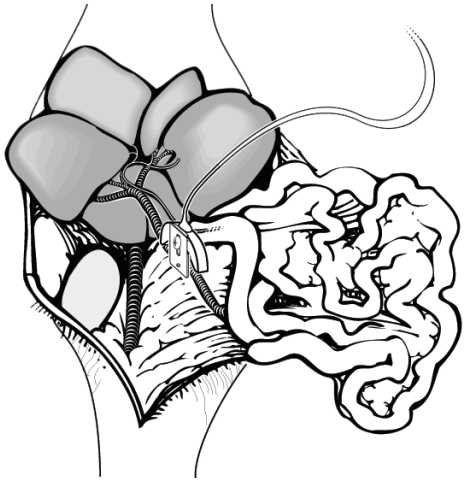


Fig. 1: Application of a Flowprobe on the portal vein of a rat.

Application

The total flux of any metabolite can be estimated from the product of blood flow and the concentration of that metabolite in the blood. This technique and similar ones have been used for measuring gastrointestinal fatty acid absorption and estimating the uptake of parasites.

Surgical Approach

Tank induction with ether or isoflurane is recommended. Place an anesthetized rat in ventral recumbency and make a small skin incision between the ears. Turn rat over and make a ventral midline abdominal skin incision. Using curved hemostats, create a subcutaneous tunnel from the abdominal incision to the cranial incision. Extend hemostats through the subcutaneous tunnel and firmly grasp the Probe. Pull the Probe through the subcutaneous tunnel, leaving the plug in the cranial incision and most of the cable in the subcutaneous tunnel.

Extend the abdominal incision through the linea alba into the abdominal cavity. Retract the lobes of the liver cranially to locate the splanchnic vessels. Gently separate the hepatic artery and the portal vein. Clean fat off portal vein for proper acoustic coupling. Place J-reflector of the Probe around the portal vein, close the slide cover. Position the Probe so that the cable is perpendicular to the portal vein and replace the hepatic lobes so that they cover the Probe (Fig. 1).

Since there is very little adjacent connective tissue, the Probe is not sutured. Probe position is maintained by the relative stability of the cranial abdominal organs. To maintain the perpendicular alignment between the portal vein and Probe cable, attach the cable of the Probe to the body wall with 4-0 suture. Close the body wall with a 4-0 simple continuous suture. The skin may be sutured or closed with wound clips. At the head, suture the CM4 connector to skin with a 2-0 suture and close the skin incision with a wound clip.

ACKNOWLEDGEMENTS

Dr. Charles Mansbach, Regenia Dowell, University of Tennessee, Health Science Center, Dept. of Gastroenterology, Memphis, TN

Dr. Robin Bell, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY.

REFERENCES

Mansbach CM, Dowell RF, "Portal Transport of Long Acyl Chain Lipids: Effect of Phosphatidycholine and Low Infusion Rates," AJP 1993; 264(6 Pt 2): G1082-9.

Ulrich PM, Tordoff MG, "Influence of Gastric Loads on Hepatic Portal Vein Blood Flow," Abstract for Eastern Psychological Association, Philadelphia, 1990

Transonic® video "Implanting the Transonic Flowprobe on the Portal Vein in the Rat."

For additional references, visit www.transonic.com

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Renal Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Renal artery
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Acute
Vessel Diameter:	0.35 - 0.55 mm
Length:	0.25 mm

PROBE

Size:	0.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-05PSL, MA-0.5PSB
FLOWMETER	TS420 Perivascular Module

0.5PSB Nanoprobe with handle

Flow Ranges Observed

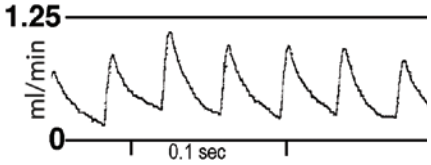


Fig. 1: Renal arterial blood flow in a 300 micron vessel in a 40 gram anesthetized mouse. Mean flow is 0.46 ml/min.

Application

The measurement of renal blood flow has an important role in research on hemodynamics, electrolyte regulation and pregnancy-induced hypertension. Flow-pressure relationships are essential in defining renal autoregulation. Other studies have focused on diuretics, cardiovascular drugs, and nephrotoxic agents. While average renal flow may also be obtained from the renal vein, the pulsatile waveform of the renal artery provides additional information and visual confirmation of a reliable renal arterial measurement.

Laparotomy surgical approaches to locating and isolating the vessel for measurement (typically used in the rat) are more challenging in the mouse. Anatomical differences from the mouse and anatomical variability among transgenic and knock out models require special consideration when choosing a surgical approach. The goals for obtaining stable data are to minimize the surgical preparation time and manipulation of the vessel and limit heat and fluid loss.

Advantages of Retroperitoneal Approach

A retroperitoneal approach to the renal artery has several advantages and is the preferred method for renal blood flow measurement. Approaching the kidney from the back allows easy visualization of the renal artery and dissection without disturbing the delicate renal vein. By laparotomy, the renal artery lies directly under the renal vein making dissection difficult.

ANATOMICAL DIFFERENCES BETWEEN MICE & RATS

Size

The renal artery of the mouse is approximately 350-550 micrometers in diameter (~60% of the diameter of the renal artery of the rat). It is ~2.5 mm long somewhat shorter that the renal artery in the rat. Less space is available for dissection than is available in the rat.

Anatomic Location

In the mouse, the renal artery differs anatomically in respect to the renal vein from the rat. In the rat, the renal artery and renal vein lie almost parallel in the same plane in the back of the animal. It is, therefore, relatively easy to dissect the renal artery away from the renal vein. In the mouse, the renal artery tends to be more dorsally positioned in respect to the renal vein. Using a conventional laparotomy, the renal artery appears to lie slightly behind the renal vein and has to be dissected free from the renal vein. This poses a challenge in that the renal vein is very thin.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Renal Artery: Acute Blood Flow Measurement

Advantages of Retroperitoneal Approach Cont.

Retroperitoneally, there is no interference with the abdominal organs. By contrast, in laparotomy the intestines and abdominal contents are exposed and must be deflected to the side to allow access to the renal artery and vein. This lengthens the procedure and exposes the mouse's abdominal cavity for additional heat loss.

There is considerable variability in renal vascular branching among mice. In some mice, exploration of the left kidney reveal insufficient vessel length to fit the Flowprobe before the vessel branches. Because a retroperitoneal approach is quicker, it is possible to move on in the same mouse to explore the right renal artery.

Protocol: Retroperitoneal Approach, Left Renal Artery

- Anesthetize mouse and position animal in right lateral recumbency.
- Make initial skin incision 1 cm lateral to midline of back.
- Cut through skeletal muscle layer to expose the hilus of the kidney.
- Gently retract kidney to the left to expose the area between the kidney and the aorta to reveal renal artery.
- A 2 mm length of vessel without visible branching is required for Flowprobe placement.
- If the vessel is too short or bifurcates, the incision may be closed and the animal turned on its left side for exploration of the right kidney.
- Use blunt dissection along the renal artery to isolate the vessel and clear off fat for proper acoustic coupling of Probe.
- Position Probe so that the renal artery is in the lumen of the Probe.
- Use a syringe with a flexible catheter tip to deposit Surgilube gel in air spaces of Probe and verify good transmission of the ultrasound signal by checking the Flowmeter "Test" mode.
- Stabilize Probe position with a micromanipulator for continuous measurement.

CAUTION: CAREFUL DISSECTION REQUIRED

In general, dissections or manipulation of vessels in mice should be approached very carefully. The renal vein and renal artery may be dissected away from each other by grabbing carefully the adventitia of the renal artery and, using very fine Dumont vessel dilators (D-5az), carefully go around the renal artery and dissect it free from the renal vein. Renal artery dissections are best performed by applying slight pressure against the renal artery and allowing the D5az forceps to spread and dissect the adventitia away from the artery itself. Do not apply any kind of dissecting force against the renal vein. Instead, apply pressure toward the artery and let the instruments themselves perform the dissection by separating the adventitia from the artery. This will result in fewer misadventures with the renal vein.

ACKNOWLEDGEMENTS

Thomas L. Smith, Ph.D., Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

John Lorenz, Murine Core Physiology Facility, University of Cincinnati, Cincinnati, Ohio

Mouse Renal Artery: Acute & Chronic Blood Flow Measurement

APPLICATION BASICS

Site:	Renal artery
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Chronic
Vessel Diameter:	0.35 - 0.55 mm
Length:	0.25 mm

PROBE

Size:	0.5 mm
Reflector:	JN
Connector:	4-pin
Cable Length:	6 cm
Catalog #:	MC-0.5PSL-JN-WC06-CA4S-GC
FLOWMETER	TS420 Perivascular Module

Measurement of Renal Arterial Blood Flow in the Mouse Protocol©

Courtesy of Michael F. Callahan, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC. ©Wake Forest University School of Medicine, Department of Orthopaedic Surgery. Used with permission.

For the complete application PowerPoint presentation see: www.transonic.com

0.5PSL
Chronic style

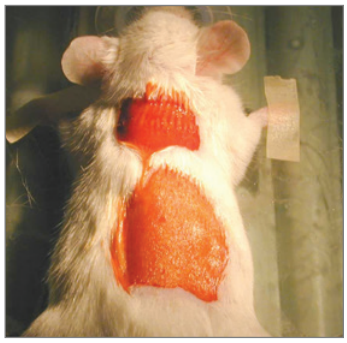


Fig. 1: Shaved, prone mouse on a warming pad prepared for surgery.

Preparation

1. The mouse is prepared for renal Flowprobe implantation by placement in a prone position on a warming pad to maintain core temperature (Fig. 1).
2. The surgical site is shaved and prepared with Betadine. Note: the midscapular area is also shaved and prepared for exteriorizing the Probe connector.
3. The mouse is anesthetized with a mixture of ketamine:xylazine (50:10 mg/kg). For the duration of the implantation procedure, the mouse is anesthetized with inhaled isoflurane (1 - 2%).

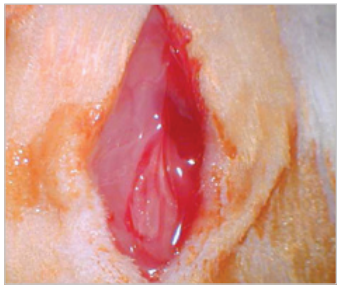


Fig. 2: Make the incision 1 cm lateral to back midline. Cut through the skeletal muscle to the hilus of the kidney.

Surgical Implantation of Flowprobe

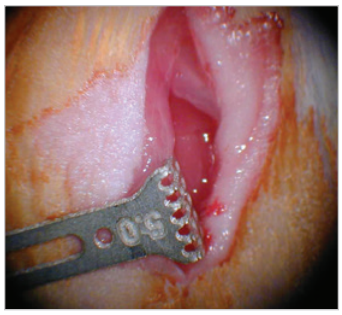


Fig. 3: Place a miniature retractor at the base of the incision to reveal the kidney beneath the muscle layer.

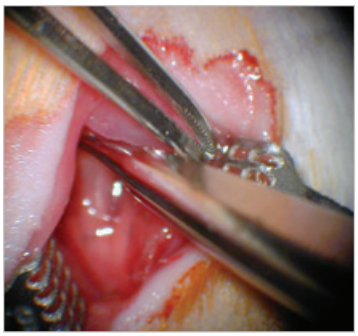


Fig. 4: Extend incision to see the renal artery. Place a second retractor and pack the kidney with gauze to keep it out of the surgical field.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Renal Artery: Acute & Chronic Blood Flow Measurement Cont.

Surgical Implantation of Flowprobe cont.

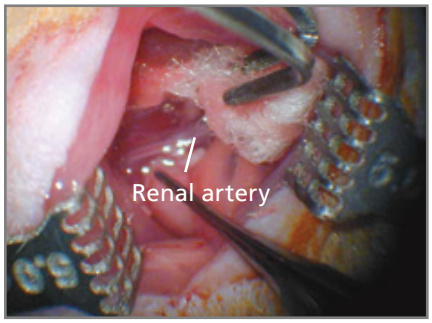


Fig. 5: Here the renal artery lies on top of the delicate renal vein. With a ventral laparotomy, the renal artery lies under-neath the vein making for a much more difficult dissection.

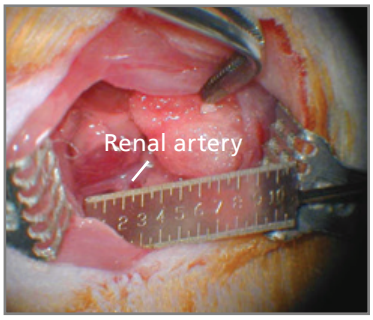


Fig. 6: This renal artery length is approximately 1.75 mm between branches, just adequate for the 0.5PSB Flowprobe.

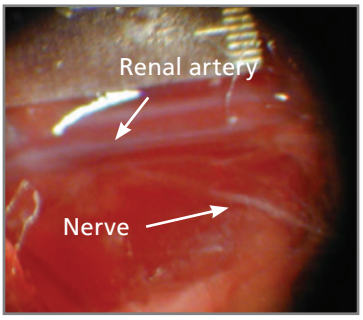


Fig. 7: Under increased magnification, the diameter of the renal artery is 0.35 mm. Note the nerve below the artery and take care to avoid damage to the nerve during dissection.

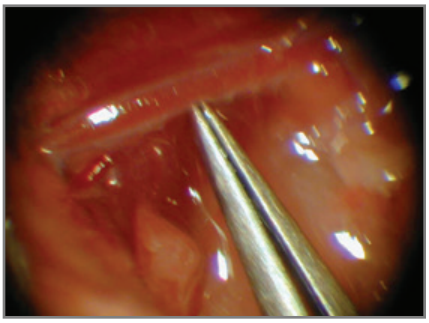


Fig. 8: Separate the renal artery from the renal vein by carefully grabbing the adventitia of the artery using very fine Dumont vessel dilators (D-5aZ) or by carefully passing microblunted 45° Dumont forceps under the vessel as shown.

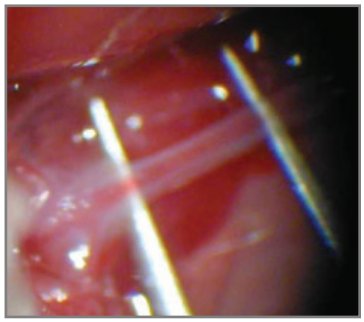


Fig. 9: Apply slight pressure against the renal artery to allow the 45° Dumont forceps to spread and dissect the adventitia away from the artery itself. Do not apply any kind of dissecting force against the renal vein.

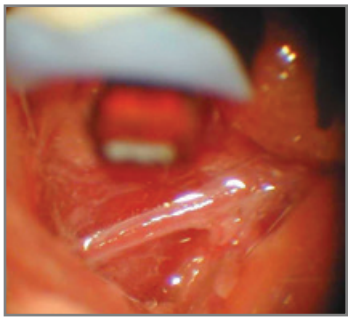


Fig. 10: Carefully go around the renal artery until a long enough segment is freed from the underlying vein to accommodate the Flowprobe reflector.

Mouse Renal Artery: Acute & Chronic Blood Flow Measurement Cont.

Acute Measurement Protocol

Flow measurements may be recorded for acute studies by using acoustic coupling gel (Surgilube) to displace the air in the Flowprobe. Use a blunt tipped syringe or angiocath to insert the gel, being careful not to impact the delicate Probe reflector.

Connect the MA-0.5PSB or MA-0.5PSL Probe to the TS420 Flow Module and check that the "Test" signal indicates "Good." A "Low" signal may indicate an air bubble. Select "Measure" to record flow.

Do not grasp the Probe by its fragile reflector.

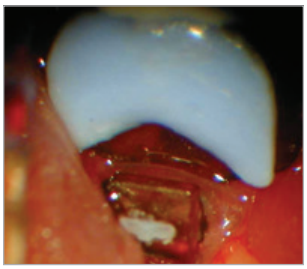
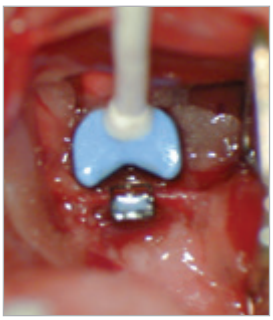


Fig. 11: Place the Flowprobe around the vessel so that the reflector hook is not tugging on or deforming the vessel and the Probe head is not placing pressure on or blanching the kidney. The vessel may be gently lifted into the Flowprobe with blunted D5aZ forceps.

Acute style MA-0.5PSB Probe. For acute experiments Nanoprobes are configured with a handle for easy maneuvering and stabilization.

Chronic Measurement Protocol



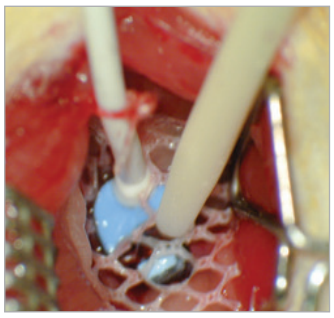


Fig. 12: The MC-0.5PSL lateral cable configuration is preferred for chronic implant. The Probe is maneuvered by gently grasping the blue Probe body with D5aZ or curved forceps. Position the Probe with the cable rostrally, so the Probe is "floating" on the renal a. Anchor the cable with a suture to the psoas muscle. A 3/4" square of Mersilene (dacron) mesh is placed over the Probe and acoustic gel is deposited into the lumen of the Probe to provide a barrier before placing the sealing agent into the retroperitoneal space. The ultrasonic pathway must remain unobstructed. This can be checked by monitoring the Probe test signal on the Flowmeter.

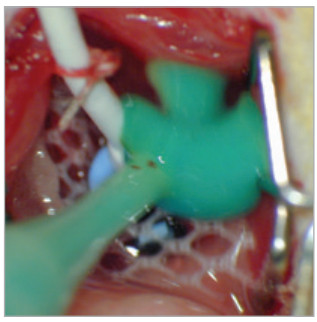


Fig. 13: Inject Kwik-Sil into the retroperitoneal cavity beginning at the corner and around the Probe to hold the Probe, cable and kidney in place.

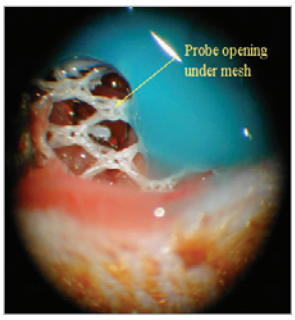


Fig. 14: Kwik-Cast is shown. Kwik-Sil has a shorter cure time (1 minute) and is less likely to obstruct the ultrasound path, which will block the ultrasound signal, and prevent flow measurement.

Kwik-Sil (clear) and Kwik-Cast (blue) are both 2-part agents used to attach electrodes in nerve recording.

Mouse Renal Artery: Acute & Chronic Blood Flow Measurement Cont.

Chronic Measurement Protocol cont.

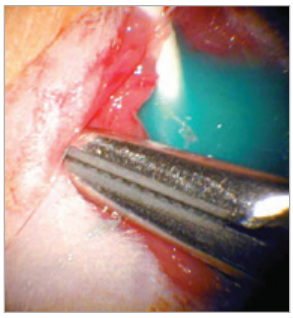


Fig. 15: Allow the cast to harden before moving the tissues. Anchor the Probe cable to the psoas major muscle with 5-0 Vicryl suture.

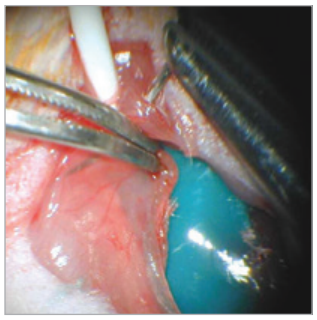
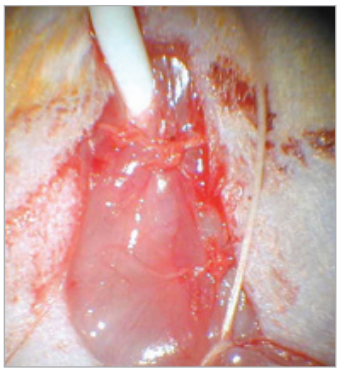


Fig. 16: Continue to close the skeletal muscles over the renal Flowprobe preparation.



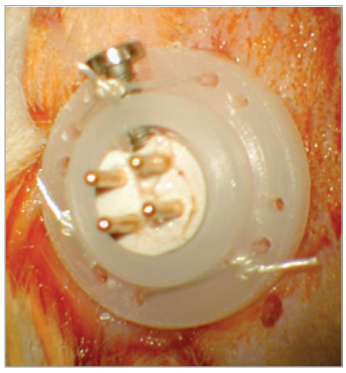


Fig. 17: Close the skin over the incision with 5-0 Vicryl sutures. The Flowprobe maintains the best position if the cable is not disturbed during subcutaneous preparation. To do this, cut and close the skin over the cable to the Probe connector at the midscapular region, leaving the Probe's CA4 connector exposed. Use a 3/4" square of Mersilene Mesh under the skin at the connector and suture the skin closed around cable. Install a skin button cuff over the CA4 connector as shown suturing through the skin and the mesh to improve long term stability of the implant.

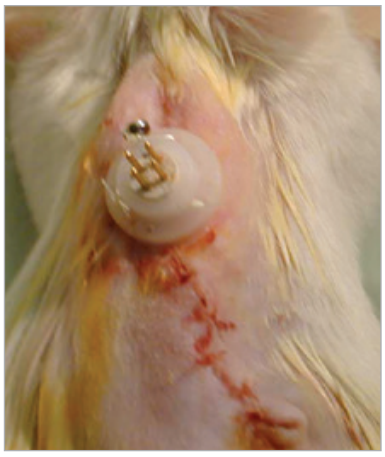


Fig. 18: The mouse is shown with the skin button positioned after surgery. The wound is cleaned and the animal is allowed to recover before measurements are made. Generally, it takes 3-5 days to achieve a stable signal as fibrotic tissue helps to encapsulate the Probe, though the flow signal may be available as soon as 1 day post surgery.

REFERENCES

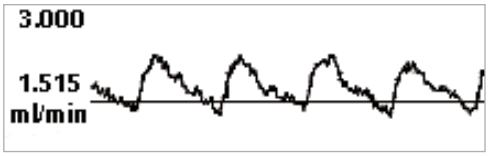
Brands MW et al, "Interleukin 6 knockout prevents angiotensin II hypertension: role of renal vasoconstriction and janus kinase 2/signal transducer and activator of transcription 3 activation," Hypertension 2010; 56(5): 879-84; 819-21.

Iliescu R et al, "Renal blood flow and dynamic autoregulation in conscious mice," Am J Physiol Renal Physiol 2008; 295(3): F734-40.

Mouse Renal Artery: Acute & Chronic Blood Flow Measurement Cont.

Chronic Measurement Protocol cont.

Fig. 19: Blood flow measurements can be continuously monitored in the conscious mouse via a tether connection to the skin button and suitable low torque electrical swivel (Dragonfly Inc.).

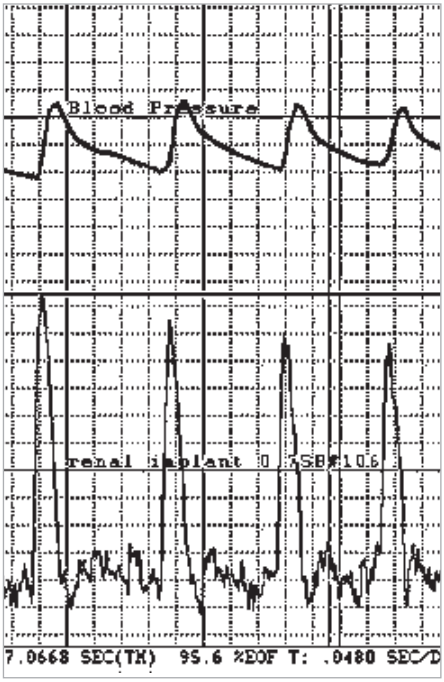


This renal blood flow recording was made 1 day after implant in the conscious mouse.

ACKNOWLEDGEMENTS

Transonic Systems Inc.® gratefully acknowledges the assistance of Michael F. Callahan, PhD, and Thomas L. Smith, PhD, Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, in the development of this protocol.

Adapted from a PowerPoint presentation produced by: Margo Sosa, Senior Product Manager, Transonic Systems, Inc., 34 Dutch Mill Road, Ithaca, NY 14850 www.transonic.com



Renal blood flow recording ~1.5 years after implant in the conscious mouse during femoral catheter implantation (isoflurane anesthesia).

Rat Renal Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Renal artery
Species:	Rat
Body Weight:	< 300 grams
Duration:	Acute
Vessel Diameter:	0.7 - 0.8 mm

PROBE

Size:	1 mm
Reflector:	V / JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.7VB, MA-1PRB

FLOWMETER

TS420 Perivascular Module

0.7VB

Flow Ranges Observed

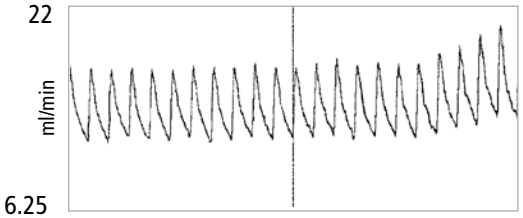


Fig. 1: Renal flow in the rat varies greatly with the anesthetic used and the plane of anesthesia. In general, protocols with ketamine will show higher flows than those with pentobarbital. Hypothermia is also a common cause of lower than expected flow measurements.

Application

The measurement of renal blood flow has an important role in research on hemodynamics, electrolyte regulation and pregnancy induced hypertension. Flow-pressure relationships are essential in defining renal autoregulation. Other studies have focused on diuretics, cardiovascular drugs, and nephrotoxic agents. While average renal flow may also be obtained from the renal vein, the pulsatile waveform of the renal artery provides additional information and visual confirmation of a measurement with a properly functioning Flowprobe.

Surgical Approach

Anesthetize the rat with Inactin anesthesia (100 mg/kg IP). If Inactin is not available, sodium pentobarbital (60 mg/kg IP) may be used instead. Note that pentobarbital anesthesia is less stable than Inactin and may significantly depress flow when compared to that of the conscious animal. The use of a heating pad or hot water bottle is recommended as hypothermia also reduces flow. In long procedures, fluid infusion (0.9% NaCl @ 1 ml/hr) through a femoral catheter is also recommended (See Anesthetic Guidelines for more details).

Place rat in dorsal recumbency and make a ventral midline abdominal skin incision. Extend the abdominal incision through the linea alba into the abdominal cavity. Deflect the intestines to the rat's right to expose the left kidney. To expose the right kidney, deflect the intestines to left. Identify the large renal vein; the renal artery is much smaller, cranial and deep to the vein. Carefully locate and dissect out the renal artery. Remove adjacent fat for proper acoustical coupling. Place the Probe around the artery (Fig. 2).

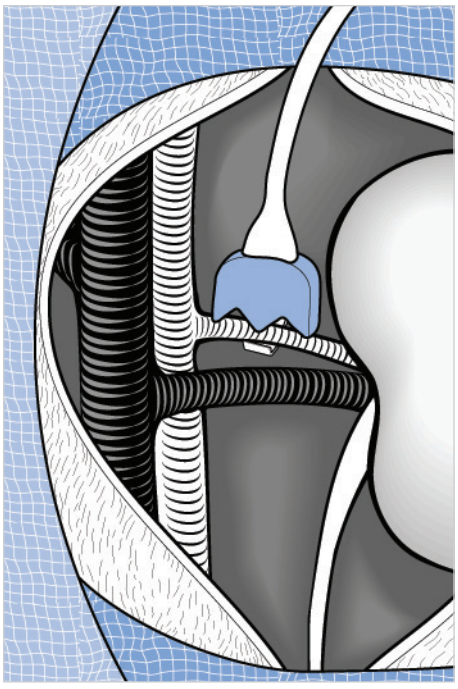


Fig. 2: Flowprobe on renal artery.

transonic
THE MEASURE OF BETTER RESULTS.

Rat Renal Artery: Acute Blood Flow Measurement Cont.

Surgical Approach cont.

Manually position the artery so that the vessel lies within the lumen of the Probe. A micromanipulator may be used to stabilize the Flowprobe position on the artery.

Remove the plunger of a 30 cc syringe and load the syringe with Surgilube acoustic gel, taking care to prevent the formation of air bubbles. Place a flexible catheter on the tip of the syringe. Insert the flexible catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The lubricating gel acts as an acoustical couplant and must replace all air space. Check the signal bar indicator on the Meter to verify that signal amplitude is about 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of lubricating gel or an air bubble.

ACKNOWLEDGEMENTS

Dr. Brian Murray, Department of Medicine, Nephrology Division, State University of New York at Buffalo, Buffalo, NY

Dr. Thomas L. Smith, Department of Orthopedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

REFERENCES

Welch WJ et al, "Validation of Miniature Ultrasonic Transit Time Flow Probes for Measurement of Renal Blood Flow in Rats," AJP 1995; 268,(1)Pt 2, F175-178.

Welch WJ et al, "The Ultrasonic Transit-time Blood Flow Meter: An Accurate Method for Measuring Renal Blood Flow in the Rat," JASN 1993; 3: 528.

Griffin KA et al, "Role of Endothelium-Derived Nitric Oxide in Hemodynamic Adaptations after Graded Renal Mass Reduciton," AJP 1993; 264: R1254-R1259.

Hatton DC et al, "Impact of Stress-Induced Hypertension on Renal Blood Flow and Vascular Resistance," JASN 1993; 4(3): 512.(336A)

Holycross BJ et al, "Adenosine Receptor Blockade Attenuates Angiotensin II Induced Decreases in Renal Blood Flow in Vivo," FASEB J 3(3): Renal Hemodynamics A109.

Hoffman A et al, "Endothelin Induces An Initial Increase in Cardiac Output Associated with Selective Vasodilation in Rats," Life Sciences 1989; 45(3): 249-255.

Rat Renal Artery: Chronic Blood Flow Measurement

APPLICATION BASICS

Site:	Renal artery
Species:	Rat
Body Weight:	250 - 400 grams
Duration:	Chronic
Vessel Diameter:	0.7 - 0.8 mm

PROBE

Size:	1 mm
Reflector:	JS
Connector:	4-pin
Cable Length:	14 cm (12 - 16 cm typical)
Catalog #:	MC-1PRB-JS-WC14-CM4S-GC

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

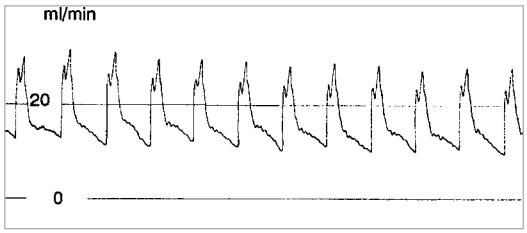


Fig. 1: Renal flow ranged from 10 to 16 ml/min in this 440 gram conscious rat.

Application

Renal blood flow measurement has an important role in hypertension research. Flow-pressure relationships are essential in defining renal autoregulation. Other studies have focused on diuretics, cardiovascular drugs, and nephrotoxic agents. While average renal flow may also be obtained from the renal vein, the pulsatile waveform of the renal artery provides additional information and visual confirmation of a functioning implant.

Surgical Approach

Mix 5 ml ketamine (100 mg/ml) and 1.6 ml xylazine (20 mg/ml). To anesthetize the rat, administer this solution at a rate of 9 ml / 100 g body weight IM. This protocol maintains cardiac output and peripheral perfusion. On long procedures, fluid infusion (0.9% NaCl @ 1 ml/hr) through a femoral catheter is also recommended.

Surgically prep the rat for the following three incisions: ventral midline, retroperitoneal, and midscapular. Glue a small oval shaped section of surgical mesh to the back side of the Flowprobe as show in Fig. 2. Place rat in dorsal recumbency and make a ventral midline abdominal incision. Extend the abdominal incision through the linea alba into the abdominal cavity. Deflect the intestines to the rat's right to expose the left kidney. After identifying the kidney and renal artery, dissect the artery free from the surrounding fatty tissue.

Slide the dacron mesh between the body wall and the kidney with the cable exiting the Probe rostrally and the open J facing ventrally. Slip the reflector under the renal artery and position the Probe so that the cable is perpendicular to the vessel and the vessel is well aligned within the Probe. Secure the Probe in place with a single

ACKNOWLEDGEMENT

Dr. Thomas L. Smith, Department of Orthopedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC 27103.

REFERENCES

Surgical Video

Blood Flow Measurement in the Rat: Implantation Techniques of the Transonic Flowprobe on the Rat Renal Artery VP-10

Validation

Welch WJ, Deng X, Snellen H, "Validation of Miniature Ultrasonic Transit Time Flow Probes for Measurement of Renal Blood Flow in Rats," AJP 1995; 268(1)Pt 2:F175-178.

Welch WJ, Deng X, Snellen H, Wilcox CS, "The Ultrasonic Transit-time Blood Flow Meter: An Accurate Method for Measuring Renal Blood flow in the Rat," JASN 1993; 3: 528.

For additional references, visit www.transonic.com

transonic
THE MEASURE OF BETTER RESULTS.

Rat Renal Artery: Chronic Blood Flow Measurement Cont.

Surgical Approach Cont.

suture around the cable. Make a stab incision through the body wall just rostral to the suture and pass the CM4 connector and cable through the incision. Examine the alignment of the Probe and vessel. If necessary, correct the alignment by suturing the Dacron mesh to the body wall. Apply a small amount of medical grade methacrylate (Exaband) to the Flowprobe and close the opening in the J reflector with a small section of Merocel sponge (Fig. 3). Replace the abdominal contents and close the ventral midline incision in two layers. Place the animal in ventral recumbency and make a 1.5 cm midscapular incision. Create a subcutaneous tunnel with straight hemostats and pull the CM4 connector through the tunnel with sutures placed through the connector cap. Close the skin incision and place the CM4 connector in the silicone cuff. Suture the silicone cuff to the skin and close the retroperitoneal incision.

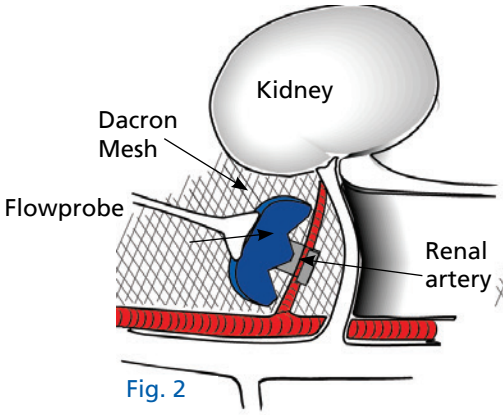


Fig. 2

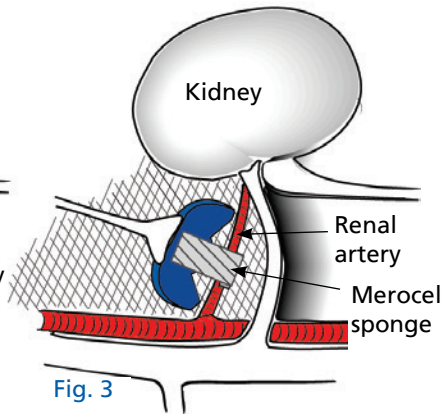


Fig. 3

Rat In Situ Autoperfused Kidney: Acute Renal Blood Flow Measurement

APPLICATION BASICS

Site:	Autoperfused Kidney Abdominal aorta
Species:	Rat
Body Weight:	300 - 375 grams
Duration:	Acute
Vessel Diameter:	1.5 - 2.0 mm

PROBE

Size:	1 mm or 1.5 mm
Reflector:	JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-1PRB, MA-1.5PRB

FLOWMETER

TS420 Perivascular Module

1.5PRB

Dose Ranges Observed

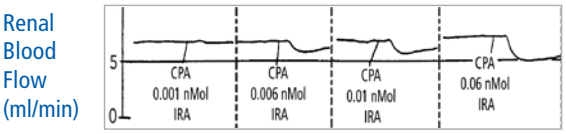


Fig. 1: Dose response effect of CPA on renal blood flow in the in-situ autoperfused kidney of an inactin anesthetized rat.

ACKNOWLEDGEMENT

David A. Dropplemann

REFERENCES

Barrett RJ, Droppleman DA, "Interactions of Adenosine A1 Receptor-Mediated Renal Vasoconstriction with Endogenous Nitric Oxide and ANG II," AJP 1993; 265: F651-F659.

Fink GD, Brody MJ, "Continuous Measurement of Renal Blood Flow Changes to Renal Nerve Stimulation and Intra-Arterial Drug Administration in the Rat," AJP 1978; 234(2): H219-H222 or Am J Physiol Heart Circ Physiol 1978; 3(2): H219-H222.

Surgical Approach

Male Sprague Dawley rats are anesthetized with Inactin (100 mg/kg,ip). Following anesthesia induction, an incision is made in the ventral cervical region of the neck. The trachea is cannulated with a 3 cm length of PE 240 tubing. The left jugular vein is cannulated with PE 10 tubing and the carotid artery is cannulated with a short length of PE 90 tubing (2.0 cm) attached to a Y connector, one arm of which is used for BP measurements and the other used to complete the extracorporeal perfusion circuit.

A midline incision is then made in the abdomen. The abdominal aorta, between the right renal artery and the posterior aortic bifurcation, is exposed and isolated from the vena cava. The left renal artery is also exposed and isolated. The animal is then heparinized with 175 units of heparin. The aorta is clamped distal to the left renal artery. The tubing from the carotid artery is allowed to fill with blood and is then inserted proximally into the aorta and secured. Renal perfusion is not interrupted during the introduction of the extracorporeal circuit. When the circuit is completed and opened, blood flows both naturally and through the extracorporeal circuit to perfuse the kidneys. The aorta is then ligated between the left and right renal arteries, so that the right kidney is perfused naturally, and the left kidney is perfused with blood from the carotid artery.

To measure blood flow, a 1PRB Flowprobe is placed on the aorta, proximal to the left renal artery. The Probe is secured in position by wrapping the aorta and Probe with a small piece of silastic sheeting (0.010 thickness) and securing with two silk sutures. Connect Probe to a Transonic® Flowmeter. Renal blood flow, systemic arterial blood pressure and heart rate are all recorded on a Grass Model 79D polygraph.

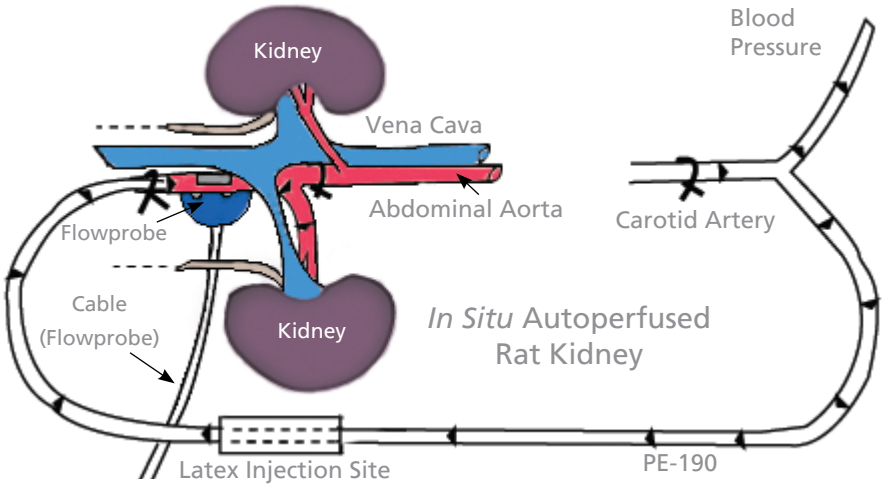
transonic
THE MEASURE OF BETTER RESULTS.

Rat In Situ Autoperfused Kidney: Acute Renal Blood Flow Measurement Cont.

Note:

The researchers initially placed the Probe around the renal artery. However, they moved the Probe to the aortic site after they found that they had to keep repositioning the Probe at the renal site and had to keep reapplying gel around the Probe. Nalco superabsorbant powder may be used to thicken coupling gel to stabilize ultrasonic coupling. Using a smaller 0.7PS Probe (not available at the time of this protocol) could alleviate the difficulties with ultrasonic coupling on smaller diameter renal arteries. In a chronic application, the renal artery site would be suitable because connective tissue would grow around the Probe and stabilize it.

At first, after moving the Probe to the aortic site, the researchers were concerned about the 1 mm Probe's tight fit on the aorta, but their results were excellent and the preparation worked well. To assess the result of the 1 mm Probe's tight fit on the aorta, flow was monitored with both a 1mm PRB Probe and a 2mm PSB Probe in 3-4 preparations. No significant differences in flow were seen. The more stable readings came from the 1PRB Probe because of its snug fit. In fact, they found that it was not even necessary to apply acoustic gel around the Probe on the aorta. A squirt of gel can be applied, however, before the silastic sheeting is sutured in place.



For Anesthesia: check what is currently available and allowed with your Institutional Animal Care & Animal Use Committee and know what affects the drugs will have on the parameters you are interested in studying. See Anesthetic Guidelines RL-67-tn for more information.

Mouse Femoral Artery: Anatomical Landmarks

The segment of the femoral artery proximal to the epigastric artery is typically isolated for acute (See Acute Protocol) or chronic (See Chronic Protocol) flow measurements. The vessel at this point measures ~250-300 µm in 25 g CD-1 mice. Small branches from the femoral are typically found at both locations where the 7-0 silk passes under the femoral artery.

Isolation of the artery from the vein in this position is very challenging. There is less fascia and connective tissue around the sheath of the artery and vein to manipulate the vessel free. The nerve runs immediately adjacent and is very easy to tear. Once isolated, avoid pulling up on the artery which would cause vascular spasm or putting pressure on the vein to cause occlusion.

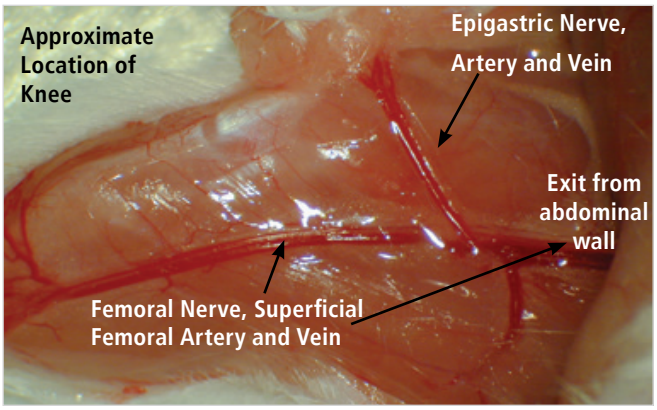


Fig. A: Figure shows femoral nerve, superficial femoral artery and femoral vein exiting from abdominal wall and running down the leg toward the knee. Note position of epigastic nerve, artery and vein.

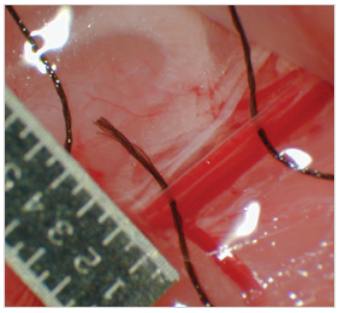


Fig. B: The femoral artery proximal to the epigastric artery, isolated from the femoral vein, measures ~400 µm diameter with a micrometer. Flow measurements can be made at this location fairly easily.

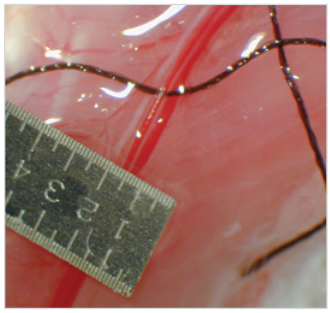


Fig. C: The superficial femoral artery, distal to the epigastric artery, measures ~200 µm diameter and is very challenging to isolate without damaging the nerve or the vein.

Measurement of Femoral Arterial Blood Flow in the Mouse©

Courtesy of Michael F. Callahan, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC. For the complete photo essay PowerPoint presentation see:

www.transonic.com

Mouse Femoral Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Femoral artery
Species:	Mouse
Body Weight:	20 - 50 grams
Duration:	Acute
Vessel Diameter:	0.20 - 0.70 mm

PROBE

Size:	0.5 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.5PSB

FLOWMETER

TS420 Perivascular Module

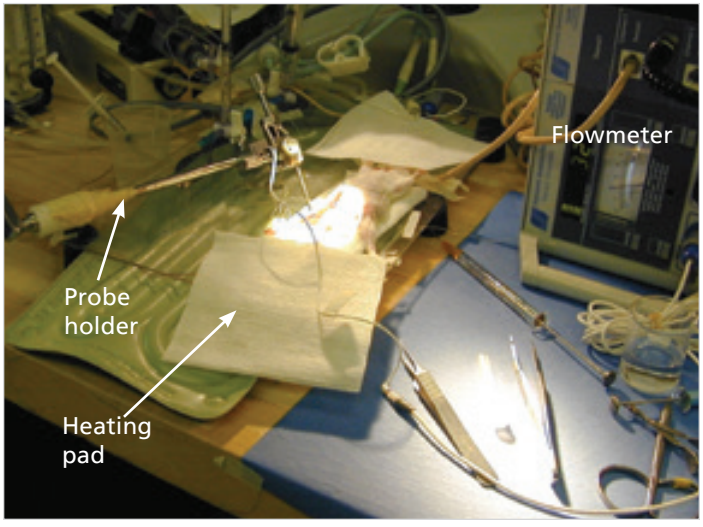
Considerations

Experimental Setup: Measurement Site; Vessel diameter; Maintenance of body temperature.

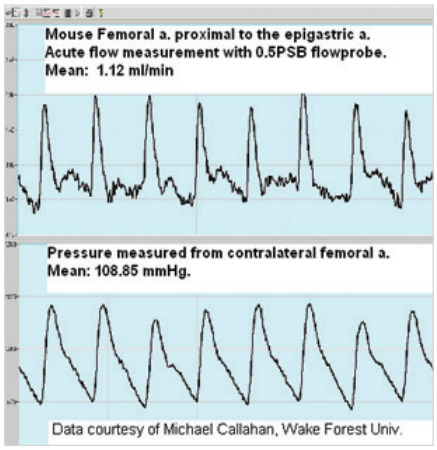
Experimental Setup

Place mouse on a heating pad with lamp with gauze cover to maintain mouse core temperature. Position Probe holder with magnetic base, adjustable arm, and micro-positioning adjustment adjacent to mouse preparation. Position the Transonic® 400-Series Flowmeter Console nearby for connecting the Flowprobe.

Administer anesthesia per protocol.



Flow Ranges Observed



Simultaneous Femoral Artery Blood Flow & Pressure Recording

Measurement of Femoral Arterial Blood Flow in the Mouse Protocol©

Courtesy of Michael F. Callahan, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC. ©Wake Forest University School of Medicine, Department of Orthopaedic Surgery; used with permission.

Fig. 1: Experimental Setup: Probe holder with magnetic base, adjustable arm, and micro-positioning adjustment. Nanoprobe handle is clamped in a micromanipulator with two-axis adjustment to fine tune and stabilize position of Probe (see close up above). 400-Series Flowmeter Console and heating pad & lamp used with gauze cover to maintain mouse core temperature. Temperature is monitored with an anal Probe.

Mouse Femoral Artery: Acute Blood Flow Measurement Cont.

Surgical Implantation of Flowprobe

PREPARE SITE FOR FLOWPROBE APPLICATION

Prepare the medial thigh for Flowprobe application with a 1.5 cm incision into the medial aspect of the thigh near the body wall. Take care to avoid the epigastric artery which exits the femoral about 7-8 mm from the body wall and travels rostrally in a fat pad attached to the skin.

ISOLATE FEMORAL ARTERY FROM VEIN & NERVE

Use a pair of 45° microblunted Dumont forceps parallel to the vessel to open the sheath covering the femoral artery, vein and nerve. Typically, one small branch of the femoral is located between the abdominal wall and the epigastric artery Removal of the fascia reveals a 3-4 mm section of the femoral artery between the small branch and the epigastric where the Flowprobe can be placed (see Anatomical Landmarks).

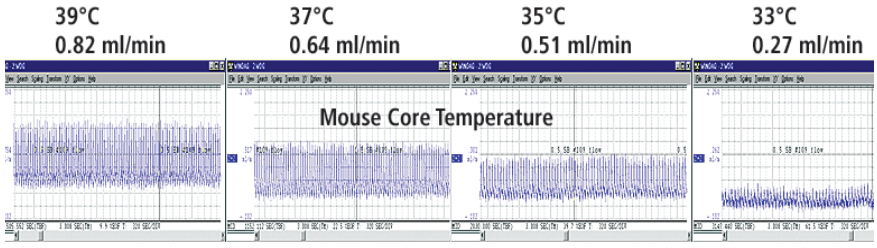
Grasp the fat/fascia located rostral to the vessels and pull rostrally. Isolate the nerve from the vessels with a 45° Dumont without touching or damaging the nerve. Pulling on the fascia caudal to the vein will allow it to be separated from the artery. A small branch of the femoral can usually be seen exiting caudally on the femoral artery immediately proximal to the epigastric artery.

POSITION FLOWPROBE & MEASURE FLOW (FIG. 2)

Using the micromanipulator, gently place the 0.5PSB Flowprobe in position over the vessel. Gently lift the femoral artery and place it into the lumen of the Probe. Use a syringe fitted with an angiocatheter, deposit acoustic gel in and around the vessel, being careful not to displace the vessel or damage the Probe reflector. With the micromanipulator holding the Flowprobe steady on the artery, measure flow.

FLOW VALUES

Blood flow in peripheral vessels can be greatly affected by core body temperature and heat loss, anesthesia, vessel spasm and Probe instability. To achieve meaningful measurements, all elements must be controlled.



Effect of core temperature on femoral blood flow in a 22 gram CD-1 mouse. Temperature has a profound effect on femoral blood flow as demonstrated by the flow traces of progressively lower core temperatures. (Courtesy MF Callahan)

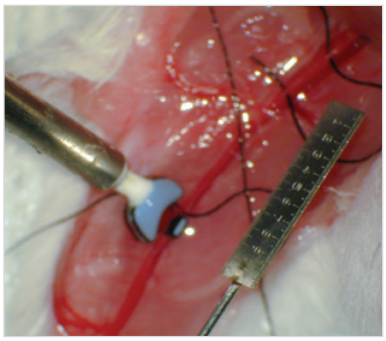


Fig. 2: A 0.5PSB Flowprobe on the superficial femoral artery, distal to the epigastric artery, must be stabilized in a micromanipulator to maintain stable Probe position during flow measurement because the vessel fills <60% of the Probe lumen. Flows at this location are ~0.6 ml/min. Recording of a femoral arterial waveform can be diagnostic of protocol difficulties. The arterial flow pulse should be apparent; an occlusion of the vessel should indicate zero flow baseline and may be nulled, as necessary to achieve true flow values.

REFERENCES

Wang CH, Chen KT, Mei HF, Lee JF, Cherng WJ, Lin SJ, "Assessment of mouse hind limb endothelial function by measuring femoral artery blood flow responses," J Vasc Surg. 2011 Jan 26.

Rickard RF, Meyer C, Hudson DA, "Computational Modeling of Microarterial Anastomoses With Size Discrepancy (Small-to-Large)," J Surg Res. 2009; 153(1): 1-11.

Fitzgerald SM, Bashari H, Cox JA, Parkington HC, Evans RG, "Contributions of endothelium-derived relaxing factors to control of hindlimb blood flow in the mouse in vivo." Am J Physiol Heart Circ Physiol. 2007; 293(2): H1072-82.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Femoral Artery: Chronic Blood Flow Measurement

APPLICATION BASICS

Site:	Femoral artery
Species:	Mouse
Body Weight:	17 - 30 grams
Duration:	Chronic
Vessel Diameter:	0.20 - 0.40 mm

PROBE

Size:	0.5 mm
Reflector:	JN
Connector:	4-pin
Cable Length:	10 cm
Catalog #:	MC-0.5PSS-JN-WC10-CA4S-GC

FLOWMETER

TS420 Perivascular Module

Before Surgery

Prepare a 0.5PSS Flowprobe with a dacron mesh attached to the underside of the Probe with silicone adhesive to help stabilize the Probe position in the implant (Fig. 1).

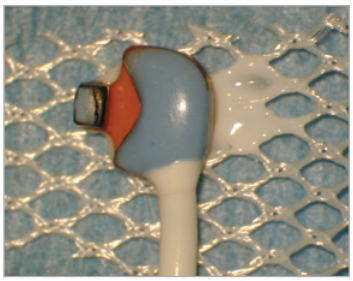


Fig. 1: A 0.5PSS Flowprobe prepared with dacron mesh attached to the underside of the Probe with silicone adhesive.

Chronic Measurement Protocol

1. Femoral Artery Isolation (See Anatomical Landmarks)

The segment of the femoral artery proximal to the epigastric artery is typically isolated for acute or chronic flow measurements. The vessel at this point measures ~250-300 µm in 25 g CD-1 mice. Small branches from the femoral are typically found at both locations where the 7-0 silk passes under the femoral artery.

2. Thigh Incision

Prepare the medial thigh and a 2 cm area on the back for surgery. A 1.5 cm incision is made into the medial aspect of the thigh near the body wall (Fig. 2). Care should be taken to avoid the epigastric artery which exits the femoral about 7-8 mm from the body wall and travels rostrally in a fat pad attached to the skin.

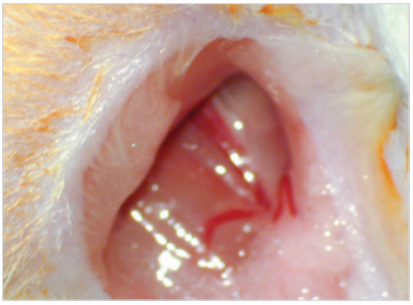


Fig. 2: Incision in medial aspect of thigh.

3. Positioning Probe Connector

Make an incision in the back to place the Flowprobe connector and then tunnel subcutaneously from the back to the posterior aspect of the thigh (Fig. 3). With a pair of forceps pull the Probe connector from the back of the thigh to the exit on the back. The length of the cable was short in this prototype Probe and prevented placement in the preferred midscapulae position. Therefore, the connector was placed on the small of the back.

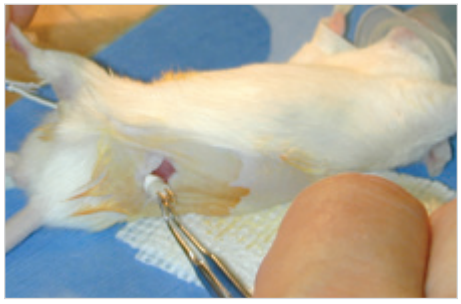


Fig. 3: An incision is made in the back to place the Flowprobe connector.

4. Isolation of the Femoral Artery

Open the sheath covering the femoral artery, vein and nerve with a pair of 45° microblunted Dumont forceps parallel to the vessel. Typically, one small branch is located between the abdominal wall and the epigastric artery. When the fascia is removed, a 3-4 mm section of the artery between the small branch and the epigastric will appear (Fig. 4).

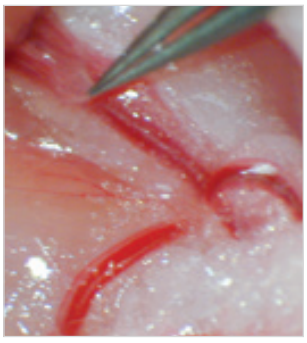


Fig. 4: Femoral artery isolated.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Femoral Artery: Chronic Blood Flow Measurement Cont.

Chronic Measurement Protocol cont.

Clear this section to place the Flowprobe. Grasp the fat/fascia located rostral to the vessels and pull it rostrally. The nerve can then be isolated from the vessels with a 45° Dumont without touching or damaging it (Fig. 5). Pulling on the fascia caudal to the vein allows it to be separated from the artery. A small branch of the femoral can usually be seen exiting caudally on the femoral artery immediately proximal to the epigastric artery (Fig. 6).

5. Placement and Securing of the Flowprobe

Gently place the Probe in position over the vessels and pull the dacron mesh under the artery (Fig. 7). The Probe cable and or mesh can be anchored to the muscle with 7-0 silk suture prior to passing the mesh under the vessel but these anchor points should be placed before positioning the Probe.

After passing the mesh, gently lift the femoral artery and place it into the lumen of the Probe. Place a section of dacron mesh over the Probe and secure the two pieces of mesh with silk (Fig. 8). Place acoustic coupling gel or lidocaine gel into the lumen of the Probe to displace air bubbles. The gel acts as a barrier to prevent the Kwik-Sil from entering the lumen of the Probe. Check the signal of the Flowprobe on the Flowmeter.

Inject 2-part Kwik-Sil or Kwik-Cast (shown) around the Flowprobe and cable (Fig. 9). The vessel can be visualized under the mesh, exiting the lumen of the Probe. Do not inject the casting material into the lumen of the Probe. This will block the ultrasound signal, and prevent flow measurement. Allow the cast to become hardened before closing wounds. Prepare skin button as previously indicated.

6. Experimental Exercise Setup

The mouse is connected to a Flowmeter via an electronic swivel connected to the skin button (Fig. 10). Femoral arterial blood flow is recorded by the Flowmeter as the mouse, implanted with a Nanoprobe on the femoral artery, runs on the treadmill.

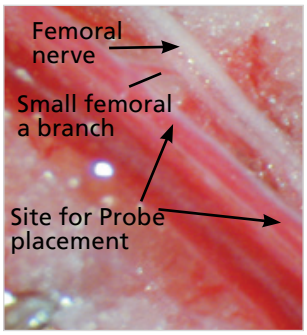


Fig. 5: Nerve isolated from the femoral vessels.

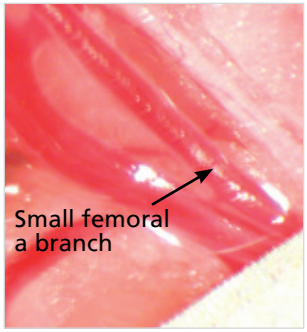


Fig. 6: A small branch of the femoral exiting caudally on the femoral a. proximal to the epigastric a.

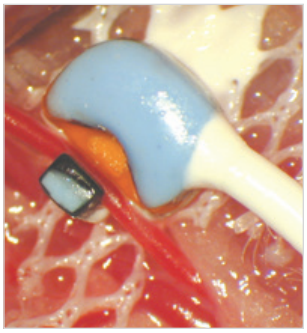


Fig. 7: Dacron mesh pulled under the artery.

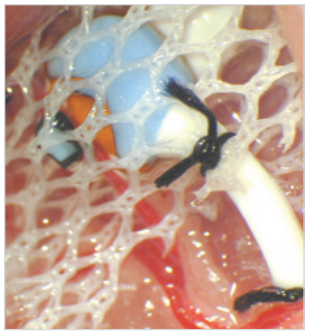


Fig. 8: Upper and lower pieces of Dacron mesh secured with two pieces of silk.

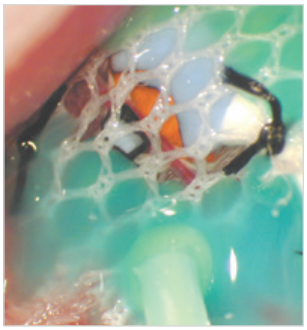


Fig. 9: Kwik-Sil or Kwik-Cast injected around the Probe and cable.

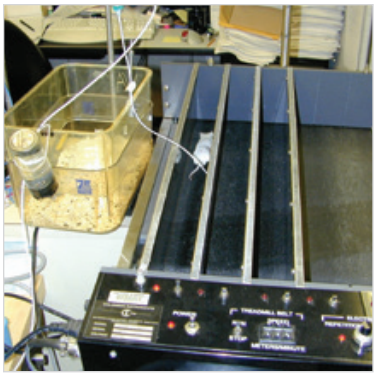


Fig. 10: Three station treadmill for rodents by Columbus Instruments.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Femoral Artery: Chronic Blood Flow Measurement Cont.

Chronic Measurement Protocol cont.

7. Flowprobe Recovery After 5 Weeks Implantation

Connective tissue and blood vessels overlie the Kwik-Cast but have not infiltrated it (Fig. 12). Tissue has infiltrated the mesh but this can be cut away (Fig. 13) to visualize the intact blood vessel exiting the Probe lumen (right). At this point one can check for "zero" flow by occluding the vessel.

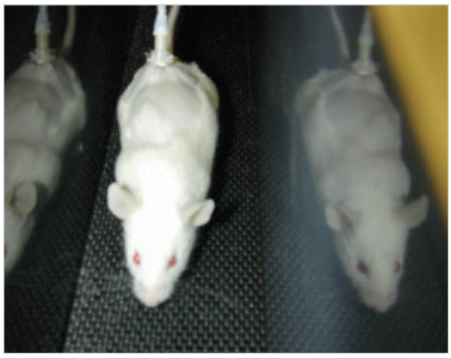


Fig. 11: Mouse with femoral Nanoprobe implant running on treadmill.

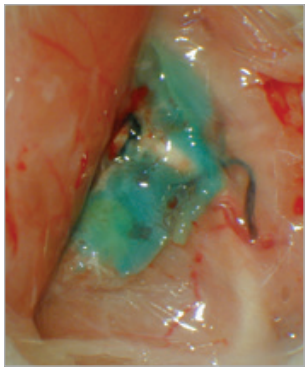


Fig. 12: Kwik-Cast with connective tissue and blood vessels.

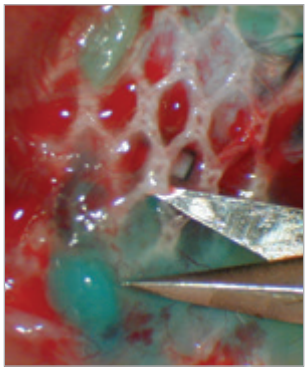


Fig. 13: Cutting away tissues from the Dacron mesh.

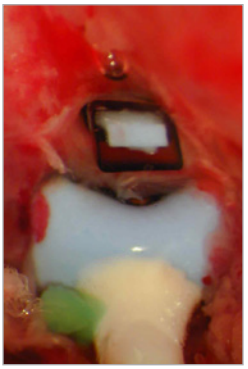


Fig. 14: Probe removed.

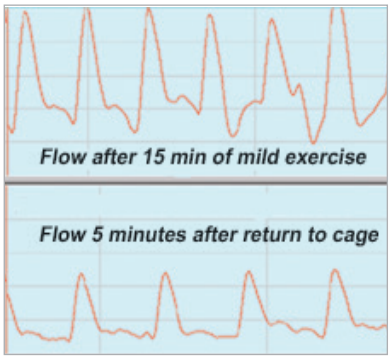


Fig. 15: Effect of brief exercise on femoral artery blood flow. Mean Flows: Top: 1.9 ml/min during treadmill exercise; Bottom: 0.8 ml/min returned to home cage.

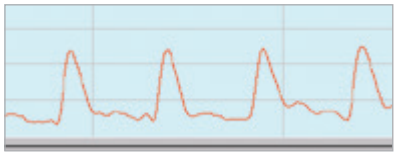


Fig. 16: Typical mouse chronic femoral artery flowtrace.

ACKNOWLEDGEMENTS

Transonic Systems Inc.® gratefully acknowledges the assistance of Michael F. Callahan, PhD, and Thomas L. Smith, PhD, Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, in the development of this protocol.

Adapted from a PowerPoint presentation produced by: Margo Sosa, Senior Product Manager, Transonic Systems, Inc., 34 Dutch Mill Road, Ithaca, NY 14850 www.transonic.com

REFERENCES

Cabou C Campistron G, Marsollier N, Leloup C, Cruciani-Guglielmacci C, Pénicaud L, Drucker DJ, "Brain glucagon-like peptide-1 regulates arterial blood flow, heart rate, and insulin sensitivity," Diabetes 2008; 57(10): 2577-87.

Cabou C, Cani PD, Campistron G, Knauf C, Mathieu C, Sartori C, Amar J, Scherrer U, Burcelin R, "Central insulin regulates heart rate and arterial blood flow: an endothelial nitric oxide synthase-dependent mechanism altered during diabetes," Diabetes 2007 ; 56(12): 287.

Rat Femoral Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Femoral artery
Species:	Rat
Body Weight:	200 - 400 grams
Duration:	Acute
Vessel Diameter:	0.70 - 0.9 mm proximal to epigastric 0.5 - 0.6 mm distal to epigastric

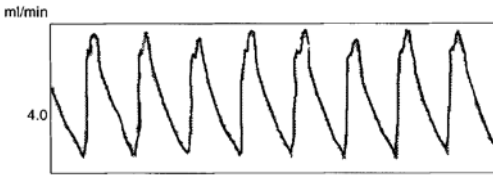
PROBE

Size:	0.7 mm or 1 mm
Reflector:	J
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-0.7PSB, MA-1PRB

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed



Flow trace courtesy of T.L. Smith, Wake Forest University School of Medicine, Winston Salem, NC.

Application

The femoral artery is a convenient site for the blood flow measurement in basic hemodynamic research as it is relatively accessible and does not require highly invasive surgery. Since access does not require opening the abdomen or the thorax, there are fewer complications from hypothermia and shock. This site is also relatively free of adipose fat.

Surgical Approach

Anesthetize the rat with ketamine/xylamine solution (0.09 ml / 100 g body weight IM thigh). Use a heating pad or heat lamp to avoid hypothermia which will reduce flow. A 0.5 ml bolus of saline placed subcutaneously every half hour is also recommended.

Note: The femoral artery tapers from 0.9 mm diameter at the iliac artery to 0.5 mm distal to the epigastric branch. Flowprobe size will depend on the chosen measurement site.

Place the rat in dorsal recumbency and visually identify the femoral vessels on the medial thigh. They should be readily visible through the shaved and prepared skin. Gently stretch the skin caudally, make a 1.5 cm incision adjacent to the femoral vessels, and release the skin so that it slides back over the vessels.

When the combined femoral artery, vein and nerve are exposed, carefully separate the vessels from the surrounding fascia. First pass a small curved needle completely under the vascular bundle and back out the other side, then use a fine pair of curved forceps to tease away the fascial layers. Continue this process until the vascular bundle is completely undermined and freely movable.

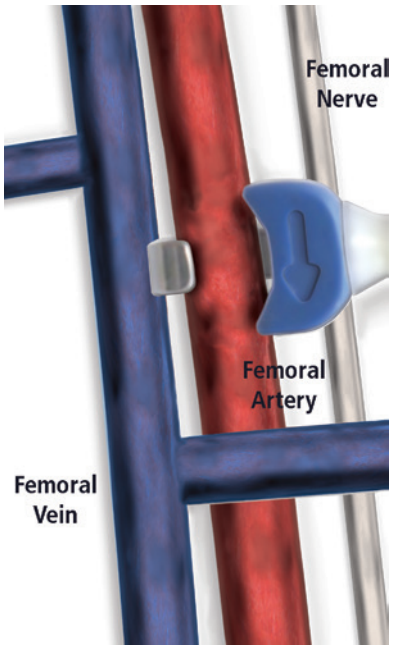


Fig. 1: Site of femoral artery in relation to femoral vein and femoral nerve.

transonic
THE MEASURE OF BETTER RESULTS.

Rat Femoral Artery: Acute Blood Flow Measurement Cont.

Surgical Approach Cont.

Separation of the vessels is often the most difficult step of the procedure. One technique is to take a pair of forceps with a very blunt tip and repeatedly open and close them in a direction parallel to the vessels. Try to make a small gap between the vessels. The vein may be identified by alternately applying pressure proximally and distally to distend and collapse the vein. When the femoral artery is isolated, place the Flowprobe around the artery. Use a micromanipulator to stabilize the Probe position.

Remove the plunger of a 30 cc syringe and load the syringe with Surgilube gel, taking care to prevent the formation of air bubbles. Place a flexible 20 gauge catheter on the tip of the syringe. Insert the catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The gel acts as an acoustic couplant and must replace the air space. Select the test mode on the Meter to verify that signal amplitude is close to 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of Surgilube gel or to an air bubble.

REFERENCES

Shimura H et al, "A New Simultaneous Method for Measuring the Blood Flow in Small Experimental Animals Using the Transit-Time Ultrasonic Volume Flowmeter," Jap J of Pharmacol 1986; 40(Suppl): 101P.

Hoffman A et al, "Endothelin Induces An Initial Increase in Cardiac Output Associated with Selective Vasodilation in Rats," Life Sciences 1989; 5(3): 249-255.

Wachter C et al,"Visceral Vasodilatation and Somatic Vasoconstriction Evoked by Acid Challenge of the Rat Gastric Mucosa: Diversity of Mechanisms," J Physiol 1995; 486(2) 505-516.

For additional references, visit www.transonic.com

Rat Femoral Artery: Chronic Blood Flow Measurement

APPLICATION BASICS

Site:	Femoral artery
Species:	Rat
Body Weight:	150 - 400 grams
Duration:	Chronic
Vessel Diameter:	0.70 - 0.9 mm

PROBE

Size:	1 mm
Reflector:	JS
Connector:	4-pin
Cable Length:	14 cm (12 - 16 cm typical)
Catalog #:	MC-1PRB-JS-WC14-CM4S-GC MC-1PRS-JS-WC14-CM4S-GC

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

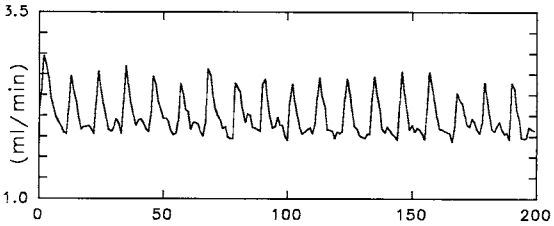


Fig. 1. Right Femoral Artery of 125 g Wistar-Furth rat, normal ambulatory posture. Flow rates observed under anesthesia, with the rat in the prone position, are considerably less than those observed in the ambulatory rat. Ambulatory femoral artery flow rates vary over the cardiac cycle from 0.5 to as much as 9 ml/min in young rats (120-200 g). Mean flow rates are generally in the range of 1.5 to 4 ml/min.

1PRS

Application

The femoral artery provides an easily accessible site for measurement of peripheral or hindlimb blood flow, and requires only superficial surgery for the implantation of the Flowprobe. Anesthesia and the prone posture of the rat during surgery both markedly decrease femoral artery flow. Thus, chronic implantation of the Flowprobe is necessary in order to fully assess the effects of posture, immobilization or vasoactive agents on peripheral blood flow.

Surgical Approach

Rats are anesthetized with an intramuscular injection of a 1:1 mixture of ketamine hydrochloride (Ketaset, 100mg/ml) and xylazine hydrochloride (Rompun, 20mg/ml) at a dose of 0.1ml/100 g body weight. Surgery is performed under nominally aseptic conditions. The Probe and all surgical instruments are chemically sterilized by immersion in Cetylcide Blue (benzalkonium chloride and cetyl dimethyl ethyl ammonium bromide) for at least 15 min. To maintain body temperature during surgery, the rat is placed over an isothermal pad. A 1 cm, dorsal incision is made in the mid-scapular region skin. A ventral incision is made at the junction of the right thigh and the body wall. A blunt hemostat is used to create a subcutaneous tunnel from the mid-scapular to the leg incision. The connector is covered with a bullet-shaped cap, and a loop of 4-0 silk suture is tied to its end. A blunt hemostat is then passed through the subcutaneous tunnel from the mid-scapular to the leg incision. The hemostat is used to grasp the suture on the bullet-shaped cap of the connector and the Flowprobe connector and cable are passed via the subcutaneous tunnel from the leg region to the mid-scapular incision.

REFERENCES

Roer RD, RM Dillaman, "Effect of simulated weightlessness on femoral artery blood flow in the rat," The Physiologist 1991; 34: 235.

Roer RD, RM Dillaman, "Effect of simulated weightlessness on femoral artery blood flow in the rat," J Appl Physiol 1994; 76(5): 2125-2129.

transonic
THE MEASURE OF BETTER RESULTS.

Rat Femoral Artery: Chronic Blood Flow Measurement Cont.

Surgical Approach cont.

The right femoral artery is exposed and separated from the femoral vein and saphenous nerve by blunt dissection of the fascia with two pairs of fine, vascular forceps. The perivascular J-reflector of the Flowprobe is then slipped below and around the femoral artery in the region just distal to the rectus abdominis muscle, with the reflector oriented toward the anterior (Fig. 2). The Probe is then sutured with two 6-0 braided silk sutures to the underlying musculature (gracilis anterior) such that the cable is directed toward the posterior (Fig. 3). One loop is placed immediately adjacent to the transducer, and the second is placed approximately 0.5 cm away. A subcutaneous pocket is created by blunt dissection posterior to the Probe, overlying the gracilis posterior muscle. A loop of the Probe cable is inserted into this pocket to allow for movement of the limb without putting tension on the femoral artery (Fig. 4).

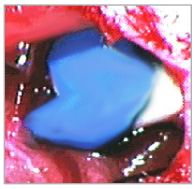


Fig. 2

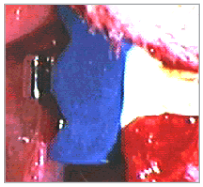


Fig. 3

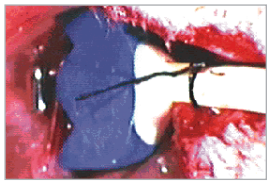


Fig. 4

The cavity containing the Probe is filled with Surgilube gel to provide acoustic coupling to the Probe. After the Probe is tested for the presence of a good acoustic signal, the ventral incision is sutured closed and the connector is sutured to the skin of the mid-scapular region (5-0 or 4-0 monofilament nylon). Both incisions are then swabbed with topical antiseptic (Betadine).

We modified the Flowmeter cable by the insertion of an electronic swivel (Braintree Scientific). By clamping the swivel to a ring stand above the cage, the animal is allowed free movement with 360° rotation. While the implant is chronic, we make our measurements periodically, detaching the cable from the mid-scapular connector and capping the connector between measurements. We have maintained the Flowprobes within rats for periods up to three weeks, but see no reason why this period could not be extended. Generally, after one or two weeks, the skin in the mid-scapular region rejects the sutures, so rats must be anesthetized for the connector to be reattached.

Rat Common Iliac Artery: Acute Blood Flow Measurement

APPLICATION BASICS

Site:	Common iliac artery
Species:	Rat
Body Weight:	300 grams
Duration:	Acute
Vessel Diameter:	0.7 - 0.9 mm

PROBE

Size:	1 mm
Reflector:	JS
Connector:	CRA10: 10-pin
Cable Length:	60 cm
Catalog #:	MA-1PRB

FLOWMETER

TS420 Perivascular Module

Surgical Approach

Anesthetize the rat with ketamine/xylazine (0.09 ml per 100 g body weight) in the thigh. The use of a heating pad or hot water bottle is also recommended as hypothermia also reduces flow. In long procedures, fluid infusion (0.9% NaCl @ 1 ml/hr) through a femoral catheter is also recommended. Place rat in dorsal recumbency and make a ventral midline abdominal skin incision. Extend abdominal incision through the linea alba into abdominal cavity.

Carefully locate the iliac artery which bifurcates at the terminal of the abdominal aorta. Use blunt dissection with forceps to isolate @ 0.7 mm of the artery from the vein. Place the Probe around the artery (Fig. 2). Manually position the artery so that it is lies within the lumen of the ultrasonic window of the Probe. Then tape down the Probe cable to help stabilize the Probe. Probes with a handle may be stabilized with a micromanipulator.

Apply acoutical couplant by removing the plunger of a 30 cc syringe and load the syringe with sterile Surgilube gel, taking care to prevent the formation of air bubbles. Place a flexible catheter on the tip of the syringe. Insert the flexible catheter through the Probe's acoustic window adjacent to the artery and deposit the gel while withdrawing the syringe. The gel acts as an acoustical couplant and must replace all air space. Select test mode on the meter to verify that signal amplitude is about 1 Volt. A low signal or an acoustic error can usually be traced to an insufficient amount of lubricating gel or an air bubble.

Flow Ranges Observed

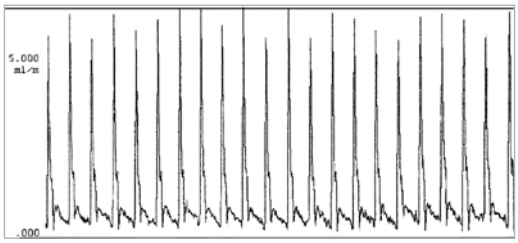


Fig. 1: Iliac flow in the rat varies greatly with the anesthetic used and the plane of anesthesia. In general, protocols with ketamine will show higher flows than those with pentobarbital. Hypothermia is also a common cause of lower than expected flow measurements.

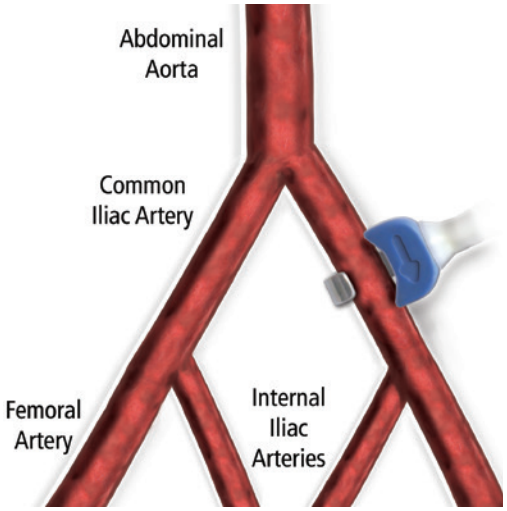


Fig. 2: Flowprobe on common iliac artery

ACKNOWLEDGEMENT

TL Smith, PhD, Department of Orthopedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC.

REFERENCES

Video: IV. Fundamental Techiques for Hemodynamic Studies in the Rat: (22 min) VP-18: Acute Volume Blood Measurements in the: Superior Mesenteric Artery; Renal Artery; & Iliac Artery of the Rat.TL Smith, PhD, , Wake Forest University School of Medicine, Winston-Salem, NC.

Unthank JL, Nixon JC, Dalsing MC, "Acute Compensation to Abrupt Occlusion of Rat Femoral Artery Is Prevented by NO Synthase Inhibitors," AJP 1995; 68 (Heart Circ. Physiol. 37): H2523-H2530.

For additional references, visit www.transonic.com

transonic
THE MEASURE OF BETTER RESULTS.

Rat & Guinea Pig Urethra: Urine Flow Measurement

APPLICATION BASICS

Site:	Urethra
Species:	Rat; Guinea Pig
Body Weight:	Rat: 450 grams Guinea Pig: 950 grams
Duration:	Acute

PROBE

Size:	3 mm
Reflector:	LS
Connector:	CRA10: 10-pin
Cable Length:	100 cm
Catalog #:	MA-3PSB

FLOWMETER

TS420 Perivascular Module

Flow Ranges Observed

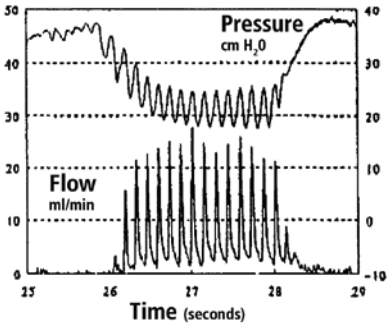


Fig. 1: Rat: A decrease in intravescical pressure on which is superimposed a series of high frequency oscillation accompanies flow.

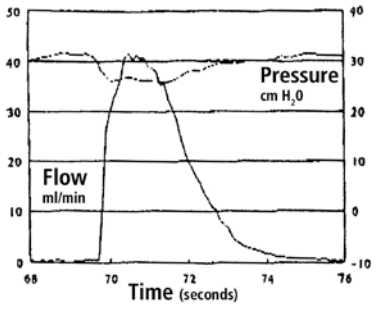


Fig. 2: Guinea pig: A small decrease in intravesical pressure is accompanied by flow. Note the absence of high frequency oscillations.

Application

Using the Transonic® Flowmeter and Probe provides the possibility to describe urinary flow patterns in terms of flow rate, flow time and voided volume. In this study, these parameters were used to compare rat and guinea pig voiding. The results show the striking difference between the intermittent and continuous flow patterns. The ability to measure flow rate will increase the insight into the intricate relationship between the urinary bladder and the urethra. It will add to a better understanding of the functioning of the lower urinary tract.

Surgical Approach

Male Wistar rats (450 g) and Dunkin Hartley guinea pigs (950 g) were anesthetized with urethane (1.2 g/kg body weight, i.p.). The animals were placed on a heated undercover. The urinary bladder and the distal part of the urethra were exposed through an abdominal incision.

A 24G needle was inserted into the bladder dome and via tubing and a T-connector was attached to an infusion pump and a pressure transducer. A 3mm Transonic® Probe was placed around the dissected distal part of the urethra and was connected to a Flowmeter. The bladder was filled with room-temperature saline at an infusion rate of 0.1 ml/min (rat) or 0.5 ml/min (guinea pig). The bladder was filled until the bladder pressure rose steeply and the animal started to void. Both flow and pressure signals were recorded by computer at a sampling rate of 100 Hz (rat) and 10 Hz (guinea pig).

Figures 1 and 2 show representative recordings of the bladder pressures and flow through the urethra. In both species, the voiding contraction consists of a steep rise in pressure, which then decreases, re-rises and finally falls down to base-line values. In the rat, the decrease is accompanied by so-called high frequency oscillations. These oscillations also occur in the flow rate of the rat which results in an intermittent flow pattern in contradiction to the continuous flow pattern in the guinea pig.

ACKNOWLEDGEMENTS

E. van Asselt, J.Groen, R.van Mastrigt, Department of Urology /Urodynamics, Erasmus University Rotterdam, Rotterdam, The Netherlands.

REFERENCES

van Asselt E, Groen J, van Mastrigt R, "High Frequency Oscillations during Voiding in the Rat; Simultaneous Flow Rate & Pressure Measurements," Neurourol Urodyn 1994.

van Asselt E, Groen J, van Mastrigt R, "A Comparative Study of Voiding in the Rat and the Guinea Pig; Simultaneous Measurement of Flow Rate and Pressure," Am J Physiol 1995; 269(1 Pt 2): R98-103.

For additional references, visit www.transonic.com

transonic
THE MEASURE OF BETTER RESULTS.

Introduction to Pressure & Pressure-Volume Measurements

Hemodynamic studies require the integration of several parameters to fully understand physiological mechanisms and relationships. No review of blood flow measurement techniques in the study of cardiovascular physiology would be complete without a discussion of the techniques to monitor blood pressure. Blood flow and pressure measurements are integrated to measure and derive vascular resistance; cardiac output and arterial pressure yield systemic peripheral resistance or TPR (total peripheral resistance).

INTRAVASCULAR ACUTE BLOOD PRESSURE MEASUREMENT

Fabrication techniques have caught up with the demand for miniaturized pressure transducers and catheters that can be used intravascularly to measure blood pressure directly. Transonic Scisense's high fidelity, solid-state pressure Catheters are available in 1.2 and 1.6 French sizes with both single and dual pressure sensors. These are small enough to be introduced into the carotid or femoral artery of the mouse for acute blood pressure measurement.

Standard pressure measurements using fluid filled catheters and external pressure transducers have also been used widely in rats and mice at lower cost. Small diameter catheters can be constructed in the lab or purchased though several companies. Instructions for preparing catheters are included in this workbook.

CHRONIC BLOOD PRESSURE MEASUREMENT

Continuous measurement of blood pressure in conscious rats and mice is also possible via telemetry. Telemetric measurements of heart rate, temperature and blood pressure were pioneered by Data Sciences International (St. Paul, MN) and miniaturized for rats and mice with implantable battery operated transmitters. These utilize fluid filled catheter technology for carotid or femoral arterial placement and have been employed for long term studies making it possible to study animals in an undisturbed environment. It is also possible to combine telemetric blood pressure measurement with implantable Transonic® Flowprobes to simultaneously monitor pressure and cardiac output, or pressure and renal flow simultaneously, though the flow measurement still requires external leads and tether connections. As systems and technologies improve, it is possible to envision improvements in telemetry systems for the future.

CARDIAC PRESSURE-VOLUME MEASUREMENT & PV LOOPS

The recognized standard for comprehensive heart performance measurement is the PV loop. By plotting ventricular pressure and volume in the XY plane, a researcher can study individual cardiac cycles or a family of loops. Ejection fraction, cardiac output, max & min dP/dt, stroke volume, and tau are just a small example of the many parameters that can be reported. Additionally, valuable measurements of contractility and elastance are obtained by creating 'pressure-volume relationship curves'. Derived only by PV loop analysis, these relationships are crucial to cardiovascular studies to generate a full report of heart function.

Transonic® Scisense is on the cutting edge of pressure-volume technology with Variable Segment Length (VSL) Catheters for flexible fit with a single adjustable Catheter and Admittance volume technology in addition to the standard Conductance method for real-time PV loop generation.

Pressure-Volume Conductance Theory of Operation

Deriving ventricular volume from a Conductance Catheter is based on a very simple electrical principle: Ohm's Law:

Voltage (V) = Current (I) X Resistance (R) V = IR

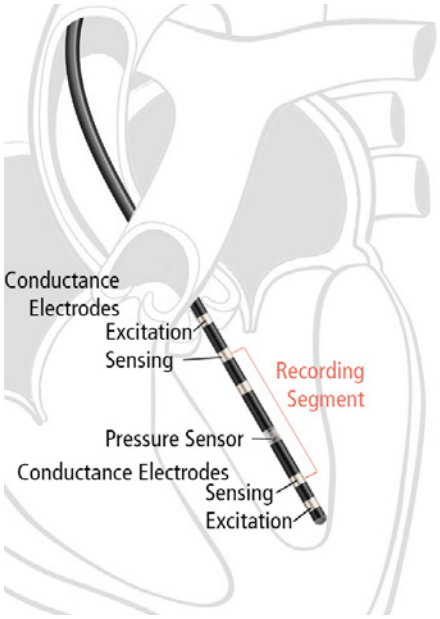
Conductance (G) rather than resistance is the parameter of interest. Since conductance is the inverse of resistance, Ohm's Law can be rewritten as:

Voltage = Current/Conductance V = I/G

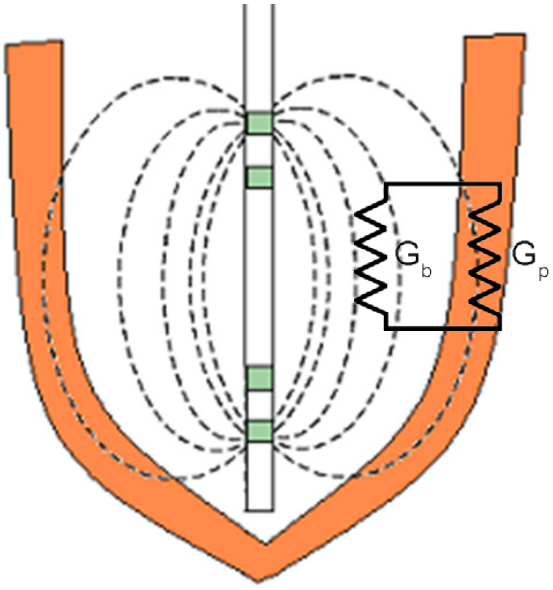
Conductance Catheters are comprised of both excitation electrodes and recording electrodes. The excitation electrodes (most distal and proximal electrodes on the Catheter) generate an electrical field inside the heart from the aortic valve to the apex. This field is generated as a result of an alternating current being applied (at a constant magnitude) between these 2 outermost electrodes. The inner recording electrodes measure voltage change which is proportional to a change in resistance.

The electrical field cannot be restricted to just the blood volume and must pass through some of the cardiac muscle. This means that the measured conductance value (G_x) is actually a combination of blood conductance (G_b) and muscle or parallel conductance (G_p).

In 1981, Dr. Baan et. al. proposed a relationship between time-varying measurements of total conductance (G_x) to time-varying changes in ventricular volume (Vol). This volume formula takes into account the distance between the recording electrodes (L), blood resistivity (ρ), and the parallel conductance (G_p). It also takes into account the non-uniform nature of the electrical field with the field correction factor, alpha (α).



Conductance excitation electrodes create an electric field wile sensing electrodes measure the voltage change which allows for the calculation of resistance and conductance.



Conductance uses a circuit model where both blood (G_b) and cardiac muscle (G_m) are conductive and measured together as a single conductance value (G_x) and phase components are ignored.

BAAN'S EQUATION

$$Vol = \frac{1}{\alpha}\rho L^2(G_x - G_p)$$

ρ = Blood resistivity

L = Measuring electrode distance

α = Baan's SV correction factor = $(\frac{SV_{conductance}}{SV_{reference}})$

G_x = Measured total conductance

G_p = Baan's parallel/muscle conductance (assumed to be negated by hypertonic saline injection)

Pressure-Volume Conductance Theory of Operation Cont.

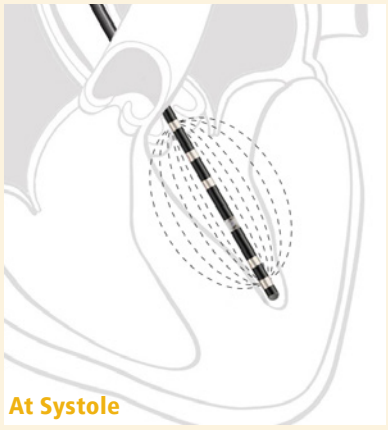
However, Baan assumed alpha to be a constant with a value of one for a uniform current field distribution (in reality electrical field strength decreases non-linearly with distance). Alpha can be calculated from the SV conductance ratio (see box below) or by cuvette calibration. Both of these methods give a single constant value for alpha. Parallel or muscle conductance (G_p) is often determined by hypertonic saline injection which temporarily changes blood conductance but not myocardial conductance; allowing for the parallel conductance value to be determined from the graph of changing conductance. This produces a single constant value for parallel conductance.

IMPACT OF PHYSIOLOGY ON CONDUCTANCE MEASUREMENTS

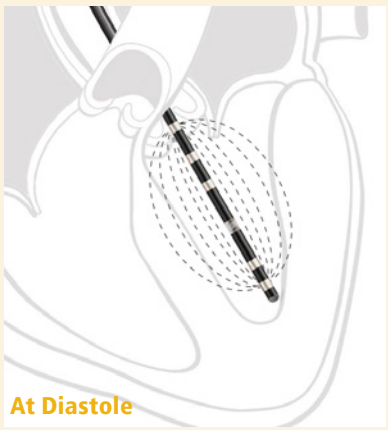
At systole there is relatively little blood in the ventricle which means that a larger portion of the electrical field passes through the myocardium. Thus, myocardial resistance contributes more to the total measured conductance value leading to an over estimation of blood volume, because the actual muscle conductance is greater than the value determined from hypertonic saline injection which represents an average muscle contribution to total conductance.

At diastole there is a large quantity of blood in the ventricle and the heart walls have expanded. This means that most of the electrical field is passing though blood with a very small contribution from the myocardium. Thus, the measured conductance value is almost entirely blood conductance. However, the same value of parallel conductance is still subtracted from the total conductance which leads to an under estimation of blood volume.

The electrical field strength decreases in a non-linear manner with increasing field size. This means measurements of blood conductance further from the Catheter do not have the same strength as that nearer to the Catheter. Without any correction this leads to an under estimation of total volume. The larger the volume which is being measured, the greater the under estimation, thus the blood volume measurement at diastole is impacted by under estimation more than those at systole where the total volume of interest is smaller. Alpha attempts to correct some of this error but fails to address the non-linearity of the electrical field or the varying strength the under estimation has at different phases of the heart cycle.



At Systole



At Diastole

Pressure-Volume Admittance Theory of Operation

Admittance technique is an extension of the Conductance method which measures capacitive properties of blood and muscle in addition to resistivity. In the electric field blood is purely resistive, but muscle has both capacitive and resistive properties. This allows separation of the muscle component of conductance from that of blood, using electric field theory.

The capacitive property of muscle causes a time (phase) delay in measured signal (see graph at bottom right). By tracking this delay known as the phase angle in real time and mathematically relating it to the resistance of the myocardial tissue, via the σ/ε ratio, the ADV500 System allows continuous tracking of muscle/parallel conductance (G_m) throughout the heartbeat. The phase angle reports heart tissue intrusion into the field as the heart contracts and expands, and as expected, it will be at a minimum at diastole and a maximum at systole. This provides a great advantage over classical conductance volumetry which treats parallel conductance as a constant, rather than a dynamic variable which changes with the cardiac cycle.

The ADV500 system employs an equation developed by Dr. Chia-Ling Wei to convert conductance to volume instead of the traditional Baan's equation. In Baan's equation the Field Correction Factor, alpha (α), is assumed to be constant despite the non-linear nature of the electrical field. However, Wei's equation corrects for the nonhomogeneous nature of the Catheter's electrical field distribution by assuming a non-linear relationship between conductance and volume, gamma (γ), thus improving accuracy over a wider volume range.

To measure blood volume in real time values are needed for myocardial conductivity and permittivity (for σ/ε ratio/heart type), blood resistivity (ρ), and stroke volume (SV). Values for heart tissue properties (σ/ε ratio) can be obtained from a tetrapolar surface probe or know values can be applied. Stroke volume can be measured via other technologies.

WEI'S EQUATIONS

$$Vol = \frac{1}{(1-\frac{G_b}{\gamma})}\rho L^2(G_b)$$

ρ = Blood resistivity

L = Measuring electrode distance

G_b = Measured blood conductance

SV = Stroke volume

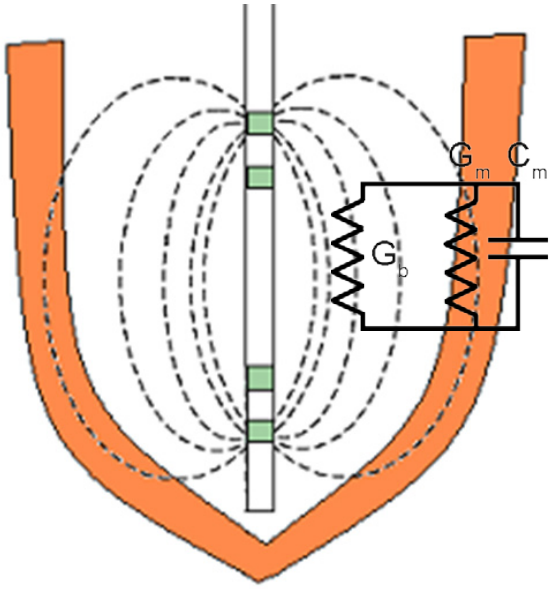
Field Correction Factor:

$$\gamma = \frac{-b \pm \sqrt{b^2-4ac}}{2a}$$

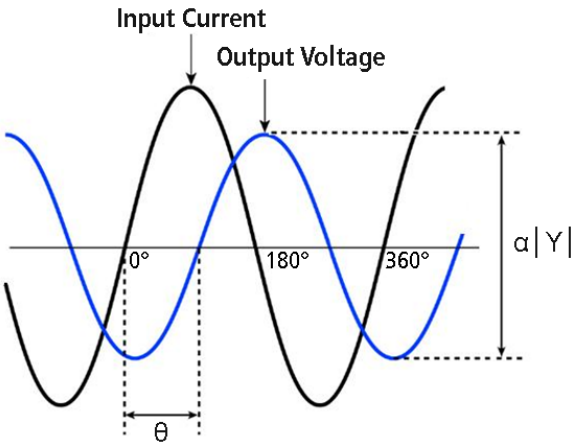
$$a = SV - \rho L^2(G_{b-ED} - G_{b-ES})$$

$$b = -SV \cdot (G_{b-ED} + G_{b-ES})$$

$$c = SV \cdot G_{b-ED} \cdot G_{b-ES}$$



Admittance uses a circuit model where blood is conductive (G_b) and cardiac muscle is both conductive (G_m) and capacitive (C_m).



The output voltage shows a "delay" compared to the input voltage signal used to generate the electric field. The signal delay, caused by myocardial capacitance, is measured in terms of degrees and is referred to as "Phase angle θ." The admittance magnitude (conductance) is impacted by both the blood and muscle.

Comparing Conductance vs Admittance

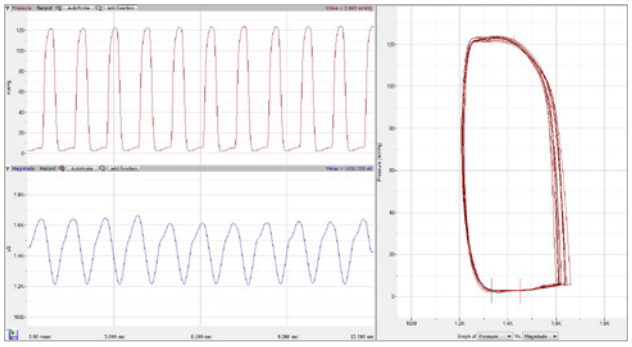
The Scisense ADV500 Pressure-Volume System is capable of being using in either Conductance or Admittance mode. Both methods have value depending on what the researcher is looking to observe.

Conductance

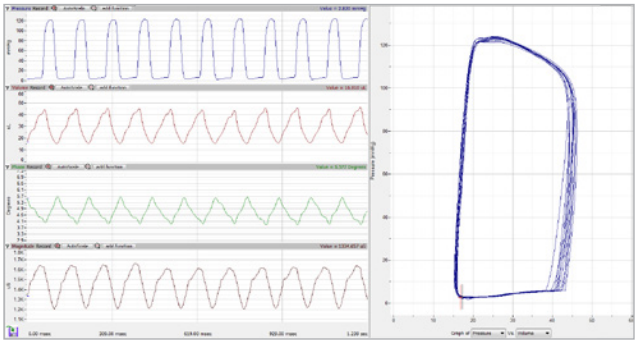
- Measures voltage magnitude
  - Difficult to determine position of Catheter in ventricle
- Uses Baan's equation to determine volume
- Parallel conductance (G_p) is constant
  - Parallel conductance determined from hypertonic saline injection after the experiment
- Field Correction Factor is constant (alpha)
  - Requires empirical stroke volume to derive α or an approximation (typically 1) can be used
- Volume calculation is done post experiment with no chance to correct for protocol or surgical errors.
- Tends to overestimate volume due to constant nature of α as observed with echocardiography.
- Accepted standard with a solid body of papers that validate the basic principle of conductance catheter volumetry.

Admittance

- Measures voltage magnitude and phase angle
  - Phase angle is useful in locating Catheter in ventricle
- Uses Wei's equation to determine volume
- Muscle conductance (G_m) varies over cardiac cycle
  - Parallel conductance determined from phase shift in real-time (no hypertonic saline injection required)
  - Requires sigma/epsilon ratio (conductivity/ permittivity) of heart muscle. Can be measured with a Surface Probe or use a typical σ/ε ratio value
- Field Correction Factor is non-linear (gamma)
  - Requires empirical stroke volume to derive γ
- Volume calculation is in real time. Corrections to experimental protocol or surgery can be made before experiment is concluded.
- Closer approximation to absolute systolic and diastolic volume as observed with echocardiography.
- Innovative technology that builds directly on the foundation of conductance catheter volumetry.



Conductance method measure pressure and magnitude in real-time, creating pressure-magnitude loops. Volume can only be calculated post-experiment.



Admittance method measure pressure, volume, phase and magnitude in real-time, creating pressure-volume loops.

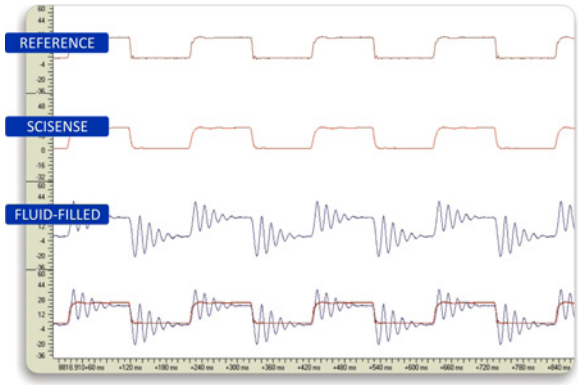
Scisense Pressure Systems, PV Systems & Catheters

SP200 PRESSURE MEASUREMENT SYSTEM

- Measure two channels of pressure simultaneously
- Permits calculation of pressure gradients and pulse-wave velocity
- Quick electronic two-point calibration
- Compatible with all data acquisition systems (± 5V range required)

SOLID-STATE PRESSURE CATHETERS

Single and Dual Pressure Sensor Catheters are available in all sizes ranging from 1.2F to 7.0F. High frequency response ensures accurate detection of pressure waveforms and resulting calculations (dP/dt, Peak Pressure, MAP, etc.). Variable placement of second pressure sensor on dual catheters is available to meet protocol needs.



Scisense Pressure Catheter compared to a reference trace and a fluid-filled pressure catheter at 300 BPM (5 Hz). The fluid-filled catheter shows large distortion and wave artifact compared to the Scisense trace which reflects the true pressure waveform of the reference sensor.

ADV500 PRESSURE-VOLUME MEASUREMENT SYSTEM

- Measure volume using either Conductance or Admittance modes
- True volume in real-time using Admittance mode
- Generate full hemodynamic reports and calculate measurements of contractility and stiffness
- Compatible with all data acquisition systems (± 5V range required)

PRESSURE-VOLUME CATHETERS

Pressure-Volume Catheters are available for all animal models. 1.2F and 1.9F catheters are ideal for mouse and rat studies, 3.5F for rabbits, and 5.0F or 7.0F for all larger animals (canine, swine, bovine, etc.)

VARIABLE SEGMENT LENGTH (VSL) CATHETERS

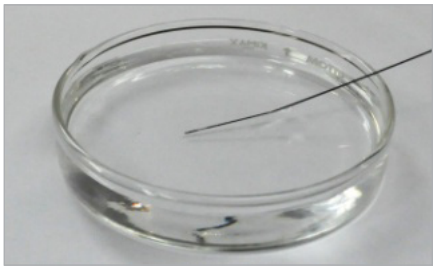
VSL Catheters have 4 volume electrode options designed to offer flexibility and ensure proper fit. Catheters are available in 1.2F and 1.9F, ideal for rodent dilation models, and are standard for all larger animal Catheters ranging from 3.5F - 7.0F. Standard segment spacings are available for all Catheter sizes while custom designs can be made to order.

transonic
THE MEASURE OF BETTER RESULTS.

Care Guidelines for Scisense Catheters

ALWAYS CLEAN CATHETERS IMMEDIATELY AFTER USE.
FAILURE TO PROPERLY AND PROMPTLY CLEAN CATHETERS MAY CAUSE SENSOR FAILURE.
DO NOT USE ULTRASONIC CLEANERS FOR THE CATHETERS.

Follow all directions for the cleaning and disinfecting agents for proper use.

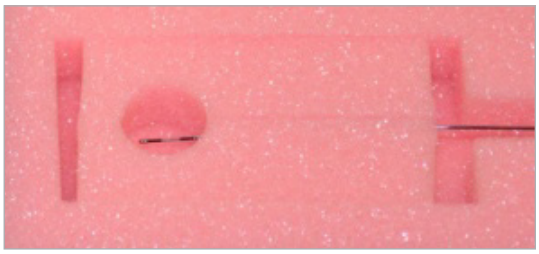


Rinse Catheter in distilled water or saline (Steps 1 & 4)

1. Immediately after use, immerse the Catheter in distilled water or saline for approximately 5 minutes.
2. Soak the Catheter in an enzymatic cleaning solution to remove all traces of biological material (i.e. Tergazyme by Alconox Inc). This should take between 0.5 and 2 hours depending on cleaning agent and level of soil on the Catheter.
3. Optional: Immerse Catheter in disinfecting agent (i.e. Cidex® by ASP) to remove all traces of viable microbes. Do not use glutaraldehyde solutions containing surfactants such as Cidex® 7 or Cidex® Plus, or solutions containing hydrogen peroxide like Sporox. Do not use Cidex® PA.
4. Rinse the Catheter by soaking in distilled water for 1 - 5 minutes to remove all traces of cleaning agents .

Immerse Catheter in cleaning agents (Steps 2, 3 & 7)

5. Dry the Catheter by gently wiping or placing on a paper towel or gauze. Do not air dry or use alternative drying methods. Never apply direct pressure to the pressure sensor membrane.
6. Before returning the Pressure Catheter to its original packaging for storage, use magnifications to check for blood or tissue residue on the Catheter tip. If any is found, repeat the cleaning process.
7. Optional: Immerse Catheter in an acid based cleaner to remove metal oxides from the ring electrodes on Pressure-Volume Catheters for 1 -2 minutes (i.e. Citranox® by Alxonox Inc).
8. Store the dry Catheter in it's original packaging. Position the pressure sensor within the foam cutout to prevent damage.



Catheter in storage foam (Step 8)

Single use Catheters should be disposed of after use according to local waste disposal regulations.

KEYS TO SUCCESSFUL USE

Follow all instructions in the Quick Start Guides and Operator's Manuals.

- To avoid baseline drift soak the Catheter tip (including pressure sensor) in sterile saline for at least 20 minutes prior to use.
- After soaking, the sensor can be zeroed by adjusting the balance control on the Pressure or Pressure-Volume System. Be sure to adjust the zero pressure only when the pressure sensor is exposed to atmospheric pressure. If pressure cannot be zeroed, inspect Catheter for tissue residue and clean with enzymatic cleaner.
- Never grasp the Catheter by the tip with your fingers or with forceps. Applying pressure directly to the pressure sensor can cause significant damage.
- Be gentle when inducing Catheters into the subject. Sudden or forceful motions can cause damage to the vessel or Catheter tip.
- Allow the Catheters time (5+ minutes) to stabilize within the body before recording measurements.

transonic
THE MEASURE OF BETTER RESULTS.

Rat Left Ventricle Acute Pressure-Volume Measurement (Open Chest Approach)

APPLICATION BASICS

Site:	Left Ventricle - Open Chest
Species:	Rat
Body Weight:	250 - 270 grams
Duration:	Acute

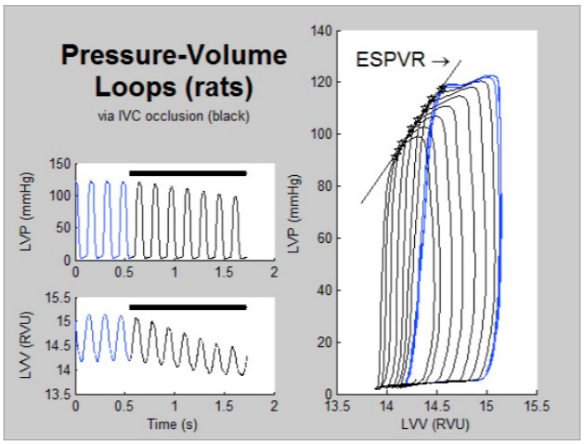
CATHETER

Size:	1.9F
Type:	Pressure Volume or VSL Pressure Volume
Catalog #:	FTH-1912B or FTH-1918B

SYSTEM

ADV500 / ADVantage

PV Loops



Application

The hemodynamic properties measured by the pressure-volume system can be used to determine cardiac function. Performing an IVC occlusion as part of the pressure-volume measurement process allows for the determination of load-independent indices.

Surgical Approach

Prior to surgery, soak the tip of the PV Catheter in 0.9% saline for ~ 20 minutes. After soaking, adjust the pressure balance to zero for atmospheric pressure.

Anesthetize rats with 3- 4% Isoflurane and maintain anesthesia with 2% Isolfurane by ventilator. Secure animal in dorsal position on the heating pad. Make V shape skin incision in the lower thorax/ upper abdomen area over the xyphoid (Fig. 1). Separate the skin from the chest wall by blunt lateral dissections. Open the abdominal wall in the proximity of the sternal manubrium (Fig. 2). Cut through the diaphragm to expose the heart apex (Fig. 3). Try to avoid any incisions around sternum to limit bleeding. Try not to artificially retract rib cage. Gently maneuver the apex, using Q-tips into the diaphragm opening.

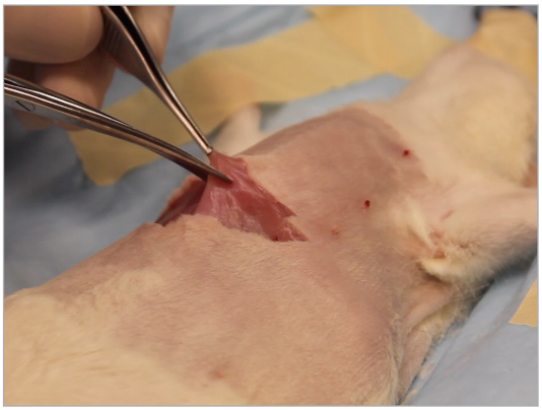


Fig. 1: Initial incision in the upper abdomen of rat in dosal position.

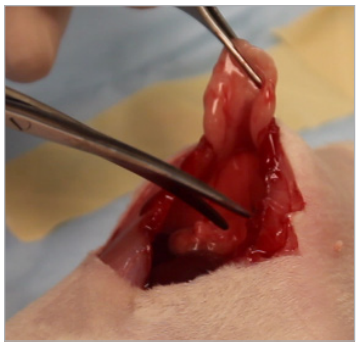


Fig. 2: Open the abdominal wall.

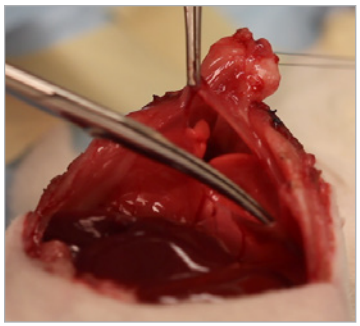


Fig. 3: Cuth through the diaphragm to expose the heart apex.

ACKNOWLEDGMENTS

Toronto General Research Institute, McEwen Centre for Regenerative Medicine, University of Toronto, 200 Elizabeth Street, MaRS 3-908, Toronto, ON, M5G 2C4, Canada.

REFERENCES

Konecny, F., Zou, J., et al. Post-myocardial infarct p27 fusion protein intravenous delivery averts adverse remodelling and improves heart function and survival in rodents. Cardiovasc Res 94, 492-500 (2012)

transonic
THE MEASURE OF BETTER RESULTS.

Rat Left Ventricle Acute PV Measurement (Open Chest) Cont.

Surgical Approach Cont.

Use the 25G needle for the LV apical stab. After successful stab, blood is found in the needle conus (Fig. 4). As needle is withdrawn from the LV myocardium with your other hand insert the 1.9F Catheter through the stab wound (Fig. 5) until the distal electrode of the catheter is fully surrounded by LV muscle (Fig. 6). This is critical step where all electrodes have to be fully submerged in the ventricle's cavity. Position the catheter to control for phase angle (ɵ) and admittance magnitude (Y) and collect pressure-volume (PV) signal in form of graphical tracing known as PV loop.

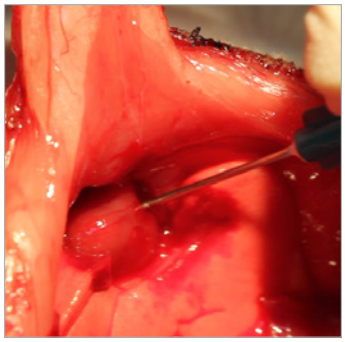


Fig. 4: Stab apex with a 25G needle.

Allow catheter to stabilize in the LV for 5-10 min before marking the data file to start protocol. Catheter positional adjustment needs to be made based on acquired signals, mostly coming from phase angle (ɵ) and admittance magnitude (Y) recordings. Both signals should measure sinusoid wave signal. In case of off-center position, acquired sinusoid signals might be distorted as e.g. (low amplitude, frequency etc.). Reposition catheter until an optimal position is found – essentially this is where magnitude waves are at their largest and phase waves are stable and devoid of noise or spikes. The researcher should also view Pressure and Magnitude in an XY plane to assist in their search for optimal catheter position. Once optimal catheter position is obtained, preform a "baseline scan" on the ADV500/ADVantage control unit - end-systolic and end-diastolic blood conductance (Gb_{ed} and Gb_{es}) values will be sampled and reported on the LCD screen. This scan is best conducted when the ventilator is turned off a few seconds prior to scanning and for the duration of the scan. Repeat the baseline scan as necessary throughout the experiment to ensure most accurate report of volume. Record load-dependent values during steady state for at least 10 min for each animal before attempting IVC occlusion.

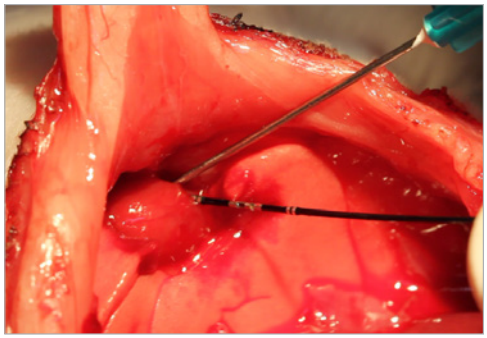


Fig. 5: Insert Catheter into the stab wound.

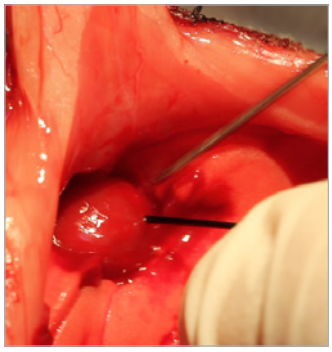


Fig. 6: Submerge all electrodes.

IVC occlusion is used to derive various load-independent indices of systolic function. During the preparation of the open chest IVC occlusion suture (5-0 silk) is placed under the vena cava as it is carefully separated from adventicia and thoracic aorta (Fig. 7). 5-0 silk is placed above the liver at close proximity of the heart. This position will ensure an immediate volume drop to better control and compare the data sets. IVC occlusions can be performed by pulling on a suture placed around the vessel (Fig. 8). Shut off the ventilation for a few seconds to acquire data without lung motion artifacts.

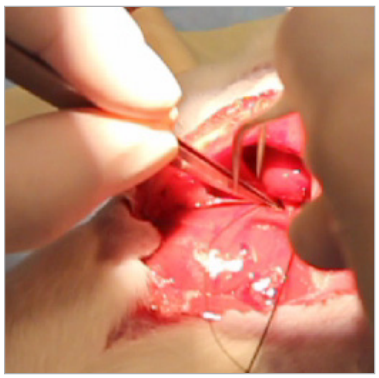


Fig. 7: Place sutures for IVC occlusion.

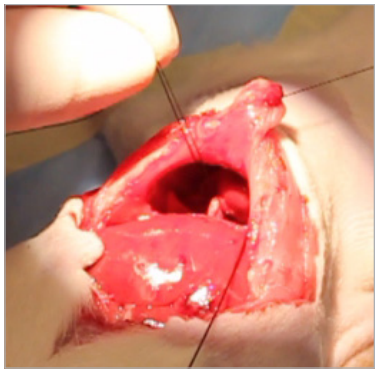


Fig. 8: Pull sutures to perform IVC occlusion.

At the end of the experiment, carefully remove the PV Catheter by gently pulling it back through the stab wound. Immediately, insert catheter tip into 5 ml saline pre-filled syringe. Clean Catheter as soon as possible according to proper care guidelines to considerably prolong the Catheter's life.

Arterial Catheter Construction

A Technique for Making Catheters to Measure Arterial Pressure in Mice

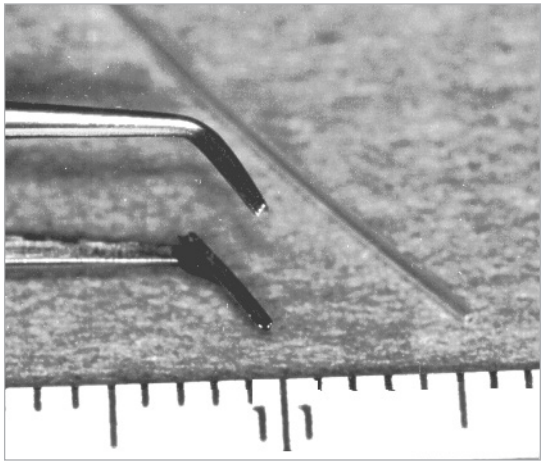


Fig. 1: Illustration of PE10 catheter size where each division is equal to 1 mm.

The catheters are comprised of two separate segments, each of which is constructed out of polyethylene tubing. The smaller tubing, which is used to cannulate the femoral or carotid artery is made of PE10 that has an inside diameter of 0.28 mm and an outside diameter of 0.61 mm. The PE10 catheter is then glued inside a 15-20 cm long segment of PE50 polyethylene tubing, approximately 0.58 mm inside diameter and 0.965 mm outside diameter.

Catheter Construction

A length of PE10 catheter of about 3 1/2-4 inches is heated using either a heat gun or a candle chimney and the catheter material is drawn down to a very thin length of tubing. The rate of taper should be such that the catheter goes from an outside diameter of 0.61 mm to an outside diameter of 0.25 mm over a distance of approximately 14 mm. When working with the polyethylene catheters use a scalpel blade or razor blade to cut the material so that it is not distorted (as it would be with a pair of scissors).

The PE10 catheter is then shortened to a length of approximately 7 mm from the end of the catheter, where it is at its widest part, to the beginning at the taper which will be introduced into the artery. This length of the PE10 catheter is then glued inside a longer segment of PE50 polyethylene tubing which is approximately 15-20 cm long. PE50 polyethylene tubing is approximately 0.58 mm inside diameter and 0.965 mm outside diameter. A two component adhesive system consisting of an activator and a bonding agent is applied to the outside of the PE10 segment which is then introduced into the PE50 catheter material. Once joined, these segments become bonded. An alternative method of joining these two pieces of material would be heat welding.

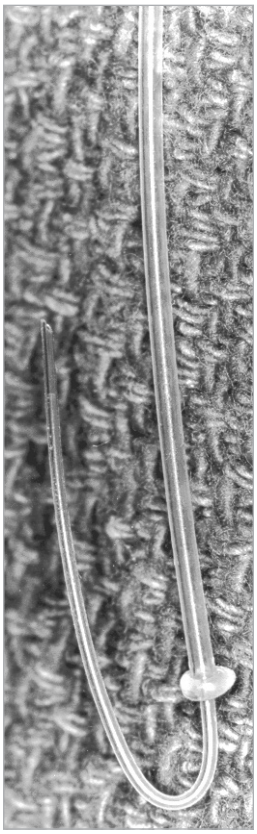


Fig. 2: Welded and bent catheter

Catheter Bending

After the PE10 and PE50 catheter is constructed, the PE10 catheter tubing is formed into a sharp 180 bend just beyond the point at which is welded into the PE50 material. The catheter can be bent by creating a mandril about which the catheter material can be formed. The mandril should be approximately 2-2.5 mm in diameter. The easiest way to bend the catheter material is to form it around the mandril and dip it into boiling water for approximately 1 1/2-2 seconds. Immediate immersion into cold water after boiling water will set the plastic catheter in the new shape.

Catheter Tip

Once formed into a 180 bend, the tip of the catheter is cut on an angle with a razor blade so that it can be introduced into the femoral artery or the carotid artery. The tip of the catheter, from the point at which the taper has begun to the end of the catheter which is first introduced into the artery should be 13-14 mm long. These catheters can be sterilized using ethylene oxide cold gas sterilization procedure or a bacteriocidal soaking solution such as

Arterial Catheter Construction Cont.

Cidex may be employed (check for compatibility with adhesive system before use).

The portion of the catheter which exits through the animal's skin should be long enough to provide manipulation by the investigator (4-5 cm in length). Sealing of the catheter after it is flushed with heparinized saline and supplied with a heparin lock should be done using heat sealing. The catheter is clamped after being flushed using covered hemostats to prevent destruction of the plastic catheter.

The open end of the catheter is heated until it flares and is then quickly collapsed upon itself (or crushed) to form a heat seal while it is still plastic and hot.

Catheter Protection

The easiest way to contain the catheter once it is outside the mouse (so that the mouse does not destroy the catheter or bite it) is to contain the catheter within some sort of protective device. The easiest and least expensive device available is found in fabric stores and consists of a two component cloth-covered button kit. These are made of nickel plated brass and consist of a 14 mm base and a 16-17 mm cover which fits upon the base. The two halves snap together providing an excellent protective device for the mouse catheter. They weigh only 750 milligrams. This button is sewn to the mid-scapular region on the mouse's back. The base is attached to the mouse and the catheter comes through the middle of the base. Excess catheter which is outside the mouse is coiled inside the snap-on cap. The cap is snapped onto the base and the catheter is protected.

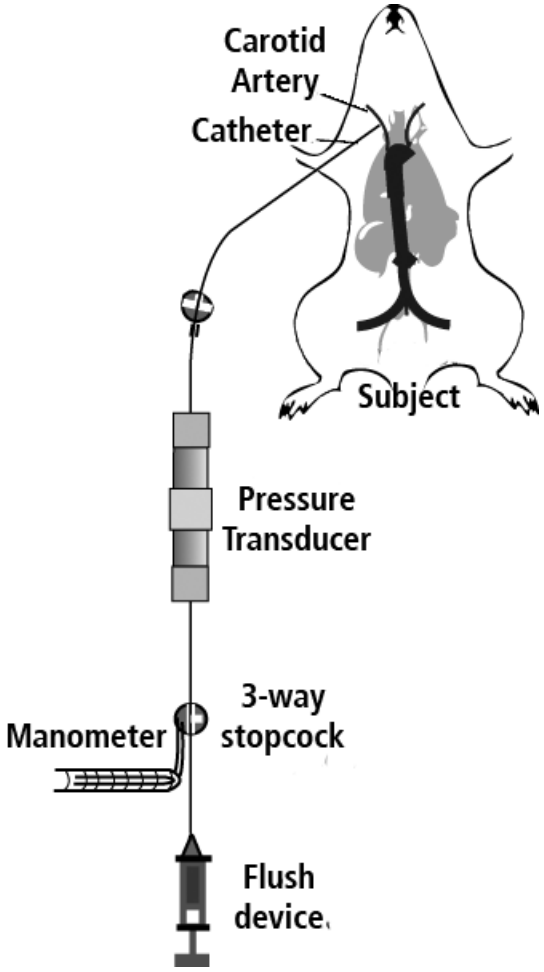
Fig. 3: "Cloth" covered metal button. Bottom has exit slot and suture holes (left) and top has teeth to snap onto the bottom to protect coiled catheter (right).

Commercially Available Catheters

Recently, excellent quality catheters for mice and rats have become available from commercial sources. ReCathCo (www.recathco.com) supplies various sizes and tubing types ready made for infusion, blood sampling and pressure cannulation.

Carotid Artery Cannulation: Acute Pressure Measurement

Cannulation



The carotid artery of the mouse can be cannulated using the same catheters constructed for use in the femoral area. Carotid artery cannulation should be performed only on an acute basis since compromise of cerebral circulation by occlusion of one of the carotid arteries generally results in some morbidity to the mouse.

The carotid artery is approached through a midline incision over the trachea and throat of the mouse. Two approaches can be used. The most common approach goes along the midline separating the two muscles overlying the larynx and bisecting the muscle plane to expose the trachea itself. Dissect laterally, usually to the animal's left trachea side. The carotid artery lies approximately 2 mm lateral and just posterior to the trachea. In isolating the carotid artery, careful dissection is required to avoid damage to the vagas nerve or the recurrent laryngeal nerve. A long straight area of artery is available at this site.

Three sutures are used for cannulation:

Distal Suture: The most distal suture (most cranial) is placed just proximal to the carotid sinus and ligated.

Proximal Suture: The proximal suture is placed as close to the chest area as possible. The intervening section of the carotid artery is cleaned of adventitia.

Middle Suture: A third suture is placed between the proximal and distal sutures and is used to prevent or reduce blood flow when introducing the catheter.

After the carotid artery is isolated, tension is placed on the proximal 7-0 silk suture and blood is removed from the lumen of the isolated portion of the carotid by gentle massage of the artery, pushing the blood back beyond the proximal ligature. No reflow of blood insures that the proximal ligature is tight enough to prevent bleeding once the carotid artery has a small cut placed in it.

The catheter is introduced into the carotid artery at the incision site, approximately 2-3 mm proximal to the carotid sinus. The carotid arterial catheter needs to be approximately 14 mm long when introduced at this site to insure that the tip of the catheter will extend down the carotid artery all the way to the aortic arch. When the animal is in the prone position with its head extended, this catheter length is empirically determined to achieve placement of the catheter tip just at the aortic arch.

Acute Experiments

For acute experiments, most of the catheter or the total length of the catheter can be reduced so that maximum catheter fidelity can be achieved when used with a conventional transducer manometer system. The PE10/50 cannular system is probably advisable for the carotid artery as well as the femoral artery in that it allows easier connection of the catheter within the animal to the external manometer systems.

ACKNOWLEDGEMENT

Thomas L. Smith, Ph.D., Dept. of Orthopaedic Surgery, Wake Forest Univ., Winston-Salem, NC.

Use of TRANSPAC® in Acute Pressure Studies

This protocol is provided as a guide to using the Transpac® IV transducer with a fluid-filled arterial or venous catheter previously implanted for the purpose of pressure measurement. Transpac® IV transducers are used with Transonic® TS430 or SP430 Pressure Amplifier Modules and other signal amplifiers.

Prepare the Pressure Sensor

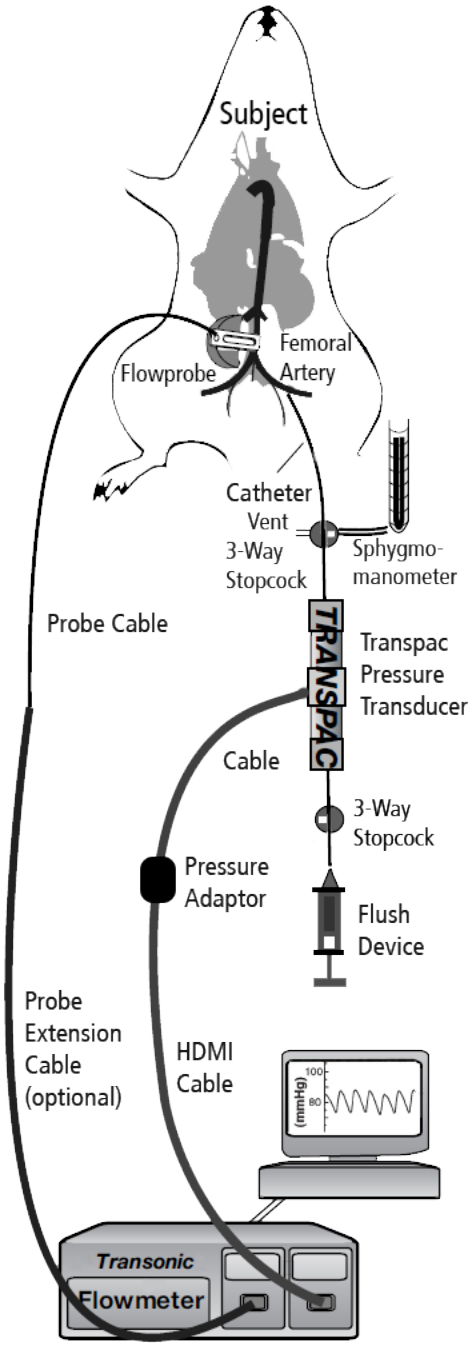
1. Open the package and remove the transducer with cable.
   a. Cut away the cup-like protector on the phone jack connector end and keep the connector dry.
   b. If you are using an extension cable, you may retain the protector; it is intended to keep the connector clean and dry from fluids or blood.
2. Assemble the tubing set as desired. (Refer to diagram on right).
   a. Connect the catheter to transducer Luer lock connector
      i. Use an IV extension set
      ii. Include a 3-way stopcock with side port for venting and calibration.
   b. Connect opposite side of transducer to stopcock/flush device as desired.

IV Line Set-Up

1. Fill lines with solution and flush air from the lines using heparinized saline.
2. Take care that no air is trapped in the transducer or connector.
3. Keep the catheter/tubing open with a constant, slow supply of heparinized saline, or by manual flushes, as required.

Calibrate the Pressure Channel

1. Connect the transducer connector to the Pressure Amplifier Module (SP430 requires a fluid filled catheter adaptor).
2. Connect a BNC cable from the pressure channel output to your data acquisition system & select the proper data channel.



Schematic of pressure cannula setup for arterial pressure via the femoral artery and simultaneous flow measurement on the terminal aorta.

transonic
THE MEASURE OF BETTER RESULTS.

Use of TRANSPAC® in Acute Pressure Studies Cont.

Calibrate the Pressure Channel Cont.

3. Adjust the zero offset of the transducer.
   a. Turn the venting stopcock so that the transducer is connected to the animal.
   b. Open the side port of the stopcock to atmosphere for “Zero” pressure reading.
      i. For TS430: Switch to Cal 0 mode & press the recessed enter button to null the offset. Use this zero voltage output as your 0 calibration point.
      ii. For SP430: Use the balance control knob for channel 1 on the SP430 Module to ensure that the output voltage is -2.86 volts.
4. Apply a known pressure (100 mmHg) to the transducer.
   a. Close the side port of the venting stopcock and apply 100 mmHg to the transducer from a calibrated source (such as a sphygmomanometer).
      i. For TS430: Switch to Cal + mode and press the recessed enter button to adjust the pressure channel gain. Use this 1 volt output signal as your 100 mmHg calibration scale.
      ii. For SP430: Check to ensure that the output voltage is -0.57 volts.

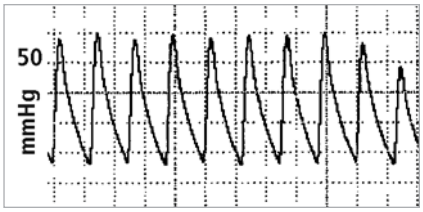
Connect to Subject

1. Pressurize the solution source or active infusion pump for continuous flush setups.
2. Use caution so as not to introduce air.

Femoral Artery Cannulation: Chronic Pressure Measurement

Preparation

The mouse is prepared for surgery by anesthetizing it with ketamine and xylazine. After anesthesia is attained, the groin area of the left rear leg and the mid scapular region behind the neck are shaved using animal clippers. The area is disinfected using Betadine scrub and Betadine solution.



Mouse femoral artery pressure from PE10PE50 tapered catheter.

Mid-Scapular Incision

A small incision is made in the mid-scapular region to allow later exteriorization of the catheter from the body. The mouse is then placed on its back with the legs extended.

Surgery

A 15 mm skin incision is made over the femoral artery and vein region. The fat pad which overlies the femoral artery and vein is then reflected from the artery and vein; clamped, and transected after clamping. Minimal bleeding occurs following this procedure.

The femoral artery is then very carefully dissected away from the vein; the surgeon should use a great deal of care to avoid damaging either the femoral vein or the femoral nerves which lie adjacent to the femoral artery. The use of Dumont vessel dilators, either D-5a or D-5az, is recommended for this procedure because of the delicate structure of the femoral artery and vein.

A 4-5 mm section of femoral artery is dissected free from the surrounding tissue between the area where the artery and vein send branches to the overlying fat pad and the peritoneal wall. Once isolated, 3-4 drops of a 2% lidocaine solution is applied to the surface of the femoral artery and vein to prevent vasospasm. This greatly facilitates cannulation of this vessel which is approximately 400 micrometers in diameter.

Three 7-0 silk ligatures are placed around the femoral artery.

1. The first ligature is placed most distally, close to where the artery branches to the fat pad overlying the femoral area. This most distal 7-0 silk ligature is tied completely, occluding the blood vessel.
2. A second 7-0 silk ligature is placed proximally on the femoral artery. It should go around the femoral artery and tied with half of a surgical knot. This ligature should be loose and it should be close to the abdominal wall.
3. A third ligature is placed between the first two, again with half of a loosely tied surgical knot.

Cannulation

To cannulate the femoral artery, apply traction to the most distal ligature which is then taped into position with masking tape, putting tension on the artery. Elevate the most proximal ligature using a pair of forceps clamped to the end of the ligature as a weight to apply traction. This occludes blood flow into the remaining middle portion of the femoral artery which is then isolated so that the blood vessel can be cut or incised and the catheter introduced.

At this point ensure that the proximal ligature completely occludes the blood vessel by pushing the blood back proximal to the ligature and verifying that no reflow occurs. A small incision should be made approximately half way across the femoral artery within two millimeters of the most distal ligature. A pair of Dewecker iris scissors works very well.

Using the tip of a pair of 5/45 sharp-pointed Dumont forceps, gently open the vessel and elevate the proximal edge of the incised artery and introduce the tip of the catheter into the artery at this point.

Femoral Artery Cannulation: Chronic Pressure Measurement Cont.

Gently advance the catheter tip until it is against the most proximal ligature and tighten the middle ligature loosely around both the blood vessel and catheter. Release the most proximal ligature and advance the catheter as far into the femoral artery as it will go. It should advance until the taper begins which is 13-14 mm in length from the tip.

At this point, both the proximal and middle ligature should be securely tied around the artery and catheter combination and the remaining ligature left on the most distal tie on the femoral artery should be brought around the catheter itself and tied again so that three separate ligatures hold the catheter within the blood vessel. A fourth ligature, consisting of either 5-0 or 7-0 silk, should be placed into the heavy thigh muscle on the inner aspect of the mouse thigh and tied around the catheter body to help hold the catheter body away from the femoral vein.

Occlusion of the femoral vein probably results in increased morbidity of the ipsilateral foot and limb. The remainder of the catheter should be brought subcutaneously from the femoral region to the mid-scapular region. This is accomplished using a 15 gauge needle which has been threaded below the skin from the mid-scapular area to the femoral area. The catheter end is brought through the 15 gauge needle to the exit at the mid-scapula region. The needle is then withdrawn from the femoral area, over the edge of the catheter, and away from the animal. This leaves the catheter in position under the skin with a bend at the femoral region.

Completion

Completion of the femoral site requires reapproximation of the femoral fat pad over the underlying femoral artery and vein and reapproximation of the skin. Both procedures utilize a 7-0 silk suture. Interrupted skin sutures are recommended at the femoral site so that if the animal chews through one of the sutures as healing progresses, the entire suture line will not become unraveled.

Place the animal on its sternum and complete the operative procedure by placing a piece of Mersilene mesh over the catheter, sliding it down to the mid-scapular area and suturing it under the skin using 4-0 Vetafil suture. The suture goes in through the skin, through an approximately 1 cm x 1 cm piece of Mersilene mesh and then back out through the skin again as it exits. The skin is reapproximated over the Mersilene mesh and the catheter itself exits through an approximately 1 cm long mid-scapular incision along the central midline at the middle of the incision.

Finally, slide the bottom half of the cloth-covered button catheter holder over the catheter, suturing it in place with Vetafil sutures, and then coiling the end of the catheter within the top half of the cloth-covered button (see Arterial Catheter Construction for details). The catheter may require shortening to allow a length of catheter outside the mouse (approximately 4 cm).

Catheter Patency

Prior to final heat sealing and closure, the catheter should be filled with 1000 units per ml pure heparin. This will not provide any long lasting antihemostasis to the mouse and will help ensure catheter patency.

Properly inserted, these catheters should require little or no maintenance for up to seven days. Arterial pressure measurements are made using materials which have been sterilized and aseptic techniques should be used in handling of all catheters and catheter materials. Sterile saline should be used for filling up the transducers and catheters. The exterior portion of the catheters should be cleaned with isopropyl alcohol prior to opening the catheter for arterial pressure measurements.

ACKNOWLEDGEMENT

Transonic Systems Inc.® gratefully acknowledges T.L. Smith, Ph.D., Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, in the gracious sharing of this protocol.

Abdominal Aorta Catheterization: Chronic Pressure Measurement

Animal Preparation

Fast animal the night before surgery.

Prepare animal for aseptic surgery using sterile instruments

Surgical Protocol

Abdominal aorta is exposed through a laparotomy and dissected free of vena cava below the left renal artery and vein from approximately 15 mm. The catheter body is taken through the psoas muscles using a trocar from the abdominal cavity to the back of the neck. The loop of the catheter within the abdomen is passed under the peritoneum on the posterior wall. Take care not to damage the ureter. Temporarily occlude the abdominal aorta for < 1 minute and make a small puncture wound about 5 mm below the left renal vein using a bent 25 or 26 gauge needle. Insert the catheter tip through the stab wound into the aorta and release the occluding ligatures (Fig.1). Advance the catheter tip up to the body of the catheter (Fig. 2). Closure is conventional.

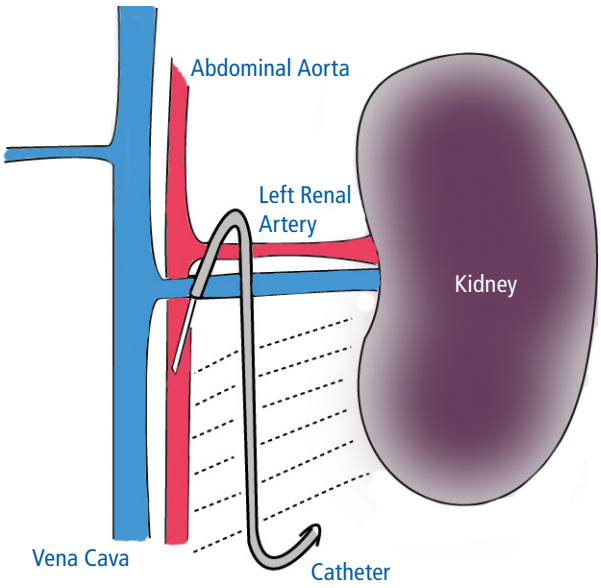


Fig. 1: Insert Catheter

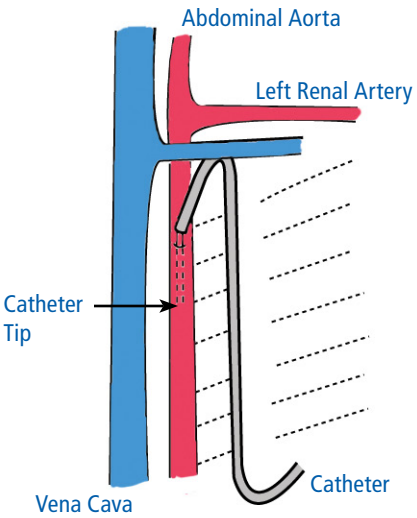


Fig. 2: Advance Catheter

ACKNOWLEDGEMENT

Transonic Systems Inc.® gratefully acknowledges T.L. Smith, Ph.D., Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC, in the gracious sharing of this protocol.

Laser Doppler Tissue Perfusion Theory of Operation

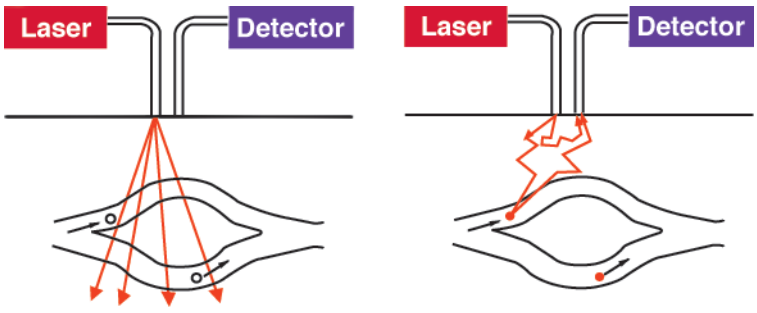
LASER DOPPLER THEORY

A low intensity beam of monochromatic light, emitted from a laser diode inside a BLF Monitor, travels via the Probe's fiber optic light guide through the Probe head to illuminate the tissue under study. There the laser beam is scattered by reflective components within the tissue. A portion of the light is reflected back, via the Probe's receiving fiber optic light guide, onto a photo detector inside the Monitor. Generally, this received light has been reflected many times by stationary structures within the tissue as well as by one or more moving particles (mainly red blood cells) within the tissue. Through the Doppler effect these moving particles change some of the received light signal's frequency.

The received signal spectrum is processed in the Monitor in accordance with algorithms derived by Dr. R.F. Bonner[1] for this type of reflective environment to calculate flow, velocity and mass of the flowing red blood cells within the tissue area sampled by the Laser Doppler Probe (velocity and mass are only available as output signals on BLF22). While the actual volume of tissue sampled by the BLF varies with the optical properties of the tissue, it is approximately one cubic mm.

TISSUE PERFUSION FLOW, VELOCITY & MASS

Dr. Bonner's theory presents a framework to calculate tissue perfusion parameters in absolute units: tissue perfusion flow in units of milliliters per minute per hundred grams of tissue (ml x min^{-1} x 100 g^{-1} of tissue), mass of the flowing red blood cells (in grams per 100 g of tissue) and their average flow velocity in m/sec. In practice this is somewhat problematic. The most significant reason is that the actual volume of tissue sampled is unknown. The tissue volume in the calculations is assumed to be 1 mm^3, but it may vary widely with differing optical properties of the tissue. The flow output is proportional to absolute flow in the tissue sampled, but since the quantity of tissue sampled maybe different from one spot to another and one patient or subject to another, the "constant" of proportionality differs for each placement of a Probe. Units, therefore, are reported as TPUs (tissue perfusion units) which are proportional to the absolute units (ml x min^{-1} x 100 g^{-1} of tissue).



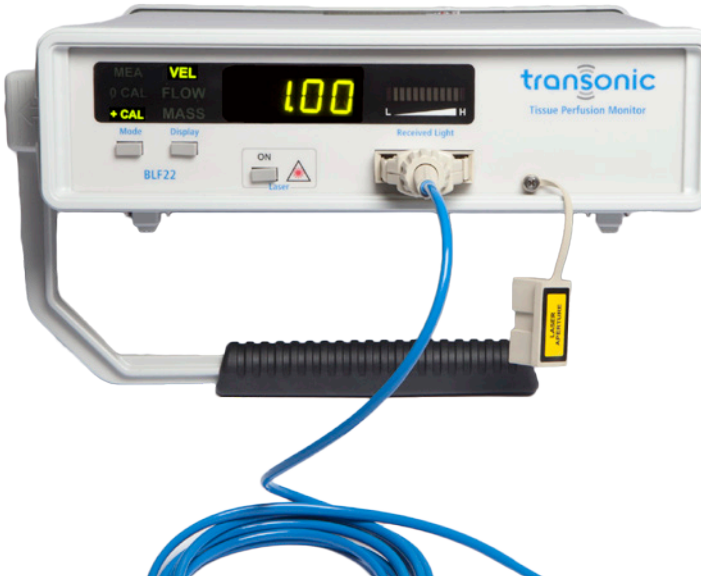
Laser Doppler emits light at a given frequency and then detects the frequency of the reflected light. Light that is reflected by moving particles, such as blood cells, experiences a frequency or Doppler shift which is proportional to the motion of the particle. That shift is used to determine the flow, velocity and mass of the blood.

[1]Bonner, RF, Clem, TR, Bowen, PD, Bowman, RL, "Laser-Doppler Continuous Real-Time Monitor of Pulsatile and Mean Blood Flow in Tissue Microcirculation, Scattering Techniques Applied to Supra-Molecular and Nonequilibrium Systems" 1981. Chen, SH, Chu, B, Nossal, R, eds. New York: Plenum, pp 685-702.

BLF22 Tissue Perfusion Monitor & Laser Doppler Probes

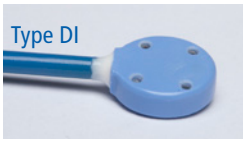
MEASURE CONTINUOUS MICROVASCULAR PERFUSION, MASS & VELOCITY

- Laser Doppler technology measures blood perfusion in 1 mm³ of tissue.
- Sensitive monitoring of small tissue perfusion changes during an experimental course.
- The recorded waveform shows heart beat synchronous microperfusion pulsatility.
- Monitor effects of exercise, drug treatments, ischemia/reperfusion, manipulations on target microvascular beds.



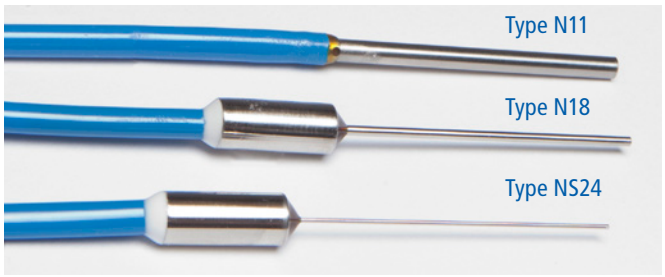
SURFACE PROBES

- Type R: Right Angle (ABLPHR) head: epoxy, diameter: 15 mm, height: 7 mm, suture holes
- Type DI: Disk (ABLPHDI) head: epoxy, diameter: 12 mm, height: 3 mm, suture holes
- Type I: Implantable (ABLPHI) head: epoxy with glass window lengths: (approximate) 14 mm, width: 6 mm, height: 3 mm
- Type S: Straight (ABLPHS): titanium tip, diameter: 6 mm, length: 6 mm



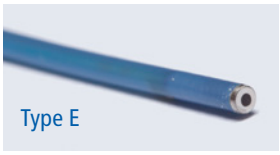
NEEDLE PROBES

- Type N: Stainless steel shaft; standard length: 40 mm, shorter or longer lengths available on request
  - 11 gauge needle: diameter: 3 mm (ABLPHN11)
  - 18 gauge needle: diameter: 1.2 mm (ABLPHN18)
  - 20 gauge needle: diameter: 0.812 mm (ABLPHN20)
- Type NS: 24 gauge needle (ABLPHNS24) diameter: 0.58 mm, length: 40 mm



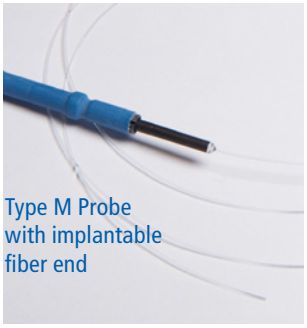
ENDOSCOPIC PROBE

- Type E: Teflon coated cable with 1 mm titanium disc at tip of endoscopy segment (ABLPHE): length: 2 m, diameter: 1.8 mm; flexible cable length: 2 m; total length: 4 m



MONOFIBER PROBE

- Type M: Two fiber cables (ABLPLM) with implantable single fiber ends; 0.5 mm diameter (AFAL1026).



CUSTOM PROBES

- MRI/NMR: Type I, R and S probes are non-magnetic. Type N can be made of brass with a 1 mm diameter. Probes can be as long as needed to isolate the instrument from the magnet.
- Curved Needles: A variety of shapes and lengths can be fabricated. Limited to 18 gauge needle.

transonic
THE MEASURE OF BETTER RESULTS.

Laser Doppler Probe Care & Laser Safety

The Probes may be sterilized by low temperature (≤ 65°C) ethylene oxide, STERRAD® or may be disinfected with 70% isopropyl alcohol.

DO NOT STEAM STERILIZE!

The Probe cable contains very fine glass fibers and must be handled with care.

- Do not disconnect the Probe from the Monitor by pulling on the cable.
- Do not bend the Probe cable to a radius less than 10 mm.

After use, wash the Probe tip and cable thoroughly in warm, soapy water. Debris on the ends of the optical fibers can adversely affect readings. Remove any visible foreign material with a soft-bristled brush or cloth. The FDDI connector should not be immersed in water but may be surface washed as needed with alcohol wipes (70% isopropyl). Use a lint free swab on the ends of the optical fibers (the two white ceramic ferrules within the FDDI connector) to remove any accumulated matter. The Probe head and cable may be rinsed and wiped in 70% isopropyl alcohol before sterilization.

When not in use:

- Cover the Monitor's Probe port with the attached cap
- Cover the Probe connector with the attached cap

MONITOR CARE

While the BLF21 & BLF22 are largely maintenance free, the Probe connections are sensitive to lint, dirt or other debris which can block the light path. Prior to each monitoring session, clean each of the two optical ports within the Monitor's Probe connector. Use the silk swab that is supplied with each Probe. Do not use cotton swabs or other lint producing cleaning materials. Moisten the swab with 70% isopropyl alcohol, insert into each port and gently rotate. The swab can be cleaned in 70% isopropyl alcohol and saved for future use.

TROUBLESHOOTING LOW LIGHT

If the instrument should report zero flow and show too low received light (Red "L"), the Probe may have come loose from the tissue, it may be improperly connected to the Monitor, it may be dirty or one or both fibers may be broken. Check for good connections to the tissue and Monitor first. Next, clean the Probe tip as described above. If received light is still too low, clean the Monitor's Probe ports and the Probe's connector. If received light is still too low, try a spare Probe and contact your Transonic® representative for assistance.

LOW POWER OUTPUT ASSURES INTRINSIC BLF SAFETY

The intrinsic safety of Transonic® BLF21 and BLF22 Tissue Perfusion Monitors originates from their low power output (less than 3 mW), and the divergence of their beam as it leaves the laser output connector and the Probe tip. This divergence exceeds the focusing capability of the human lens. Probes may be moved from one site to another without turning off the laser's power. Even though, at continuous intraocular exposure, the Monitors emit less than the FDA safety limits for Class 3R devices (IEC60825-1-2007), common sense dictates that the Probe should always be directed away from the eyes. The laser light power that would reach the retina is comparable to the light of a 160 W incandescent bulb at the same Probe-to-eye and lamp-to-eye distance. However, the eye's retina is much less sensitive to infrared than to visible light.

DO NOT BEAM LASER LIGHT DIRECTLY INTO THE EYE!

transonic
THE MEASURE OF BETTER RESULTS.

Considerations for Measurements by Laser Doppler

MOTION

In order to quantify the flow of blood, care must be taken to eliminate other sources of motion. Doppler devices measure relative motion. A BLF Monitor measures the shift in frequency between the light it emits and that which it receives. This shift is imparted when the light strikes an object which is moving, relative to the Probe head. The shift is described by the Doppler effect. Ideally, wavelength shifts are imparted only by moving blood cells; however, motion between the tissue and Probe head, within the tissue under the Probe head; or bending motion of the Probe's fiber optic cable can also produce a signal (frequency shift). This is an artifact rather than blood flow. Of these motion artifacts, by far the most important is Probe-to-tissue motion. Avoid this type of motion through careful selection of a Probe holder. If the tissue to be studied has moving filaments crossing the path of the laser light (ex. heart tissue), a significant artifact signal can result. Experiments must be performed to determine if the Monitor's signal is meaningful in cases where tissue motion is significant. Finally, Laser Doppler Probes are not designed to allow for large fiber bending motion artifacts. However small, this artifact signal is worth eliminating, so fixing the Probe cable in several points is appropriate.

APPLICATION PRESSURE

Since the laser Doppler is measuring a relatively shallow portion of tissue (about 1 mm deep), a very light application must be used so as not to occlude the small underlying vessels. In order to determine the correct pressure of application, it is best to apply the Probe and take a reading. Then move the Probe back from the tissue slightly and take a second reading after 15 seconds. If the second reading is higher, the first application was likely done with too much pressure. Additionally, it is best to use a chart recorder or computer interface to display the waveform. This very readily shows the difference between tissue occluded by pressure and non-occluded tissue. Additionally, pressure applied to tissue proximal or distal from the Probe can influence the perfusion and, therefore, the instrument readings.

AMBIENT TEMPERATURE

Room temperature has a significant effect on peripheral blood flow. In some studies, the maximal flow in tissue is important (this may be measured by warming the tissue to 42°C). In other studies, the blood flow at room temperature may be most important. In either case, careful control of the temperature is essential. Time should be allocated to allow subjects coming in from other temperature levels to acclimate before measurements are taken.

POWERFUL LIGHTING

Strong light applied to the tissue under study can affect the Monitor. While normal room lighting has minimal effects, strong overhead illumination such as operating room lights or microscope lights can flood the tissue with light in the wavelength of interest causing erroneous readings. Switch off bright lights, if possible, before taking readings or drape the area of the Probe.

RECORDING CONSIDERATIONS

You should decide if you will use hand recording of the data from the front display, or an analog or digital recorder connected to the output(s) on the back panel. The time constant switches on the rear panel control the filters for data going to any external recorder.

TO STERILIZE

Prior to using the Probes intraoperatively, they must be sterilized using cold gas (ethylene oxide at 65°C) or STERRAD®. Do not autoclave Laser Doppler Probes. Following use, any blood or tissue adhering to the Probe may be removed in a warm, soapy water bath (do not dip the connectors). See, Sterilization & Care for Transonic® Laser Doppler Probes (LD-115-tn) for more details.

WHEN NOT TO STERILIZE

When using the Probe for cutaneous measurements, an alcohol wipe may be sufficient disinfection between subjects. When making readings on multiple subjects, a disposable clear plastic Probe covering is recommended. For needle type Probes, use an oral thermometer sheath; for larger Probes other types of plastic sleeves will be useful. Always conform to appropriate health and safety regulations regarding Probe cleaning and sterilization.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Local Cerebral Perfusion Measurement

APPLICATION BASICS

Site:	Cerebral cortex
Species:	Mouse (CFW)
Body Weight:	30 grams
Duration:	Acute
PROBE TYPE	N: 18 gauge needle

TYPE N (18 gauge needle probe: ABLPHN18)

Diameter: 1.2 mm

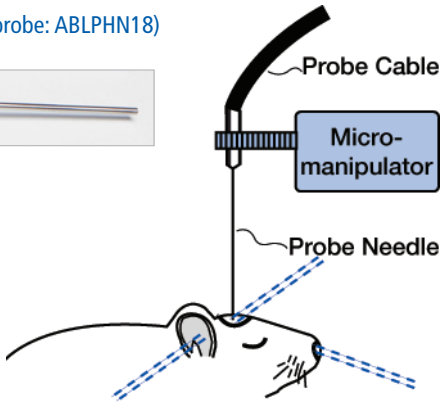


Fig. 1: Type N probe shown clamped in position for perfusion measurements in the mouse forebrain. The mouse head is stabilized in a sterotaxic device.

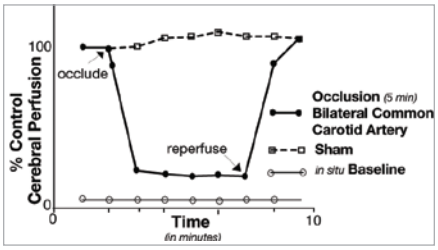


Fig. 2: Bilateral occlusion of the common carotid artery, followed by reperfusion

Application

Effects of transient forebrain ischemia. Effects of various drugs on forebrain perfusion

Surgical Protocol

1. Induce anesthesia in the mouse using chloral hydrate (100 mg/100g, IP).
2. Make a ventral incision in the neck for tracheostomy (1.27 mm OD). For bilateral occlusion of the common carotid artery, place snares (5-0 silk) around each carotid artery.
3. Place the mouse on a heating pad to maintain rectal temperature at 37°C. Secure the head in a stereotaxic apparatus.
4. Make a midline incision and reflect the scalp. Prepare a small circular craniotomy in the parietal bone taking care not to disrupt the dura.
5. Using a stereotaxic micromanipulator, position the Laser Doppler Needle Probe on the dural surface of the parietal cortex. Avoid areas directly over large blood vessels. The Probe must be mounted so that it touches the dura, without applying pressure, as this would occlude the vessels and reduce perfusion in the area of interest. This is accomplished by seeing that neither the dura nor cortex are visibly indented by the Probe. If there is any question of too much pressure, observe the perfusion readout on the Tissue Perfusion Monitor. Then withdraw the Probe slightly and observe the perfusion readout again. A sustained increase indicates the previous pressure was too great.
6. After data recording has begun, tighten the snares to occlude the carotid arteries.

Perfusion Ranges Observed Tissue Perfusion Units (TPU)

NORMAL PERFUSION	COMMON CAROTID OCCLUSION	
	Unilateral	Bilateral
20 TPU	10 TPU	3 TPU

ACKNOWLEDGEMENT

Protocol and data courtesy of Robert N. Willette, Ph.D., SmithKline Beecham Pharmaceuticals, Division of Pharmacology, King of Prussia, PA.

REFERENCES

Willette RN, Clark RK, Lee EV, Barone FC, "Transient Forebrain Ischemia in the CFW Mouse," Proceedings of the 1992 Federation of the Am Soc Exper Biol1992; 13: Abst. 500.

Barone, FC et al, "Murine Models of Cerebral Ischemia, in Central Nervous System," Trauma, ed by Ohnishi ST, Ohnishi T, CRC Press, New York, pp. 147-167, 1995.

transonic
THE MEASURE OF BETTER RESULTS.

Mouse Local Cerebral Perfusion (MCA Occlusion Model)

APPLICATION BASICS

Site:	Cerebral cortex
Species:	Mouse (C57Bl/6)
Body Weight:	22 - 30 grams
Duration:	Acute
PROBE TYPE	M: monofiber EZ Fine or JF (OmegaWave, Japan)

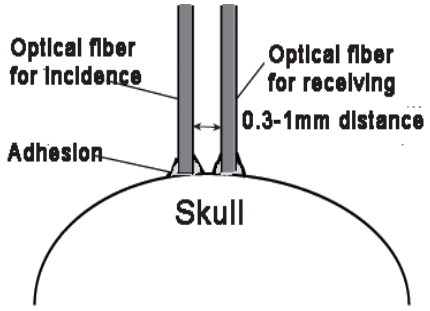


Fig. 1: Type EZ Fine Laser Doppler Probe (OmegaWave, Inc.) affixed with superglue to the skull of a mouse.

Middle Cerebral Artery Occlusion Model

Transgenic manipulations make the mouse an ideal model for stroke. Experimental data is easily compared with control data in dose response studies where researchers can assess the effects of various pharmacological agents and dosages on cerebral blood flow.

ACKNOWLEDGEMENT

Protocol courtesy of S. Kashima, OmegaWave, Inc.

Laser Doppler technology is used to assess cerebral perfusion during middle cerebral artery occlusion by confirming a sudden drop in cerebral blood flow, and subsequent reperfusion of the ischemic area. Laser Doppler Monitors give high resolution perfusion measurement in approximately 1 mm^3 of tissue at a depth of 1 mm just below the surface of the Probe. Therefore, when using Laser Doppler Monitors for ischemia/reperfusion or dose response studies, it is critical that the measurement site is the same throughout the entire measurement sequence. If the site changes during a treatment, reliable data cannot be obtained and compared.

This becomes a challenge in Middle Cerebral Artery (MCA) occlusion studies where the animal must be positioned on its back with the neck extended for insertion and advancement of the occluding filament into the carotid artery to produce the stroke. The fiber cannot be easily advanced into the internal carotid via standard protocols with the mouse fixed in an upright position; however, turning the animal over can displace the position of the Laser Probe. This is resolved in the mouse with a flexible fiber Probe.

Because the skull of the mouse is very thin, the laser light will penetrate through the skull layer without effect. Therefore, cerebral tissue perfusion can be measured over the skull without performing a craniotomy which also requires that the animal be in an upright position as described in a previous protocol (LD-105-sp). A Laser Doppler Probe using bare fiber (Type EZ Fine or Type JF, Omegawave, Inc., Japan), can be affixed to the skull using super glue, thus ensuring that the measurement site will not change during the experiment. The mouse can be repositioned on its back to advance the filament in the carotid after gluing the Laser Doppler Probe fibers in place.

The EZ Fine Probe uses an acrylic resin coated silica glass fiber that is 0.25 mm diameter. It can be cut with a special cutting tool set, the glued portion removed after each experiment and the remainder of the fiber reused. Alternatively, a Monofiber Probe (Type M, Transonic Systems Inc.®) may be used similarly, though the received light signal is stronger from a two fiber Probe.

Mouse Local Cerebral Perfusion (MCA Occlusion Model) Cont.

Surgical Procedure

- Anesthetize the animal as required.
- With the animal in the prone position, expose the skull by a midline scalp incision.
- Position the two optical fibers (Incidence fiber and Receiving fiber) on the skull separated 0.3 mm – 1 mm apart. Glue in place approximately 2 mm posterior to the bregma.
- Turn the animal to a supine position and expose the carotid complex through a ventral midline incision. Ligate the common carotid artery and the external carotid artery and its branches with 5-0 silk suture.
- Make a transverse incision in the artery and introduce the nylon filament into the external carotid artery. Gently advance it into the internal carotid until there is a slight resistance (approximately 10 mm) indicating the origin of the middle cerebral artery at the Circle of Willis.
- Observe a sudden drop in cerebral blood flow recorded by the laser Doppler confirming occlusion of the MCA.
- Occlusion may be observed for 1 to several hours.
- For reperfusion, withdraw the filament and remove the sutures on the common carotid artery and internal carotid artery, keeping the external carotid permanently occluded.

REFERENCES

Masuki S et al, Increased cerebral activity suppresses baroreflex control of heart rate in freely moving mice. J Physiol 2009; 587(Pt 23):5783-94. (L7927)

Iwai M et al, Possible inhibition of focal cerebral ischemia by angiotensin II type 2 receptor stimulation. Circulation 2004; 110(7):843-8. (L7928A)

Murozono M et al, Neuroprotective and neurotoxic effects of cyclosporine A on transient focal ischemia in mdr1a knockout mice. Eur J Pharmacol 2004; 498(1-3):115-8. (L7929A)

Namura S et al, Ebselen reduces cytochrome c release from mitochondria and subsequent DNA fragmentation after transient focal cerebral ischemia in mice. Stroke 2001; 32(8):1906-11. (L7930A)

Tabrizi P, Wang L, Seeds N, McComb JG, Yamada S, Griffin JH, Carmeliet P, Weiss MH, Zlokovic BV, "Tissue plasminogen activator (tPA) deficiency exacerbates cerebrovascular fibrin deposition and brain injury in a murine stroke model: studies in tPA-deficient mice and wild-type mice on a matched genetic background," Arterioscler Thromb Vasc Biol 1999; 19(11): 2801-6.

Lavine SD, Hofman FM, Zlokovic BV, "Circulating antibody against tumor necrosis factor-alpha protects rat brain from reperfusion injury," J Cereb Blood Flow Metab 1998;18(1): 52-8.

Guo Q, Wang G, Namura S, "Fenofibrate improves cerebral blood flow after middle cerebral artery occlusion in mice," J Cereb Blood Flow Metab 2010; 30(1): 70-78.

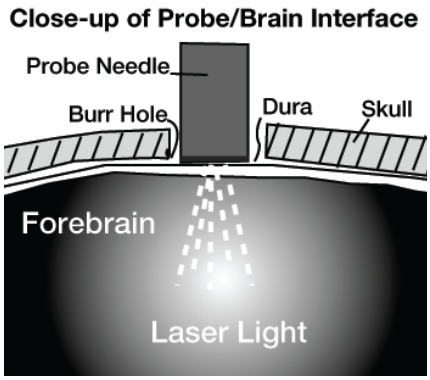
Rat Local Cerebral Perfusion Measurement

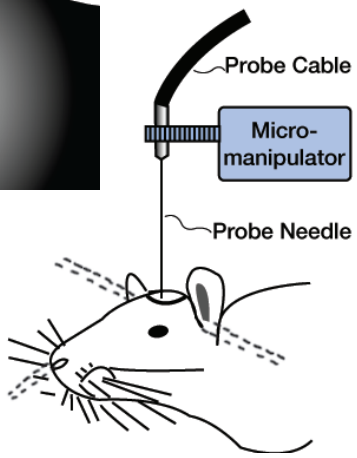
APPLICATION BASICS

Site:	Cerebral cortex
Species:	Rat (Spraque-Dawley)
Body Weight:	370 - 390 grams
Duration:	Acute
PROBE TYPE	N: 18 gauge needle

Application

To study the effects of transient forebrain ischemia and to study the effects of various drugs on forebrain perfusion.





Type N Probe clamped into position for rat forebrain perfusion measurements such that there is no visible dent in the dura.

Surgical Approach

1. Induce anesthesia in the rat using 2.5% isoflurane in 40% 0_2, 60% N_2. Maintain anesthesia by slowly administering pentobarbital (40mg/kg i.v.) over ten minutes followed by pentobarbital (10 mg/kg i.v.) every 30 minutes. Stabilize the position of the animal in a stereotaxic device. Prevent hypothermia with a heating pad or water bottles.
2. Prepare the site for perfusion monitoring at a point 8 mm lateral and 2 mm anterior to the bregma (where coronal and sagittal sinuses join).
3. Make incisions in the scalp exposing enough of the skull to bore a 2.5 mm hole. Use a slow 2.5 mm trephine with a gentle saline drip (0.9%, 25°C) over the drill to prevent thermal injury. Stop drilling when a very thin bone layer remains in order to avoid physical damage to the cortex.
4. Carefully remove the remaining bone with forceps taking care not to disrupt the dura.
5. Using a micromanipulator, clamp the probe in position over the burr hole so as to minimize movement artifact. Avoid area directly over large blood vessels. The probe must be mounted so that it touches the dura, without applying pressure, as this would occlude the vessels and reduce perfusion in the area of interest. This is accomplished by seeing that neither the dura nor cortex are visibly indented by the probe. If there is any question of too much pressure, observe the perfusion readout on the laser Doppler monitor; then withdraw the probe slightly and observe the perfusion readout again. A sustained increase indicates the previous pressure was too great.

PERFUSION RANGES OBSERVED

Normal Perfusion: 20 TPU (tissue perfusion units)

Common carotid artery occlusion plus hypotension: 2 TPU

Type N (18 gauge needle: ABLPHN18)

Diameter: 1.2 mm

ACKNOWLEDGEMENT

Protocol and data courtesy of Robert N. Willette, PhD, SmithKline Beecham Pharmaceuticals, Division of Pharmacology, King of Prussia, PA

REFERENCE

Willette RN, Sauermelch C, Ezekiel M, Feuerstein G, Ohlstein EH, "Effect of Endothelin on Cortical Microvascular Perfusion in Rats," Stroke 1990; 21(3) 451-458.

transonic
THE MEASURE OF BETTER RESULTS.

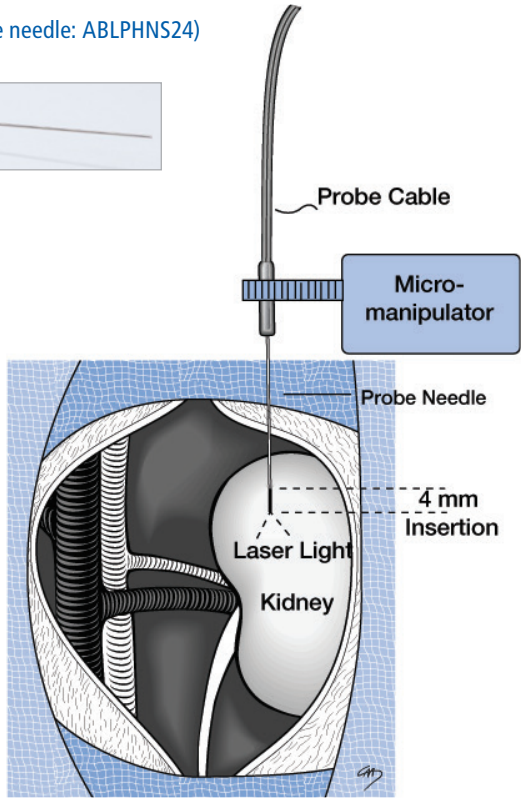
Rat Renal Medulla Acute Perfusion Measurement

APPLICATION BASICS

Site:	Kidney: outer medulla
Species:	Rat
Body Weight:	330 - 500 grams
Duration:	Acute
PROBE TYPE	NS: 24 gauge needle (0.15 mm fiber spacing)

Type NS (24 gauge needle: ABLPHNS24)

Diameter: 0.58 mm
Length: 40 mm



Application

Studies on the effect of various drugs on regional renal blood flow. In this experiment L-N^G-mon-methyl-arginine (LNMMA, an inhibitor of nitric oxide formation) was infused (40 mg/kg) over 3 - 4 minutes, intravenously. The reported systemic vasoconstrictor effect was reflected in a blood pressure increase from 110 to 122 mmHg. The Laser-Doppler trace (Fig. 1) shows a 20% decrease from baseline flow, to around 7.0 TPU. The recording was made using the Laser Doppler's time constant switch set a 3 seconds (averaging). Interestingly, the cyclic oscillations become more prominent after LNMMA infusion.

Surgical Protocol

Anesthetize the rat with Inactin (100 mg/kg). Place in dorsal recumbency and make a ventral midline abdominal skin incision. Extend the abdominal incision through the linea alba into the abdominal cavity. Deflect the intestines to the right to expose the left kidney. Mechanically fix the kidney as for a micropuncture experiment. The kidney is decapsulated to reduce tissue compression during penetration. Using a micromanipulator, insert the Laser-Doppler Probe perpendicularly into the kidney to a depth of 4 mm (i.e., the outer medulla). Maintain the kidney temperature at 37°C with a heat lamp and warm saline/mineral oil drip. Catheterize the femoral artery and vein with PE-50 catheters for infusion of normal saline with bovine serum albumin (4.5g/dl) at a rate of 0.08 ml/min and for monitoring of blood pressure with a pressure transducer.

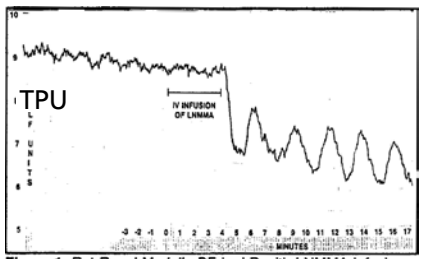


Fig. 1: Rat Renal Medulla Perfusion with LNMMA infusion

Perfusion Ranges Observed

Initial flow readings were on the order of 8.5 - 9.5 tissue perfusion units (TPU). The flow is stable, except for cyclic oscillations at 1 - 2 minute intervals.

ACKNOWLEDGEMENT

Protocol and data courtesy of: Mayer Brezis, MD Associate Professor of Internal Medicine & Nephrology, Hadassah-Hebrew University, Medical Centers-Jerusalem, Israel.

REFERENCE

Brezis M, Heman SN, Dinour D, Epstein FH, Rosen S, "Role of Nitric Oxide in Renal Medullary Oxygenation," J Clin Investig 1991: 88: 390-395.

transonic
THE MEASURE OF BETTER RESULTS.

Rat Renal Cortex & Medulla Acute Perfusion Measurement

APPLICATION BASICS

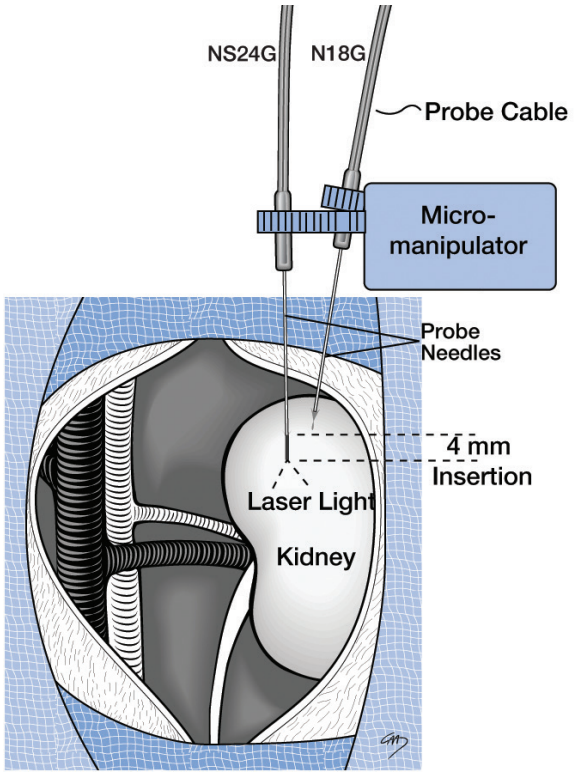
Site:	Kidney: cortex & medulla
Species:	Rat (Sprague-Dawley)
Body Weight:	320 - 400 grams
Duration:	Acute
PROBE TYPE	N: 18 gauge needle NS: 24 gauge needle

Type N
(18 gauge needle: ABLPHN18)

Diameter: 1.2 mm

Type NS
(24 gauge needle: ABLPHNS24)

Diameter: 0.58 mm
Length: 40 mm



Application

This study demonstrates the importance of studying renal microcirculation in the functional recovery of the kidney from post ischemic acute renal failure.

Surgical Approach

Rats were anesthetized with sodium pentobarbital administered intraperitoneally (50mg/kg). The kidneys were accessed through an abdominal midline incision and renal ischemia was produced by occlusion with smooth vascular clamps of both right and left renal arteries for 60 minutes.

Twenty-four hours later, the rats were again anesthetized with intraperitoneal sodium pentobarbital. The left kidney was exposed through a midline incision, decapsulated and immobilized. The exposed kidney was maintained at 37°C by bathing it in mineral oil warmed by a heating lamp. Regional blood flow was measured by a Tissue Perfusion Monitor.

Two Laser Doppler Probes were mounted on micromanipulators. Cortical flow was measured by resting the Probe on the kidney surface, and medullary flow was measured by inserting the Probe 3.0 to 4.5 mm into the renal parenchyma with placement guided by a separate needle puncture.

After one hour equilibration, animals were infused with zaprinast (0.03 or 0.30 mg/kg/min). Blood flow was measured every 30 minutes during four hours of drug infusion. Animals receiving zaprinast experienced a significant increase in their renal function. Following sacrifice, the left kidney was dissected and visually inspected to verify Probe placement in the medullary tissue.

Perfusion Ranges Observed

Both high dose and low dose zaprinast increased cortical blood flow in the post-ischemic kidney by approximately 17% compared to control. At four hours of drug infusion, low dose and high dose zaprinast increased medullary blood flow by 60% and 40%, respectively, compared to control.

KIDNEY	PRE-ISCHEMIA (TPU)	24-HOUR POST-ISCHEMIC INSULT (TPU)
CORTEX	39.0	5.6
MEDULLA	35.4	11.8

ACKNOWLEDGEMENT

Protocol and data courtesy of Steven B. Miller, Ph.D., Washington University School of Medicine, Department of Medicine, Molecular Biology and Pharmacology, St. Louis, MO.

REFERENCES

Guan Z, Miller SB, Greenwald JE, "Zaprinast Accelerates Recovery from Established Acute Renal Failure in the Rat," Kid Int 1995; 47: 1569-75.

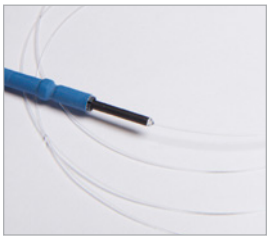
transonic
THE MEASURE OF BETTER RESULTS.

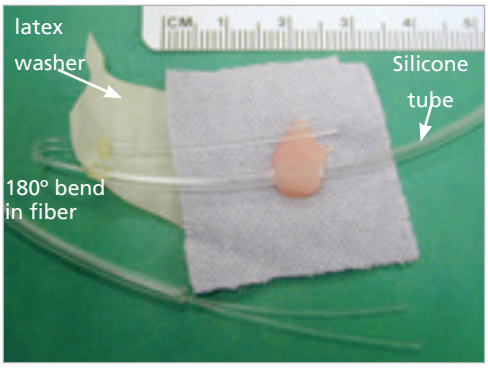
Rat Renal Cortex & Medulla Chronic Perfusion Measurement

APPLICATION BASICS

Site:	Kidney: cortex & medulla
Species:	Rat (Sprague-Dawley)
Body Weight:	340 - 420 grams
Duration:	Chronic
PROBE TYPE	M: monofiber

Type M (ABLPHM) with implantable single fiber ends (AFAL1026).





Two Fiber Implantable Assembly:
Two monofibers prepared for renal implantation: the fibers have a 180 degree bend with a latex washer to affix them at the proper depth in the kidney. They are sheathed in silastic tubing. A Dacron fiber patch is epoxied to the assembly to stabilize the position of the unit under the skin before exiting the two-fiber unit at the midscapular region and threading it through the spring tether.

Application

This methodology established a way for the kidney to be chronically implanted with optical fibers, thus providing an innovative tool for monitoring regional blood flow. This capability enables detection of either increases or decreases in rMBF and rCBF, independently and continuously, over periods of at least 11 days (6-16 post-surgical). This technique will make significant contributions in hypertension research.

Surgical Approach

Rats were anesthetized with a mixture of acepromazine (5mg/kg im) and ketamine (50 mg/kg im) and were maintained with 1.0% halothane. A flank incision was used to expose the left kidney and facilitate the placement of two Laser Doppler Probes. A 26 gauge needle was inserted to the desired depth in the kidney allowing insertion of the 0.5 mm plastic fiber to a depth of 2 mm for superficial regional cortical (rCBF) blood flow and 5 mm for regional medullary blood flow (rMBF).

Beforehand, a latex washer (surgical glove material) is epoxied around the optical fiber at the predetermined distance from the tip, thus insuring its precise placement in the cortex or medulla. The latex washer is also used to fix the fiber in place with cyanoacrylate glue. The Probe fiber was then sheathed in silastic tubing. The tubing was attached to muscles near the kidney and, after tunneling subcutaneously, to the rat's neck, using Dacron mesh. Finally, the fibers in the tubing were exteriorized through an incision at the midscapular region and were led through a protective spring to the top of the animal's cage.

A six-day recovery period allowed healing of the renal tissue before beginning the hypertension study. It was necessary to minimize movement so that the laser signal could measure small changes. To do this, the rats were restrained in cages. Both cortical and medullary measurements were stable and reproducible between days 6 and 16.

Perfusion Ranges Observed Example taken from Day 10

PROBE SITE	N	MEAN BF VALUE (ANALOG OUTPUT)		
		CONTROL (VOLTS)	**ANGIOTENSIN II** (VOLTS)	**RECOVERY** (VOLTS)
CORTEX	9	1.20	0.58	1.12
MEDULLA	8	1.04	0.64	1.03

In a typical study, rats are given 12.5ng bolus IV injection of Angiotensin II. Following this injection, the rCBF shows a sharp drop (by 50 - 75% of the baseline value) while rMBF drops by 40 - 50%.

ACKNOWLEDGEMENT

Protocol and data courtesy of Allen B. Cowley, Jr., Ph.D., Physiology, Medical College of Wisconsin, Milwaukee, WI.

REFERENCES

Lu S, Mattson DL, Roman RJ, Becker, CVG, Cowley Jr AW, "Assessment of Changes in Intrarenal Blood Flow in Conscious Rats Using Laser-Doppler Flowmetry," AJP 1993; 264: F956-F962.

Rat Gastric Mucosal Perfusion Measurement

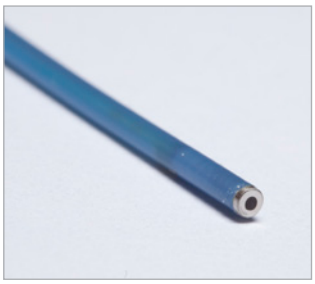
APPLICATION BASICS

Site:	Gastic corpus mucosa
Species:	Rat (Wistar Body)
Body Weight:	270 - 300 grams
Duration:	Acute
PROBE TYPE	E: endoscopic N: 11 gauge needle

Type N (ABLPHN11)

Length: 40 mm

TYPE E (ABLPHE)



Diameter: 1.8 mm
Head: Teflon coated cable with 1 mm titanium disc at tip of endoscopy segment;
Length: 2 meters
Cable: flexible; 2 meters
Total length: 4 meters

Application

Includes gastric damage studies from infusion of various agents or from hypotension/reperfusion.

Surgical Approach

1. Withhold food from the rat for 24 hours prior to the study. Anesthetize with sodium pentobarbital (50/mg/kg, I.P.).
2. Perform a tracheotomy and insert a polyethylene airway tube.
3. Place the rat on a heating pad and maintain 37 ± 0.5°C rectal temperature.
4. Place a polyethylene tube in the right carotid artery for continuous infusion of saline $0.6 ml x hr^{-1} x 100g$ body $weight^{-1}$.
5. Open the abdomen with a 3 cm long incision.
6. Place a cannula in the lower abdominal cavity and supply additional doses of anesthesia as needed.
7. Dissect connective tissue and displace the viscera in order to access the stomach. Make a small incision (4 mm) in the anterior greater curvature of the forestomach using electric microcautery. Gently lavage the contents with saline at 37°C.
8. Insert the Laser Doppler Probe through this incision, making gentle contact with the corpus mucosa. Take care that the Probe is not pressing on the mucosa so as to occlude the underlying vessels.
9. Cover the abdominal incision with a film wrap to prevent tissue dehydration.
10. Record gastric mucosal blood flow (GMBF) with the Tissue Perfusion Monitor for a minimum of 30 minutes to confirm a steady resting state. After establishing baseline GMBF, begin further experiments.

Probe Selection

Although an endoscopic laser Probe was used in this protocol, a more economical 11 gauge needle Probe may be used in the same procedure. The flat surface tip of the Probe should rest against the corpus mucosa and may be held with a balance arm for greater stabilization without pressure.

transonic
THE MEASURE OF BETTER RESULTS.

Rat Gastric Mucosal Perfusion Measurement Cont.

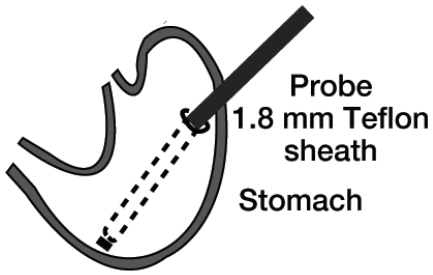


Fig. 1: Probe shown positioned in the rat stomach through a small incision.

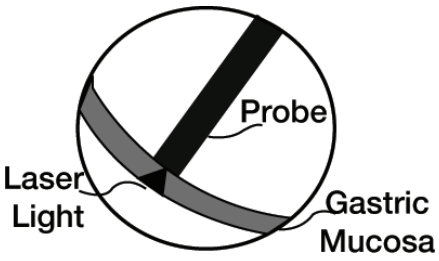


Fig. 2: Magnified cross sectional view of stomach showing the end of the Probe against the gastric mucosa.

Perfusion Ranges Observed

BASELINE	**GMBF = 30 TPU** (Tissue Perfusion Units)
After infusion of Platelet Activating Factor (100ng x kg x min^{-1})	GMBF = 40% of baseline GMBF
WITH HYPOTENSION (20 MIN, BP 20-30 MM HG) & REPERFUSION	
During hypotension	GMBF = 35% of baseline GMBF
After reperfusion	GMBF = 145% of baseline GMBF
60 minutes after reperfusion	GMBF = 65% of baseline GMBF

ACKNOWLEDGEMENT

Protocol and data courtesy of Takashi Yamaguchi, 3rd Dept of Internal Medicine, Kansai Medical University, Osaka 570, Japan.

REFERENCES

Binnaka T, Yamaguchi T, Kubota Y, Hirohara, J, Mizuno J, Sameshima Y, "Gastric Hemodynamic Disturbances Induced by Hemorrhagic Shock in Rats," Scand J of Gastroenterology, 1990; 25: 555-562.

YamaguchiT, "Relationship between Gastric Mucosal Hemodynamic and Gastric Motility," Gastroenterologia Japonica, 1990; 25(3): 299-305.

Rat Sciatic Nerve Acute Perfusion Measurement

APPLICATION BASICS

Site:	Sciatic nerve
Species:	Rat (Sprague-Dawley)
Body Weight:	Study 1 & 2: 240 - 260 grams Study 3: 200 grams
Duration:	Acute
PROBE TYPE	N: 18 gauge needle

Type N (18 gauge needle: ABLPHN18)

Diameter : 1.2 mm

Surgical Approach

The rats were anesthetized with nesdonal and ketamine (Studies 1, 2), or pentobarbital (Study 3) at 60 mg/kg im and were placed in dorsal recumbency with legs extended for access to the inner portion of the leg. The skin was retracted and an incision made into the femoral fascia. At this point, the sciatic nerve was visualized lying along side of the saphenous vein. A micromanipulator was used to position a Laser Doppler Probe perpendicular to and 0.1 to 0.2 mm above the nerve. To do so, it was necessary to dim the room lights and visualize the red illumination of the nerve by the Tissue Perfusion Monitor and Probe.

Study 1: Homogeneity of the nerve was established by recording three readings at one point on the left side and three readings at different sites on the right side nerve.

Study 2: The effect of ischemia was examined. Sciatic nerve perfusion was again measured at a middle site. The nerve was then slightly compressed with a soft plastic Probe held in a micromanipulator. Resulting ischemia was maintained for 60 seconds. After compression release, the hyperemic response reached 183% of baseline.

Study 3: The experiment was repeated with smaller rats and different anesthesia with very similar results. Mean hyperemia maximum was 179% of mean baseline and perfusion returned to baseline 120 to 180 seconds after the release of pressure.

Perfusion Ranges Observed

Study 1: n = 10

POSITION LEFT SIDE	MEAN % BASELINE	POSITION RIGHT SIDE	MEAN % BASELINE
(Baseline)	100	1 cm distal	107*
2	104*	center	109*
3	98*	2 cm prox	100*

*Not significantly different.

Study 2: n = 10

TIMING	PERFUSION TPU	% BASELINE
pre-ischemic	25.9 ± 8.2	100
post-ischemic	47.3 ± 13.0	183

REFERENCES

Desvaux B, Bourdel A, Jardel A, Saumet JL, Trouvé R, "Basic and Post-Ischemic Laser Doppler Flowmetry of the Sapehnous nerve in the rat," Experimental Biol, 1996; Abstract 312.

Personal communication with R. Trouvé.

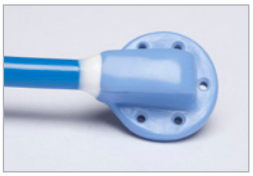
transonic
THE MEASURE OF BETTER RESULTS.

Rat TRAM Flap Acute Perfusion Measurement

APPLICATION BASICS

Site:	Skin over TRAM flap
Species:	Rat (Sprague-Dawley)
Body Weight:	360 - 520 grams
Duration:	Acute
PROBE TYPE	R: right angle

Type R (Right Angle: ABLPHR)



Head: epoxy,
Diameter: 15 mm
Height: 7 mm

Cable: heavy duty

Surgical Approach

Rats were anesthetized with enflurane induction and a mixture of ketamine and acetylpromazine given intramuscularly. The rat's hair was removed with a depilatory agent. A rectangle was marked out on the abdomen from the tip of the xiphoid to just above the pubis, overlying the rat's rectus abdominis muscle. TRAM flaps were then raised. Flaps were bipedicle with both a superior pedicle (supplied by the cranial epigastric artery - a continuation of the internal thoracic artery), and an inferior pedicle (supplied by the caudal epigastric artery - excluding anastomosis to the deep circumflex iliac artery). Only certain ipsilateral rectus abdominis musculocutaneous perforators were left intact to connect the abdominal wall. Contralateral muscle perforators, superficial epigastrics and other collateral vessels were ligated. The linea alba was incised and lifted to observe the blood supply to the flap. The pedicles were individually cross clamped with micro clamps to observe the contribution to the tissue perfusion by each individual pedicle. The flaps were isolated with a Silastic sheet to prevent neo-vascularization and reset into its original position after dividing one of the pedicles. The survival of the flaps were evaluated at 48 hours post surgery.

Perfusion Measurements

The Right Angle Probe was placed in the middle of the flap's quadrant overlying the ipsilateral rectus abdominis muscle and was fixed in place with two-sided adhesive tape. The analog signal was recorded and averaged over five minutes to obtain values. Readings were made prior to incision (initial), following incision with both pedicles perfusing the tissue (baseline), and with each pedicle cross-clamped, in turn.

Application

For studies of the physiology of flaps, the rat provides a safe, inexpensive, but comparable model for some flaps, notably the transverse rectus abdominis musculocutaneous (TRAM) flap. The Tissue Perfusion Monitor provides a useful tool for continuous monitoring of the blood flow to the tissue of the flap in the rat as it does in human flap surgery to identify compromised perfusion post surgical reconstruction.

Perfusion Ranges Observed

N	INITIAL	BASELINE	SUPERIOR PEDICLE	INFERIOR PEDICLE
10	185	100	92	44

Mean TRAM Perfusion as
a % of Baseline Flow

REFERENCE

Hallock GG, Rice DC, "Physiologic Superiority of the Anatomic Dominant Pedicle of the TRAM Flap in a Rat Model," Plastic & Reconstruc Surg 1995; 96(1): 111-8.

transonic
THE MEASURE OF BETTER RESULTS.