

Introduction

The isolated perfused heart system, as originated by Oscar Langendorff more than a century ago, has become a predominant technique in pharmacological and physiological research. The technique allows the examination of cardiac contractile strength (inotropic effects), heart rate (chronotropic effects) and vascular effects without the complications of an intact animal model. From its simple beginning, the technique and equipment has evolved to include both constant pressure and constant flow models in both recirculating and non-recirculating modes. The Radnoti perfused heart system has the capacity to function in any of these configurations, allowing flexibility in experimental research and design.

Basic Cardiac Principles

At the base of the aorta is an ostium (hole) which feeds blood under pressure into the coronary arteries. Langendorff maintained the isolated heart through the use of a reservoir containing a physiological solution that was elevated above the heart to create a pressure head. This reservoir was connected to the heart via a tube to the aortic cannula. When the reservoir was opened, the perfusate was forced through the ostium into the coronary bed. This is often termed as a "retrograde" perfusion, as the perfusate flows directly into the aorta rather than in the normal situation where blood enters the aorta from the left ventricle (Figure 1).

In the Langendorff technique, perfusate solution does not flow via the normal ventricular circulatory pathway. Therefore, this system does not permit the left ventricle to generate pressure-volume work that is representative of typical cardiac function. A modern modification that allows the heart to pump fluid via the normal left ventricular circulatory pathway is the "working" heart model developed by Neely and colleagues. In this preparation, the perfusate enters the heart via the cannulated left atrium, passes through to the left ventricle and is ejected out the aorta (Figure 2).

Selection of the Donor Heart

To maximize the capabilities of the perfused heart system, it is important to decide on the design of the experiment, the donor heart, its maintenance and instrumentation. The selection of the heart is often based on the typical or unique response of the donor organ to pharmacological or physiological stimuli, or on selected metabolic or biochemical events. The most common donors are mice, rats, rabbits, guinea pigs, ferrets, hamsters, frogs and goldfish. Note: In the latter two species there are no defined coronary vessels, merely a porous ventricle and atria. Any of these donor hearts may be accommodated in the Radnoti apparatus by the selection of appropriate cannulae and heart chambers.

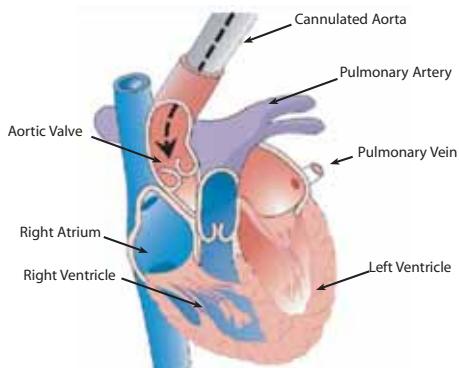


Figure 1. In Langendorff mode the perfusate flows in a retrograde fashion into the aorta and exits via the coronary arteries.

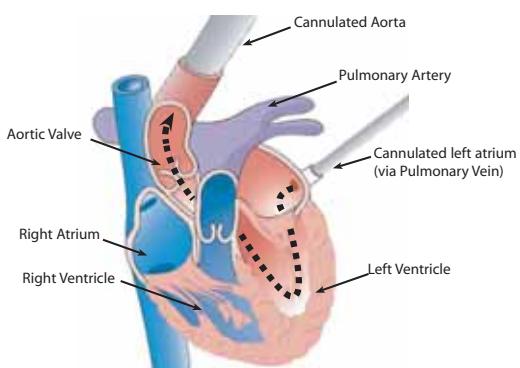


Figure 2. In a Working Heart Preparation the perfusate enters the left atrium and is ejected from the left ventricle into the aorta.

Selection of Perfusate

Once the donor heart is selected, an appropriate perfusate solution is required to provide the isolated heart with nutrients and oxygen. The heart is a metabolically demanding organ and, therefore, the choice of solution is important for the survival of the tissue and successful investigation. All solutions will have to be aerated, as the oxygen consumption of most mammalian hearts is considerable. As the solubility of oxygen in saline solutions is much lower than that of blood, the oxygen tension is normally about 550-600 mmHg at 37 °C to ensure adequate delivery to the cardiac cells. These elevated oxygen tensions are not needed if using whole blood, washed erythrocytes, hemoglobin or fluorinated hydrocarbons as the perfusate solution.

The standard Radnoti system is designed primarily for use with saline solutions that do not contain cellular or proteinaceous (containing protein) components. The optional membrane oxygenator can maintain adequate oxygen levels using erythrocytes whilst protecting the proteins and erythrocytes from damage by the oxygenation process. If using erythrocyte/cellular containing solutions, careful consideration should also be made on the type of peristaltic pump used.

If a system other than whole blood is used, the solution must be buffered, either with the traditional carbonate buffers such as Krebs-Henseleit, Locke's or Tyrode's or with variations of these formulas using HEPES or MES buffers. Due to the lower viscosity of blood-free media, the flow rates used in a Langendorff system are nearly twice as large as with whole blood. Glucose (normally 5-10mM) is necessary and, depending on the experimental design, other substrates can be used such as pyruvate, lactate, fatty acids, amino acids, etc. The ionic components of the solutions are also important and vary with the species; potassium and calcium are the most variable and critical of the ions. Calcium carbonate or calcium carbonate crystals may form in the solution if it is not maintained at the correct pH. The bubbling of the solution with carbogen is not only important in oxygenating the solution but is also important in maintaining the pH. A plasma expander such as dextran, polyvinylpyrrolidone (PVP) or albumin may be used to maintain oncotic pressure (normally about 8-25 mmHg) and prevent tissue edema.

Isolated Heart Preparation Selection

The constant flow system requires the use of a pump to perfuse the heart at a set flow rate. Once the flow is constant, syringe pumps can be used to conveniently titrate the heart with drugs or other agents. The experimental effects on coronary vessel diameter are measured as a change in perfusion pressure. This type of system setup is often used when conducting experiments in which a limitation of substrate or oxygen availability to the cardiac tissue is provided. The coronary vessels can dilate or constrict, i.e. auto-regulate, but the total supply of oxygen and substrate is controlled by the flow rate.

In the constant pressure system, the pressure head is kept constant by adjusting the reservoir level or through the use of a pump and overflow system. These types of experiments will result in changes in flow that can be measured volumetrically, or with fraction collectors, electromagnetic flow probes, drop counters, ultrasonic flowmeters etc. In the constant pressure system, changes in vascular resistance can increase or decrease the supply of oxygen and substrate supplied to the heart.

In the working heart system, both the aorta and the left atrium are cannulated and the atrial pressure (preload) and aortic resistance (afterload) are regulated experimentally as the heart circulates the perfusate solution.

In using any of these systems, the experimenter may choose to have a non-recirculating (single pass) system or a recirculating system. A single pass system is useful when applying several agents in sequence and then allowing their effects to dissipate as the agent is washed out of the heart. This approach is also useful when measuring the uptake or release of various drugs, neurotransmitters or metabolites. A recirculating system is useful when it is necessary to reduce the total volume of perfusate when using expensive drugs or substrates. If recirculation continues for 15-30 minutes or more, denatured proteins released from the heart will accumulate in the perfusate. The Radnoti perfused heart system has removable filters placed in the circuit to remove denatured proteins, thereby preventing blockage of the cardiac vessels.

Cardiac Pacing

Rather than having the heart beat spontaneously, the heart can be artificially paced using suitable electrodes. Pacing is used to maintain a standard contractile response and metabolic demand, while spontaneous beating allows for measurements in changes of heart rate and rhythm that will occur with various drugs or manipulations. To pace a heart, the stimulus rate must exceed the natural cardiac pacemaker rate. Often the sinoatrial node is crushed or the right atrium excised to eliminate contribution of the primary intrinsic pacemaker. Pacing voltage is determined as a set percentage (normally 110-150%) above the voltage required to capture (pace) the heart and should not have to exceed 3-5 V, with a duration of 0.1-1 msec.

Hearts may be paced using electrodes inserted into the cardiac tissue by running Teflon-coated wires into needles, exposing the tips of the wires and bending the wires over the tips of the needles. The needles are then pushed into the heart and withdrawn, leaving the wire embedded in the tissue. Another technique is to attach a single electrode and use a stainless steel aortic cannula as a ground. The wire can be a fine gauge Teflon coated stainless steel or platinum. Various other electrodes have been used, such as suction, wick and sewn-on contacts. Pacing may also be used to induce arrhythmias in attempts to measure changes in fibrillation threshold.

Measurement of Contractile Force

The simplest measurement of contractile force is made using a force transducer attached to the apex of the heart with a pulley in between the heart and the transducer. In this system, a measurable amount of force is lost in a rotational motion as the heart contracts, which can be compensated with a three-point mount. A common technique to measure ventricular contractile force is a saline-filled balloon catheter inserted into the left ventricle which measures iso-volumetric work. Balloons should be slightly larger than the maximum expanded volume of the ventricle to avoid effects of measuring the resistance of the balloon to stretch. Balloons may be made of plastic wrap, condoms or latex cast on models formed from the ventricle. The balloon is secured to a plastic or stainless steel tube that is connected to a pressure transducer. The balloon is inserted through the left atrium or by passing the catheter through the wall of the left ventricle for pressure measurements. In this case, a one-way valve must be placed in the aortic cannula if the intraventricular pressure exceeds the perfusion pressure. In the working heart model, contractile function can be measured by the initial ejection pressure at the aorta and at the same time the ability to pump against an afterload as adjusted via the compliance chamber and/or reach a set ejection pressure with a preload set by adjusting the height of the atrial reservoir. Pressure-volume work is determined by the total volume of fluid ejected by the ventricle over time. In any of these cases, the appropriate amount of resting force or pressure required to maintain the heart on the ascending limb of the Starling curve should be ensured to avoid overstretching the heart muscle.

Other useful functions derived from contractile measurements include the first derivative, dP/dt , a determinant of the rate of change of developed pressure and the integral of pressure as an index of work. Heart rate can be monitored from force measurements or monitored independently with electrodes measuring cardiac electrical activity and a biopotential amplifier.

Other Experimental Options

There are a number of physiological parameters that can be measured in the perfused heart preparation. Cardiac electrical activity can be measured using surface monopolar or bipolar electrodes. Microelectrodes implanted in the surface myocytes can also be used for electrical measurements. Oxygen consumption can be measured with dual oxygen electrodes, one placed in the perfusate stream entering the heart, the other monitoring the effluent leaving the coronary sinus. This effluent can be bypassed through the use of a peristaltic pump and then transferred to the second oxygen electrode. Similarly, ion selective electrodes can be placed in the effluent or perfusate stream or oxygenation chamber of the Radnoti perfused heart apparatus, to measure pH and other cations and anions. Radiolabelled compounds can be used for metabolic studies, the release or uptake of various ions or substrates. Optical studies have been performed on the fluorescence of endogenous or exogenous fluorescent compounds.

Anesthesia and Cardiac Removal

Preparation of the Donor

The anesthetic(s) used will depend on the donor, potential side effects, extent of the surgical procedures and the regulations of local Animal Care, Ethics and Use Committees. The most common anesthetics are barbiturates, such as nembutal or thiopental, ethyl ether and common volatile surgical anesthetics (the latter two which can present potential personnel hazards due to fire or intoxication). Due to the danger of an overdose causing severe or prolonged cardiac impairment or hypoxia, carbon dioxide or euthanasia solutions should not be used. Unless there is an experimental reason, it is recommended that the donor should be heparinized prior to surgery to reduce the formation of emboli/clots in the cardiac vasculature.

Removal of the Heart

The following is a guide to the efficient removal of the heart from the donor. The technique can and should be modified according to experimental conditions, researcher/technical expertise and laboratory conditions. As the heart is a highly metabolically active organ, it requires a constant supply of oxygen and nutrients. Therefore, the time in which it takes to remove and mount the heart is very important. An extended period (> 30 seconds) of reduced oxygen and/or nutrients will significantly affect cardiac tissue (at body temperature), in particular its survival and experimental responses.

After inducing anesthesia, the donor should be placed in a dissecting tray near the isolated heart apparatus. To facilitate fast removal and mounting of the organ, extra sets of sutures and instruments should be positioned close at hand. Cardiac removal may be performed as a surgical procedure by intubating the donor and artificially ventilating the animal. Ventilating the animal provides more time to prepare and remove the heart as the animal and heart will continue to be oxygenated once the thorax has been opened. After exposing the heart by a sternotomy and cutting and retracting the rib cage, two loose ties are placed around the aorta. One tie is used to manipulate the aorta and the other to secure the aorta to the cannula. An extension catheter with perfusate solution is placed on the cannula for ease of preparation. This cannula can be connected to the perfusion apparatus and a slow stream of perfusate is permitted to flow through the aortic cannula, removing any air bubbles. The vena cava is then clamped above the diaphragm and the heart flooded with ice cold perfusate to arrest/reduce its activity. The pulmonary artery is then cut, followed by a cut across the aorta. The cannula is then inserted and secured with the ties. The tip of the aortic cannula should not be inserted below the base of the aorta, as the ostium may be occluded (coronary perfusion restricted) or the aortic valve may be damaged. Once perfusion has commenced, the heart may be removed from the animal and the cannula disconnected from the extension tube and placed in the apparatus.

For the more experienced researcher/technician and when the animal is not artificially ventilated, cardiac removal may also be performed by simply making an incision (median sternotomy) with scissors to open the thoracic cavity. The heart is exposed by cutting the pericardium and removed by cutting across the arch of the aorta and the vena cava. Care should be taken that the aorta is not cut too short as to restrict mounting on the cannula. The heart is then immediately placed in a beaker of chilled, heparinized perfusate to arrest/reduce beating of the heart and then mounted on the aortic cannula as perfusate is flowing from the cannula. Some researchers prefer to use two pairs of tweezers to position the aorta onto the cannula, but care must be taken to avoid puncturing the aorta. The heart can be held on the cannula with a blood vessel clamp such as Dieffenbach serafine, while tying the heart onto the aortic cannula with sutures.

Perfusion of the Heart

Once mounted on the apparatus, the heart should begin to beat strongly within seconds of reperfusion. The pressure of the perfusate, if a constant flow system is used, should be carefully monitored to avoid underperfusion or overperfusion. Perfusion rates are about 3-15ml/min/g heart weight for constant flow systems using Tyrode's, etc. For both constant pressure and constant flow systems, the initial pressure should be about 50-60 mmHg for most mammalian hearts (dependent on the donor, heart rate [pacing], oxygen delivery and work output). Physiologically-normal perfusion pressures of 80-100 mmHg as in blood-perfused hearts are not used in saline-perfused hearts as this leads to tissue edema and potential valve damage. A healthy heart will stabilize rapidly and most experiments can begin within 10-15 minutes after the preparation has been mounted and the various monitoring systems attached. With all conditions optimized, the heart should be experimentally viable for several hours (approximately 3-4 hours), although it is advisable to reduce the experimental time as much as possible. Preparations will suffer edema if uncompensated by a plasma expander concomitant with protein loss from the heart.

In a working heart preparation, the same procedure is used for the removal and mounting of the heart on the apparatus as described above. The left atrium is cannulated after the heart has been perfused through the aorta. To test that the left atrial cannula is secure, the atrial reservoir is opened prior to switching from aortic perfusion. Once secured, the atrial pressure head is adjusted (normally 2-5 mmHg) and the perfusate switched from the aorta to the atria. Aortic pressure development can be monitored via a pressure transducer connected to a T-connection from the aortic cannula (*). Aortic compliance is determined by adjusting the amount of air in the compliance chamber. Afterload is also adjusted by the height of the outflow of the aortic cannula (60-70 mmHg).

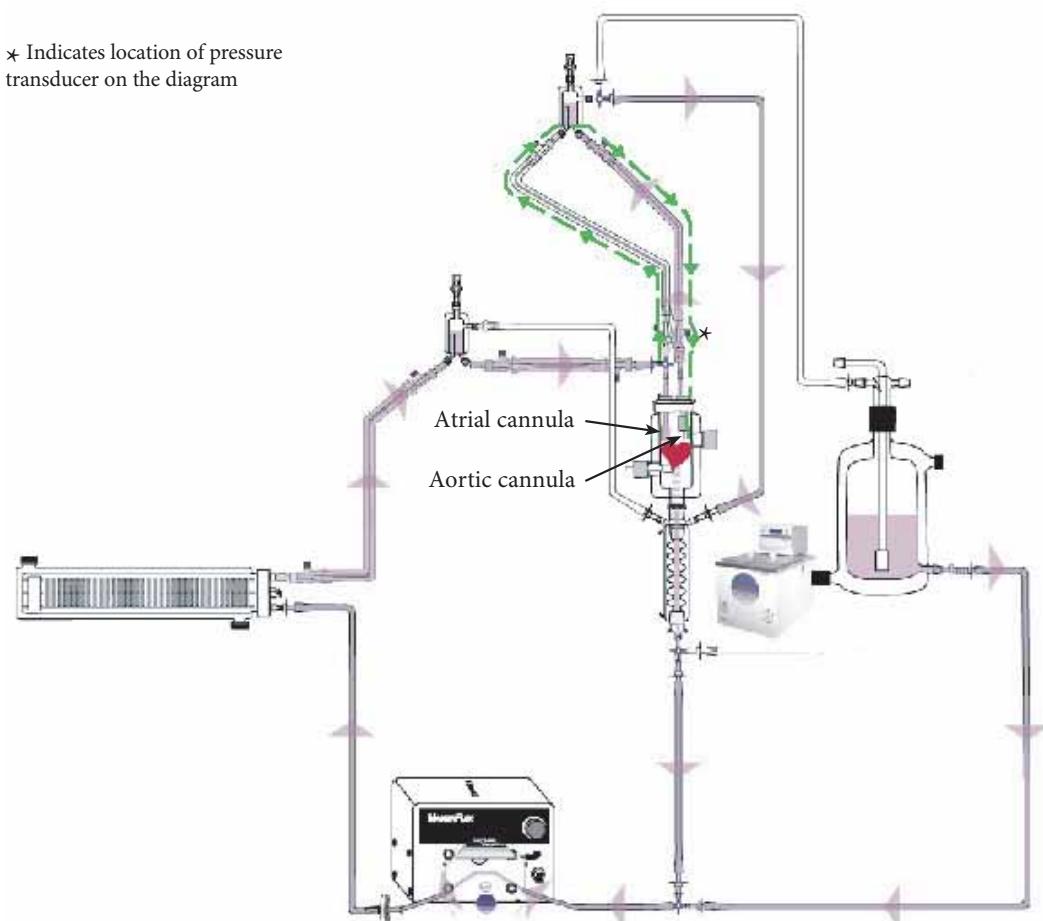


Figure 3. ADInstruments/Radnoti Working Heart System for Mice in recirculation mode. Pink (solid) lines indicate perfusate flow path in the recirculating "working heart" mode. The green (dashed) lines indicate perfusate flow path when system is used for a "langendorff" preparation.

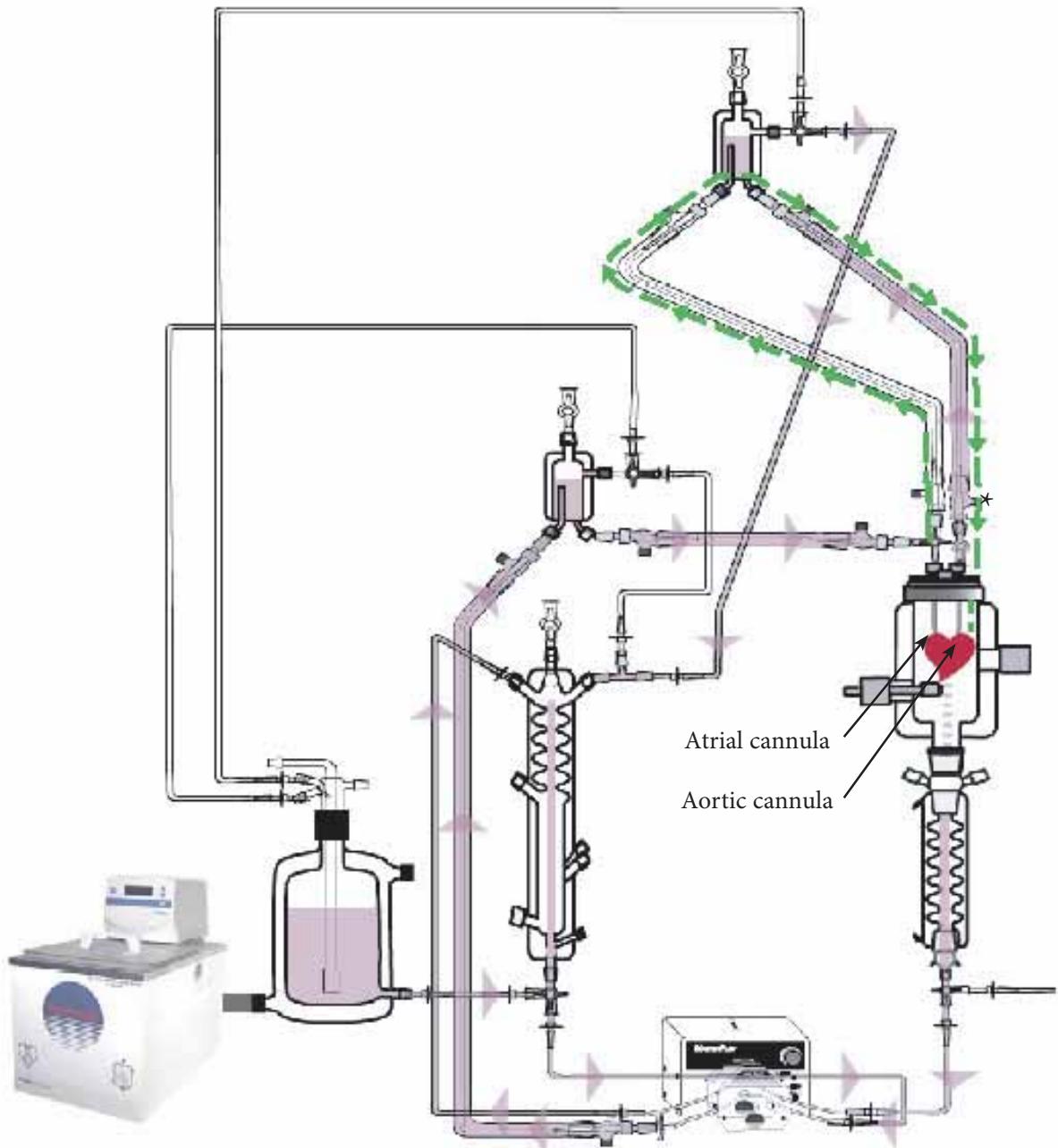


Figure 4. ADInstruments/Radnoti Working Heart System for Rats/Rabbits in recirculation mode. Pink (solid) lines indicate perfusate flow path in the recirculating “working heart” mode. The green (dashed) lines indicate perfusate flow path when system is used for a “Langendorff” preparation.

Post Experimental Cleaning

After the experiment has been completed, all the equipment should be carefully cleaned to reduce the accumulation of any biological deposits within the tubing and glassware. Most of the Radnoti apparatus is borosilicate glass, which can be cleaned with a wide range of soaps, dilute HCl or other cleaning solvents. Great care should be taken not to wash or expose non-glass components of the apparatus to acids or solvents used in the cleaning procedure. Chromic acid is not recommended for cleaning the apparatus due to possible heavy metal contamination of the system. Non-glass portions should be cleaned with aqueous soap solutions. The entire equipment/system should be rinsed multiple times in distilled water after cleaning. Areas to be especially well cleaned are the aerator, tubing, syringe ports, cannula, pressure transducer fittings as well as balloon catheters and electrodes inserted in the heart. The tubing should be inspected at the pump head for wear.

Troubleshooting

The isolated heart preparation is normally very stable and data collection reproducible once the setup parameters/conditions have been optimized and experimental protocols defined. If two consecutive preparation failures occur, this is a strong indicator of a problem. Many times this failure is due to the growth of bacteria and the release endotoxins into the perfusate. Initial corrective measures should include:

- A thorough cleaning of the apparatus and replacement of tubing.
- Replacement of solutions (which have a limited storage life in the refrigerator).
- A check of the water purity/source.
- A check of aeration, the appropriate gas mixture and pH of the aerated buffer at the normal operating temperature.
- A review of the heart removal technique.

Certain toxic agents used in some experimental protocols may be difficult to clean from the system and may require the use of organic solvents or the removal of tubing after each use as well as the use of a separate reservoir.

- Tubing should be thoroughly pre-rinsed to remove plasticizers
- The use of a high-quality silicone or Tygon tubing is recommended.
- Note that silicone tubing is extremely gas permeable; oxygen and other gas losses can be considerable.
- The use of high quality water is essential. The use of small amounts of EDTA (0.1mM) to chelate trace heavy metals in water (this is less of a problem with modern multiple cartridge ion-exchange systems) is recommended.

Recommended Reading

If the researcher/technician is not familiar with cardiovascular pharmacology and physiology, there are a number of excellent texts that will introduce this area. General principles can also be obtained from basic medical physiology and pharmacology textbooks. The following text is recommended:

- Pharmacologic Analysis of Drug-receptor Interaction by Terrence P. Kenakin (Raven Press, NY 1987) is a compact text with practical emphasis on isolated tissues and organs in pharmacological research.
- Determination of function in the isolated working mouse heart: Issues in experimental design. Gauthier, N.S., Matherne, G.P., Morrison, R.R. & Headrick, J.P. (1998). *J Mol Cell Cardiol* 30, 453-461.

The following table provides a summary of values obtained from a variety of sources as a guide to the approximate ranges of normal physiological parameters for a variety of adult animals. Heart rate and blood pressure are taken at rest. Cation values are from serum. Left ventricular volume (LVV) is given for a balloon inserted into the left ventricle. CF (coronary flow) is given for a saline solution at 50-60 mmHg.

Animal	Heart Rate bpm	Blood Pressure mmHg	Na ⁺ (mM)	K ⁺ (mM)	Ca ²⁺ (mM)	Mg ²⁺ (mM)	LVV (ml)	CF ml/ min/gm/ heart
Cat	110-140	125/70	163	4.4	1.3	0.7	0.7-2.4	2-3
Rat	330-360	129/91	140	5.7	2.6	1.1	0.1-0.2	8-10
Guinea Pig	280-300	120/170	145	7.4	2.6	1.2	0.1-0.2	5-8
Mouse	600-655	135/106			2.1	0.7		
R. pipens	37-60	31/21						
Carp	40-78	43						
Rabbit	205-220	110/73	155	4.6	3.5	1.6	0.4-0.7	2-5

The following table is only intended as a guide for expected values during a Langendorff experiment. The values are approximates only and may vary according to the perfusion method and type of solution. For further information see:

- The Isolated Perfused Heart by H.J. Döring, H. Dehnert (1988). English Edition, Biomesstechnik-Verlag March GMBH, ISBN3-924638-04-7

Animal	Body Weight (g)	Heart Weight (g)	Heart Rate (bpm)	Perfusion Pressure (mmHg)	CF (ml/min)	Perfusion Solution (K-H = Krebs - Henseleit)
Mouse	25-35	0.14-0.18	250-400	50-60	1.6-2.9	K-H
Rat	200-300	0.8-1.2	260-450	70-80	7-9	K-H, KHB-Neely
Guin.Pig	300-500	1.3-2.0	220-330	50-80	7-14	K-H
Hamster	120	0.6	N/A	70-80	5-6	K-H
Rabbit	2.5-3.5 kg	9-14	120-150	70-80	20-40	K-H, Tyrode, Blood
Cat	3-5 kg	15-18	110-240	70-80	25-29	K-H, Blood

A good estimation of the coronary flow/heart weight relationship can be calculated as:

- Coronary flow = 7.43 * heart weight (g)^{0.56}

The material presented here is believed accurate at the time of writing and is only intended as an introduction to isolated perfused heart principles. ADInstruments and Radnoti Glass Technology assume no liability for the use or misuse of this information.