



SI-LANGWH

"In-vitro" Perfusion System for Isolated Working Heart

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INSTRUCTION MANUAL

Serial No. _____

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World Precision Instruments

SI-LANGWH



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ABOUT THIS MANUAL

The following symbols are used in this guide:



This symbol indicates a **CAUTION**. Cautions warn against actions that can cause damage to equipment. Please read these carefully.



This symbol indicates a **WARNING**. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

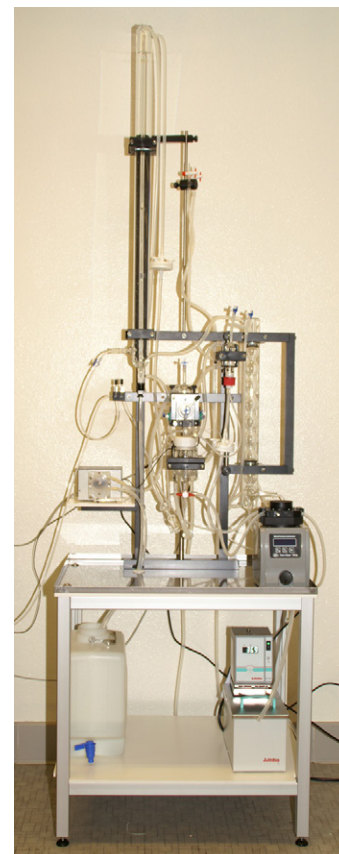
NOTES and **TIPS** contain helpful information.

INTRODUCTION

Studies on coronary circulation can easily be accomplished using an apparatus known as a Langendorff system. These devices perfuse isolated heart preparations from small mammalian species through a cannula inserted into the aorta of the heart. The perfusion buffer flows into the heart through the aorta and puts pressure on the aortic valves to close. The perfusion fluid has nowhere to go but through the coronary arteries that come off the side of the aorta. Since the flow of blood into the heart during this type of perfusion is the reverse of normal blood flow, Langendorff methods are known as retrograde perfusions. Also, the hearts subjected to Langendorff perfusion are described as being non-working since the heart does not work as it does *in vivo*. Langendorff systems are useful for measuring the effectiveness of drugs on the vasodilation and vasoconstriction of coronary arteries, and the resultant effects of increased or decreased cardiac perfusion.

Fig. 1–(Right) The SI-H Working Heart Langendorff.

By adding glass chambers, tubing and pumps to isolated heart perfusion systems, the mechanical abilities of hearts to perform work can be measured. Over 40 years ago, Neely and his associates added components that mimic parts of the body to a Langendorff system for the purpose of recreating the circulatory system of the body in a controllable environment. For example, a secondary aeration chamber is used as the lungs, and the right atria and ventricle are recreated by a peristaltic pump. Even a small glass chamber, which is the analog of the left atria, is used to control the preload on the left ventricle. Finally, adjustable valves and tubing that function as arteries, capillary beds and veins can be set to control the afterload on the isolated heart. Since perfusion fluid is



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flowing through the heart and the components of the system in the normal direction and the heart is the driving force behind the movement of this fluid, hearts in Neely systems are often described as being working since the heart works as it does *in vivo*.

The **SI-LANGWH** isolated working heart system is actually a combination of Langendorff non-working and Neely working heart systems. As soon as the heart is isolated and mounted on the cannulas, the heart is perfused using the Langendorff method. After the heart has equilibrated to the experimental conditions of the system, the system can be switched to the working heart mode for additional experiments.



CAUTION: Unpacking and installing the system requires more than one person! Beware of the heavy weight of the heavy-duty parts (for example, stands, larger glass or Plexiglas units, etc.).

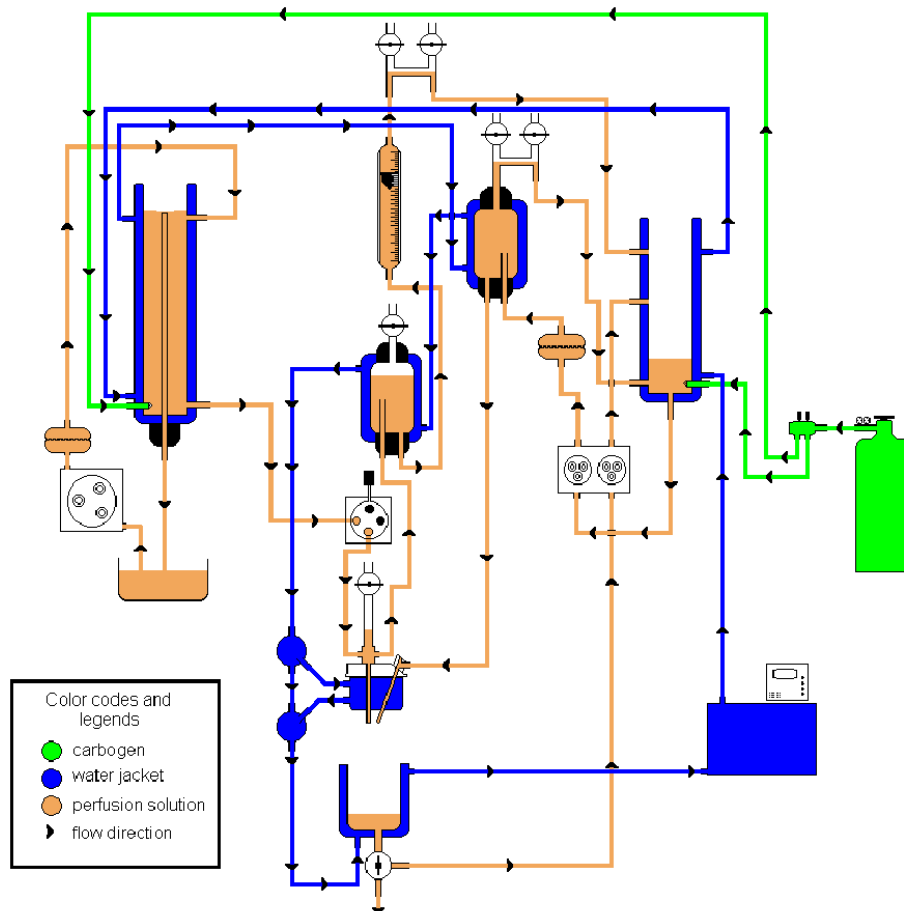


Fig. 2—This plumbing diagram shows how oxygen, water and perfusion buffer run through the Langendorff system.

**Unpacking**

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 46 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 46 of this manual.

INSTRUMENT DESCRIPTION

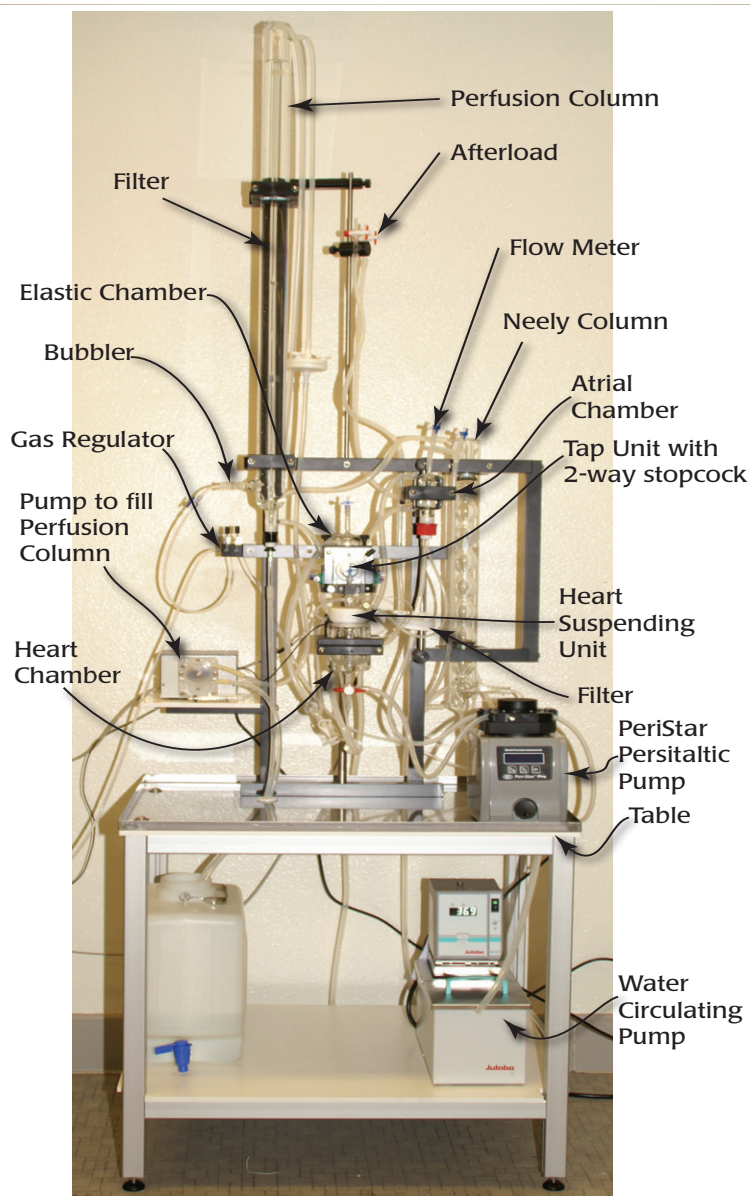


Fig. 3–The components of this Langendorff are labeled.



Setup

1. The table that supports this working heart system is delivered in pieces with the legs, cross beams, shelf and table top packed separately. Carefully remove the wrapping from the components. Keep the foam pad in the stainless steel tray on top of the table to prevent damaging the edges of the table. The table top is designed to collect any fluids that might drip from the system. Lay the table top face down on a work table or the floor (Fig. 4).

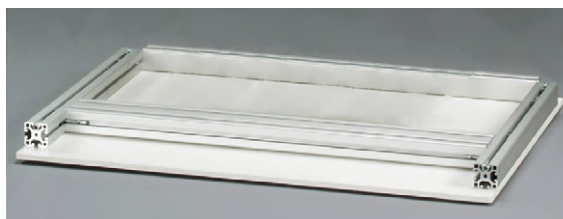


Fig. 4—Turn the table upside down.

2. Install the table legs.

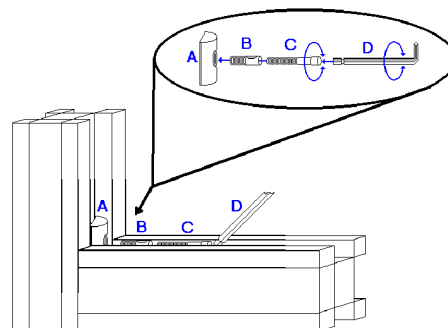
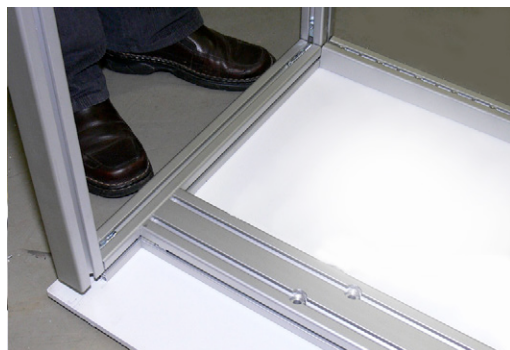


Fig. 5—(Left) Position the H-shaped legs of the table.

Fig. 6—(Right) Schematic shows how to lock the table legs into place.

- A. Align the top of one of the "H" shaped legs of the table against the cross beams mounted on the bottom of the table (Fig. 5).
- B. Slide the spring-loaded nuts (A) into the tracks of the legs and align them with the threaded tubes (B) in the table frame (Fig. 6).
- C. Push the hex screws (C) into the threaded tubes with the Allen wrench (D) (Fig. 7).
- D. Thread the screws into the nuts and rotate them until they are snug (Fig. 8).
- E. Attach the other leg to the table top in the same manner as the first leg.

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Fig. 7–(Left) Push the hex screws into place.

Fig. 8–(Right) Tighten the screws.

3. Attach the lower cross beams between the two sets of legs in the same manner as the legs are attached (Fig. 9).



Fig. 9–(Left) Attach the cross bars.

Fig. 10–(Right) Place the shelf under the table.

4. Setup the table.
 - A. Turn the table over and place it where the system will be used. Check the stability of the table. Adjust the height of the feet on the table to level it.
 - B. Place the shelf on the lower crossbars of the table (Fig. 10).
 - C. Remove the foam pad from the tray top of the completed table.
4. Attach the frame rack of the system to the table.
 - A. With the assistance of another person, align the holes on the bottom of the frame rack with the mounting holes on the table top (Fig. 11).

- B. As one person holds the frame rack in position, the other person should use four Allen screws and flat washers to fix the frame rack to the top of the table. Tighten the screws firmly.



Fig. 11—Attach the frame rack.

NOTE: Assemble the perfusion column and the overflow tube before mounting them on the frame rack.

5. Carefully remove the perfusion column and the overflow tube from the packing box. Lay them down on a work table and make sure that they are secure.
- The overflow tube has already been fitted with parts (1-5, 7 in Fig. 12) that are needed for mounting it in the perfusion column, and a silicone grommet near its top to protect the tube if it touches the inside of the column.
 - The perfusion column has already been fitted with the blocks that mount it to the frame rack, and a hollow nut (6 in Fig. 12) on the bottom of the column that is used to secure the overflow tube in the column.

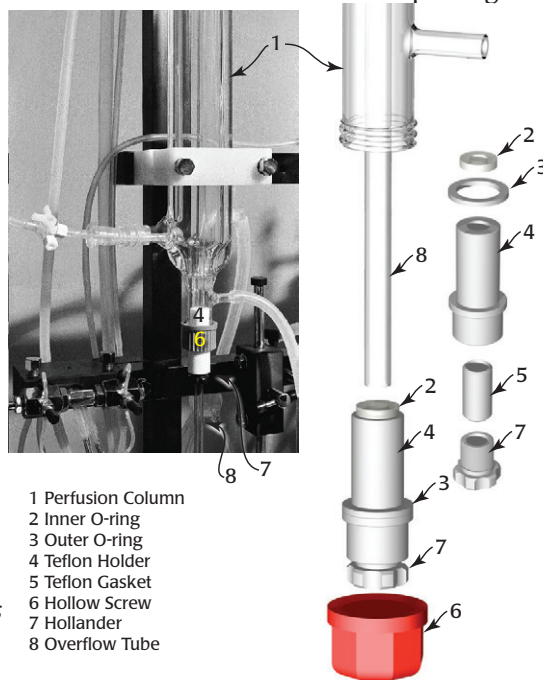


Fig. 12—(Right) The exploded view shows how the perfusion column fits together.

6. Assemble the perfusion column.

- A. Remove the hollow nut from the column.
- B. Carefully feed the upper end of the overflow tube, with its grommet, through the opening in the bottom of the perfusion column until the shoulder of the Teflon tube support (4 in Fig. 12) touches the bottom of the column.
- C. Reinstall the hollow nut over the end of the overflow tube and onto the bottom of the perfusion column.
- D. Finger-tighten the hollow nut to secure the overflow tube in the column.



CAUTION: Be sure the inner (2) and outer (3) O-rings and the Teflon gasket (5) are installed on the tube support (4) before assembling the perfusion column.

NOTE: The height of the overflow tube sets the pressure used to perfuse the heart in Langendorff mode. To adjust the height of the overflow tube, loosen the knurled nut (7 in Fig. 12) and carefully slide the overflow tube to the correct position. If the tube does not slide, loosen the knurled nut a little more. Once the tube is in position, finger-tighten the knurled nut to secure the overflow tube.

7. Mount the assembled perfusion column to the left side of the frame rack (Fig. 13). The mounting blocks for the perfusion column are already positioned on the column and should align with the mounting holes on the frame rack. With the assistance of another person, attach the perfusion column and its mounting blocks to the frame rack using the four (4) cap screws provided.



Fig. 13—Mount the perfusion column to the left side of the frame.

8. With the assistance of another person, mount the Neely column and its attached brackets to the back-right side of the frame rack (Fig. 14) in the same manner the perfusion column was mounted. The inner chamber of the Neely column, which is the analog of the lungs, has an undulating shape to increase the oxygen of the perfusion fluid flowing through it.

NOTE: Make sure the Neely column is installed so the water jacket outlet at the top of the column points to the left (Fig. 14), and the aeration port at the bottom of the column points to the right.

Fig. 14—(Right) Mount the Neely column.

9. Mount the valve tap assembly (Fig. 15), which also includes the elastic chamber, to



the central crossbar of the frame rack.

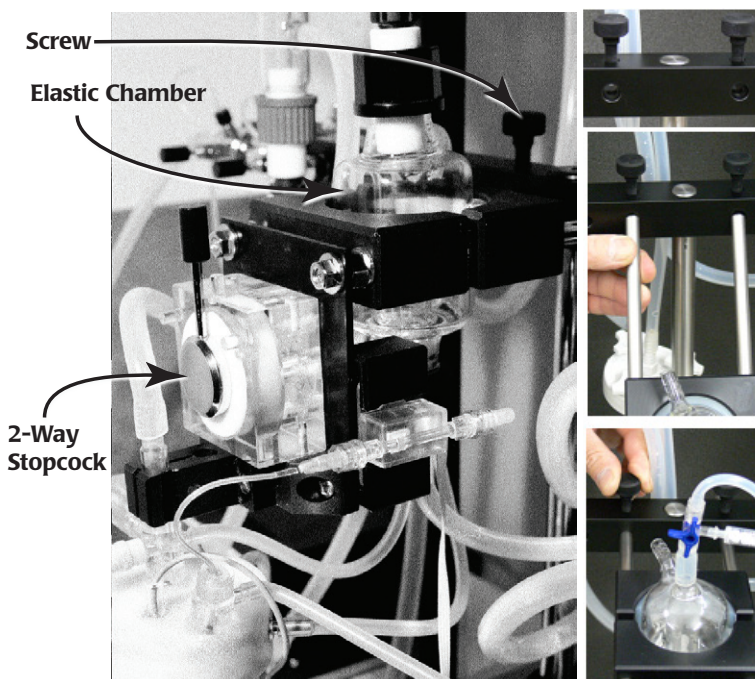


Fig. 15–(Left) Mount the valve tap assembly

Fig. 16–(Right) Secure the rods of the tap valve support to the crossbar.

- A. Loosen the two knobs on the holes in the center of the crossbar (Fig. 16).
 - B. Push the rods of the tap valve support into the holes on the crossbar.
 - C. Tighten the knobs to secure the tap valve assembly to the frame rack.
10. Mount the carbogen manifold assembly (Fig. 17) to the left end of the central crossbar. The regulators and the tubing are already mounted to the manifold. The manifold is mounted using a single cap screw.

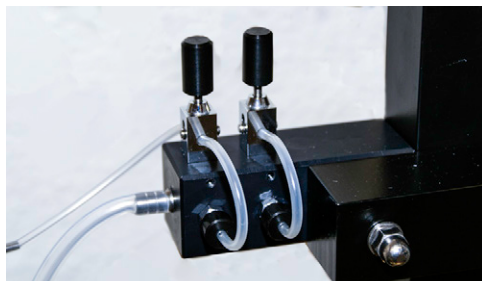


Fig. 17–Mount the carbogen manifold.

11. Mount the shelf for the pump that fills the perfusion column to the left side of the

frame rack (Fig. 18).

- A. Align the mounting hole of the shelf platform with the hole on the left upright of the frame rack. This hole is about 25cm above the tray top. Secure the platform with the screw and nut provided.
- B. Place the filling pump that is supplied with system on the shelf

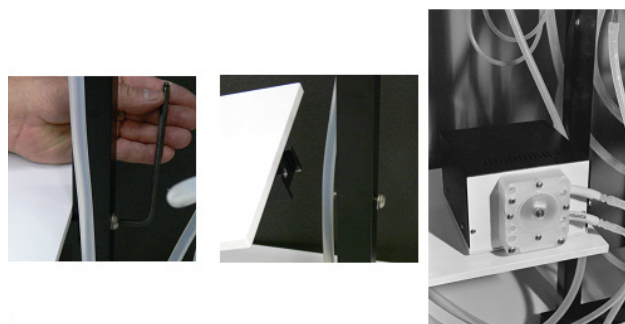


Fig. 18–Mount the pump shelf.

12. Locate the bracket for the heart chamber on vertical stainless steel rod mounted between the middle and lower crossbars of the frame rack.
 - A. Loosen the T-bar screw on the back of the bracket to adjust the position of the bracket.
 - B. Align the bracket so that it faces forward and is high enough for the heart chamber and its drain to safely clear the top of the table.
 - C. Tighten the T-bar screw to lock the bracket in position.
13. Mount the heart chamber and its support to the bracket using the cap screws provided (Fig. 19). Use the upper sets of holes on the bracket and the chamber support.

NOTE: Loosen the T-bar screw to rotate and raise/lower the heart chamber so that it aligns with the heart suspension unit mounted on the tap valve assembly. Likewise, the tap valve assembly and the heart suspension unit can be moved forward and back to align with the heart chamber by loosening the knobs on the middle crossbar.

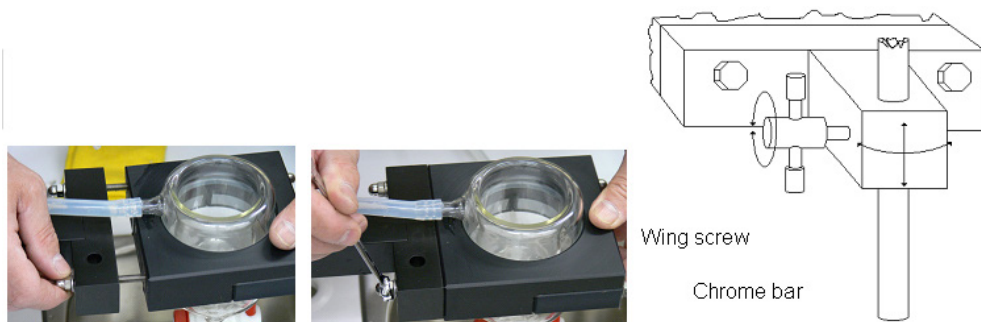


Fig. 19–Mount the heart chamber.

14. Locate the clamp and support rod for the atrial chamber to the right of the tap valve/elastic chamber assembly (Figure 20).

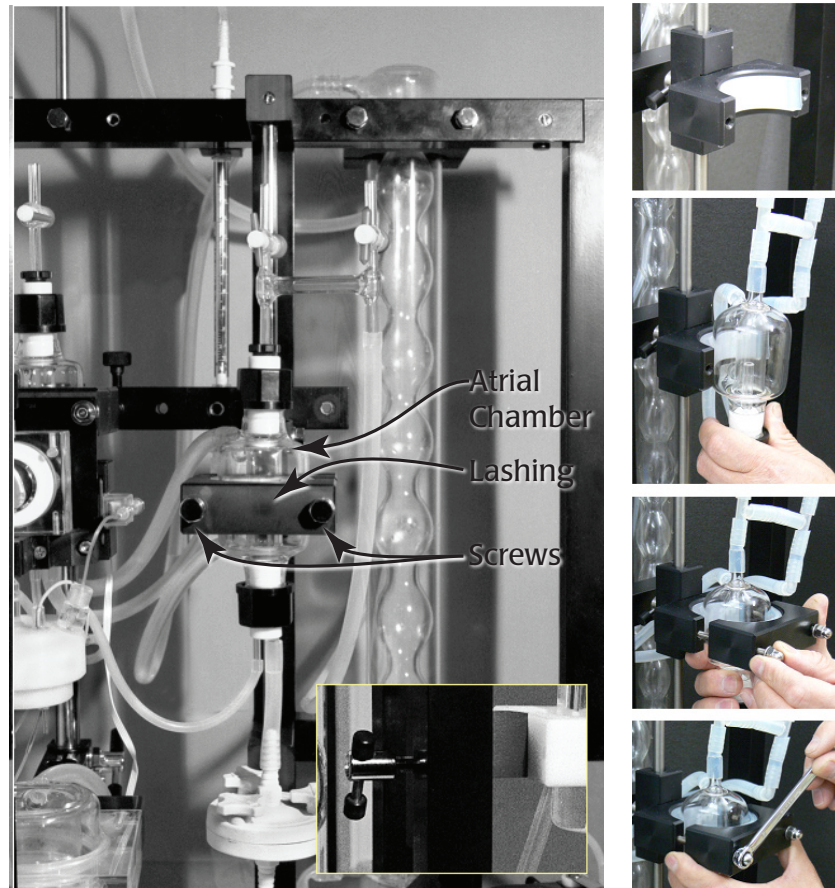


Fig. 20–(Left) Install the atrial chamber to the right of the tap valve and elastic chamber.

Fig. 21–(Right) Instal the atrial chamber.

- A. Remove the screws from the lashing on the front of the clamp to open the clamp (Fig. 21).
- B. Position and hold the atrial chamber against the back of the clamp. Make sure the water jacket fill ports have enough clearance for tubing to be attached, and that the H-tubing is on the right side of the chamber.
- C. With the assistance of another person, install the lashing and its screws to the front of the chamber clamp. Tighten the screws until the chamber is held snugly. *Do not overtighten these screws.*

NOTE: Loosen the T-bar screw on the clamp to raise/lower the atrial chamber and set the preload on the left ventricle when the system is used in Neely working heart mode.

15. The flow meter was packed separately. Carefully unwrap it and mount it on the frame rack.
 - A. Locate the two brackets for the flow meter on the back of the frame rack (Fig. 22), between the upper and middle crossbars.
 - B. Remove the screws that hold the lashings to the brackets.
 - C. Place the fittings at the top and bottom of the flow meter in the grooves of the brackets.
 - D. With the assistance of another person, install the lashings and their screws to the fronts of the clamps.
 - E. Tighten the screws so that the meter is held in place, but can still be rotated.

CAUTION: Do not tighten the screws on the clamps of the flow meter until the tubing is attached to the inlet and outlet of the meter and the meter is aligned so that scale is visible. After the flow meter is in the correct position, tighten the clamp screws until the flow meter is held snugly. Do not overtighten the screws.

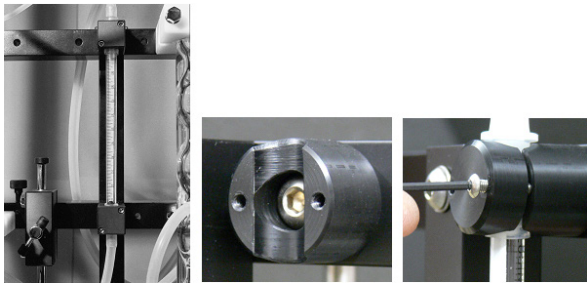


Fig. 22—Install the brackets for the flow meter.

16. Mount the afterload assembly to the frame rack.
 - A. Carefully unwrap the H-valve assembly (Fig. 23) and place it on its support rod.
- Fig. 23—(Right) The H-valve assembly is on the support rod.
 Fig. 24—(Far Right) Mount the support rod.
- B. Mount the support rod in the hole on the left side of the upper crossbar (Fig. 24).
 - C. Secure the rod in place with an Allen screw.
 - D. Stabilize the top of the support with the beam attached to the top of the frame rack. If the support rod has already been mounted on the frame rack, open the clamp on the stabilizing beam, and slide the H-valve over the top of the support rod.
 - E. Place the beam back on the support rod.
 - F. Adjust the height of the H-valves to determine the afterload on the heart in Neely working heart mode (Figure 25).

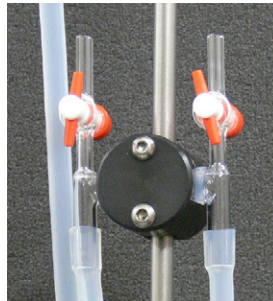
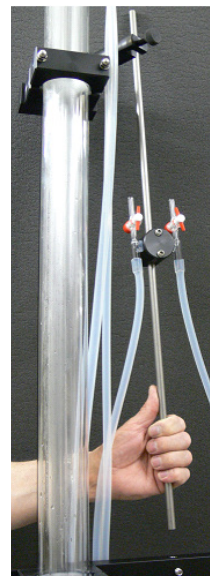
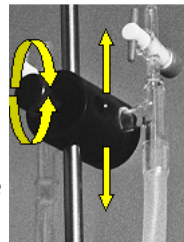


Fig. 25—(Right) The height of the H-valves can be adjusted.

NOTE: If the H-valves need to be cleaned or changed, remove the two screws on the cover plate of the bracket. Remove the cover plate and perform the required maintenance or replacement of the valves.



G. Reinstall the valves and cover plate and screws on the bracket.

17. Mount the heart suspension unit to the front of the valve tap assembly (Fig. 27). Make sure there is enough clearance between the valve tap assembly and the heart chamber to mount the suspension unit.

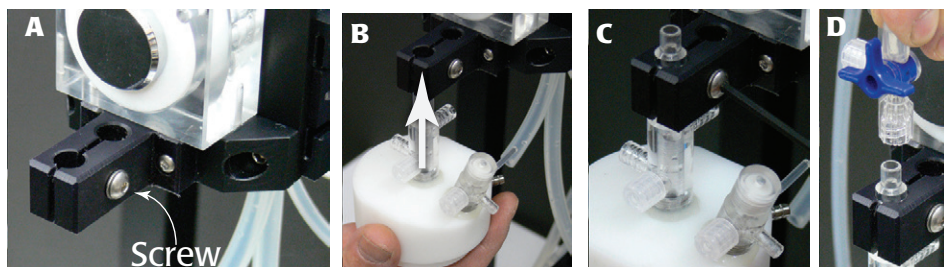
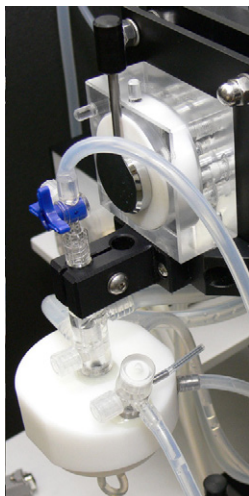


Fig. 26—Follow the steps to mount the heart suspension unit.

Fig. 27— (Left) The heart suspension unit is mounted.

- A. Loosen the screw on the side of the mounting bracket in the front of the valve tap (Fig. 26, A).
- B. Carefully push the stem of the heart suspension unit into the front mounting hole (Fig. 26, B and C).
- C. Gently tighten the mounting screw to hold the suspension unit snugly in place.



CAUTION: Do not overtighten the mounting screw! Too much pressure on the stem of the suspension unit will cause it to crack.

- D. Attach a three-way stopcock, with an attached piece of silicone tubing, to the top of the stem for the purpose of flushing and draining the heart suspension unit. (Fig. 26, D)

Plumbing the System

The plumbing of Langendorff and working heart systems are organized into three distinct circuits (Fig. 28):

- The regulation of the temperature of the organ and the perfusion buffer
- The movement of perfusion fluid in the system
- The oxygenation of the perfusion fluid and the organ.

The circuits are coded in different colors and the arrows indicate the direction of water, perfusion buffer and oxygen movement.

Diagrams of the individual circuits (Fig. 29, Fig. 30, Fig. 32) that show only the components that are related to each circuit are useful for completing the tubing connections. Many of the components in a circuit may already have tubing attached to them. In some cases, completing sections of the circuit are as simple as connecting the open end of the tubing on one component to another component. A table that lists the lengths and diameters of the tubing pieces in all three circuits, and a numbered diagram that pinpoints the location of each piece of tubing in each circuit, can be found at the end of this manual.

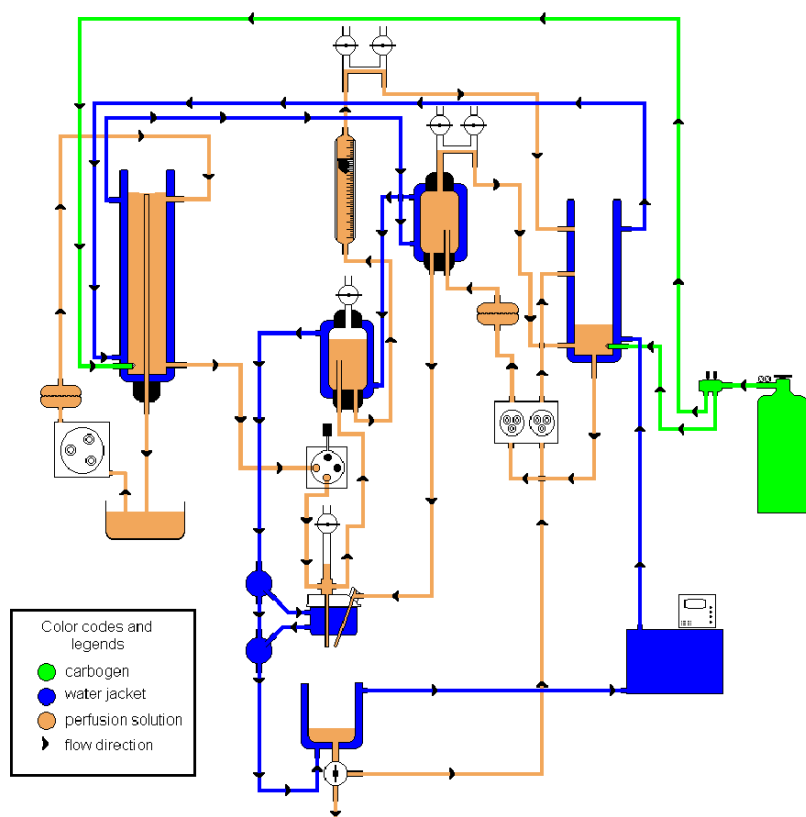


Fig. 28—Plumbing diagram showing all three circuits.

Temperature Regulation Circuit

Complete the connections between the water jackets of the required components and the recirculating water bath using the following directions (Fig. 29).

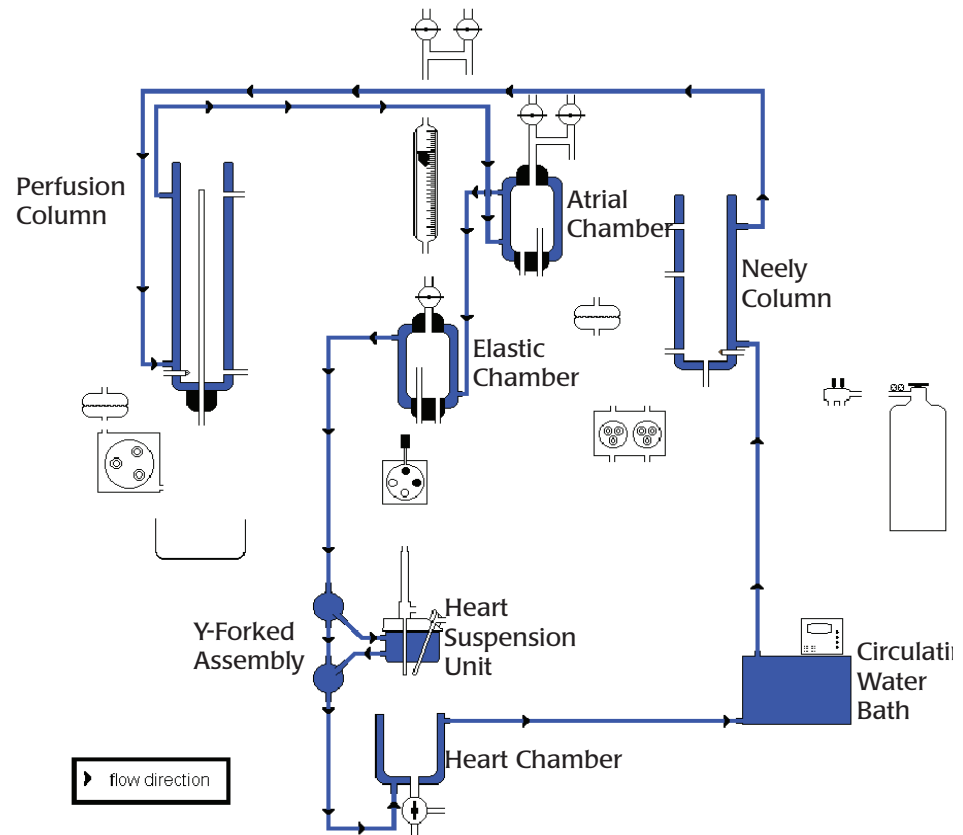


Fig. 29—Water Jacket plumbing diagram.

1. Connect the outlet of the recirculating water bath to the water jacket inlet at the bottom of the Neely column with a piece of silicone tubing.
2. Connect the silicone tubing on the water jacket inlet at the bottom of the perfusion column to the water jacket outlet at the top of the Neely column.
3. Connect the water jacket outlet at the top of the perfusion column to the water jacket inlet at the bottom of the atrial chamber.
4. Connect silicone tubing between the water jacket outlet at the top of the atrial chamber and the water jacket inlet at the bottom of the elastic chamber.
5. Connect a large open end of the Y-forked bypass assembly to the water jacket outlet at the top of the elastic chamber.

6. Connect the small tubes of the Y-forked bypass assembly to the water jacket inlet and outlet on the back of the heart suspension unit.
7. Connect the remaining large open end of the Y-forked bypass assembly to the water jacket inlet at the bottom of the heart chamber.
8. Connect the water jacket outlet of the heart chamber to the inlet of the recirculating water bath.

CAUTION: When the circuit is complete, make sure it is filled with deionized water only.

Carbogen Circuit

Complete the connections between the carbogen supply and the aeration frits in the reservoirs using the following directions (Fig. 30).

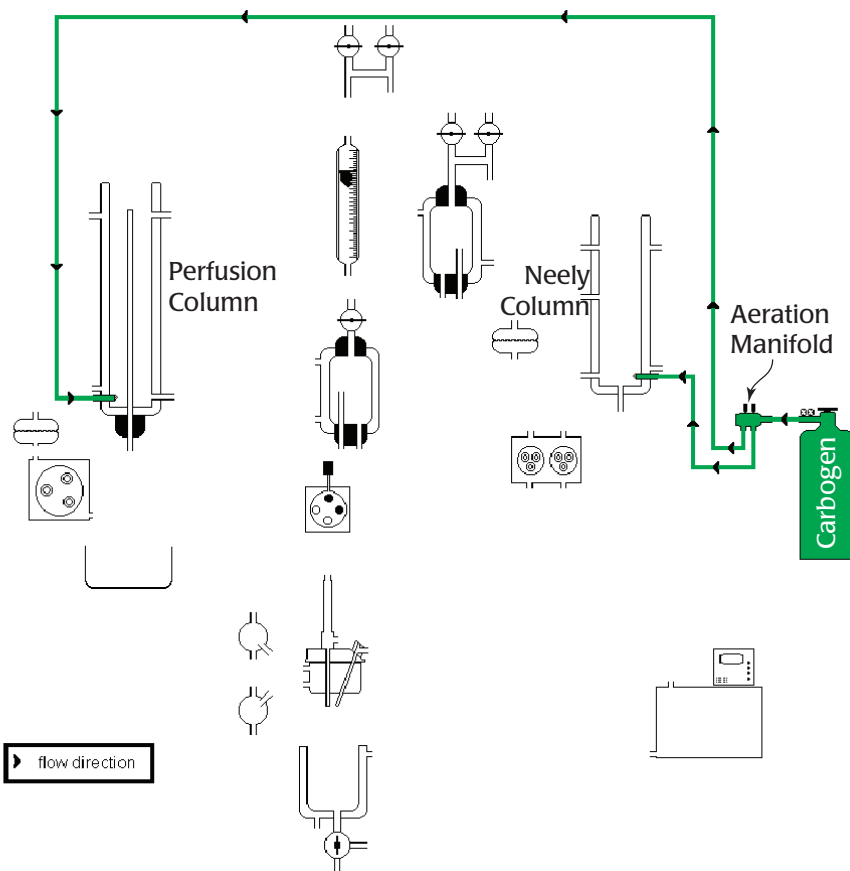


Fig. 30–Carbogen circuit plumbing diagram.

1. Connect the pressure regulator and valves on the carbogen cylinder to the manifold and regulators on the left side of the frame rack using the thin silicone tubing already attached to the inlet of the carbogen manifold.
2. A piece of silicone tubing has already been attached to the outlet of each carbogen regulator of the aeration manifold on the left side of the frame rack. Connect the longer piece of silicone tubing on a regulator to the Luer fitting and 3-way stopcock on the aeration frit of the Neely column.
3. Connect the shorter piece of silicone tubing on the other regulator to the Luer fitting and 3-way stopcock on the aeration frit of the perfusion column (Fig. 31).



Fig. 31—(Right) Carbogen connection.

Perfusion Circuit

Complete the connections between the components that perfuse the heart in either Langendorff or working heart mode using the following directions (Fig. 32).

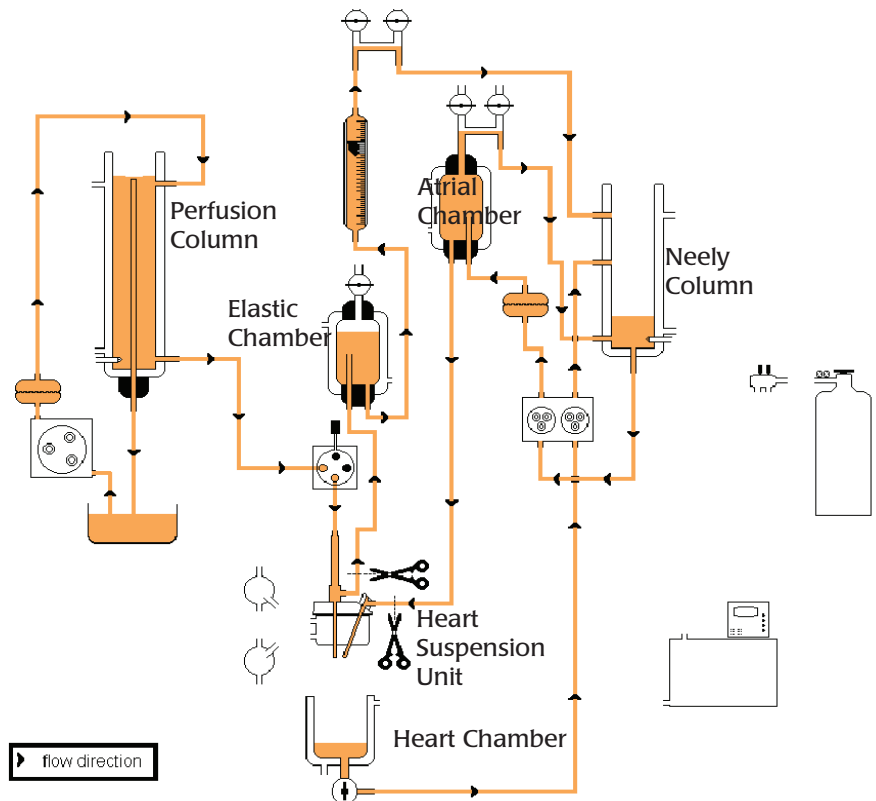


Fig. 32—Perfusing circuit plumbing diagram.

1. Place a carboy, which will eventually hold fresh perfusion buffer, on the left side of the shelf under the tray top.
2. Place the open end of the silicone tubing on the bottom of the overflow tube through the hole on the left side of the tray top and into the carboy that will hold perfusion buffer. Make sure the end of this tubing does not go below the surface of the buffer when the carboy is filled.
3. Connect a piece of silicone tubing to the inlet tube of the pump used to fill the perfusion column. Put the open end of this tubing into the buffer carboy. The tubing should be long enough to reach the bottom of the carboy.
4. Attach another piece of silicone tubing between the outlet of the fill pump and the filter and tubing that is already mounted on the upper fill port of the perfusion column.
5. Use a short piece of silicone tubing to connect the outlet at the bottom of the perfusion column to the open inlet port on the back of the valve tap. Two of the other ports on the back of valve tap are plugged and not in service in this configuration. Another piece of silicone tubing has already been mounted on the remaining open port.
6. Connect the silicone tubing on the remaining open port on the back of the valve tap to the inlet on the back side of the stem of the heart suspension unit.
7. Attach the silicone tubing on the inlet of the elastic chamber to the outlet on the left side of the stem of the heart suspension unit. The inlet of the elastic chamber is the taller of the glass tubes inside the chamber.
8. Connect the silicone tubing on the outlet of the elastic chamber to the inlet at the bottom of the flow meter. The outlet of the elastic chamber is the shorter of the glass tubes inside the chamber.
9. Attach the silicone tubing on the left side of the afterload assembly to the outlet on top of the flow meter.
10. Connect the silicone tubing on the right side of the H-valve afterload assembly to the highest buffer inlet on the Neely chamber.
11. Use a silicone tubing to connect the side port on the drain tap of the heart chamber to the inlet of one of the channels on the constant flow pump. Use another piece of silicone tubing to connect the outlet of the same pump channel to the middle buffer inlet on the Neely chamber.
12. Connect the right side of the other H-valve assembly, which is on top of the atrial chamber, to the lower buffer inlet on the Neely chamber.
13. Use a piece of silicone tubing to connect the outlet on the bottom of the Neely chamber to the inlet of the second channel on the constant flow pump. Use another piece of silicone tubing to connect the outlet of the same pump channel to the filter and tubing on the inlet of the atrial chamber. The inlet of the atrial chamber is the taller of the glass tubes inside the chamber.



14. Connect the outlet of the atrial chamber to the inlet of the left atrial cannula on the heart suspension unit. The outlet of the atrial chamber is the shorter of the glass tubes inside the chamber.

Filling The System

Filling the Temperature Regulation Circuit

1. Follow the directions for operating the circulating heated water bath that are provided by its manufacturer.
2. Fill the reservoir of the circulating water bath with deionized water.
3. Turn on the water bath and set the temperature needed for the experiment.
4. Activate the pump of the bath to move water from the reservoir into the water jackets of the columns and chambers. As water moves into the system, replenish the water in the reservoir until the water jackets are full.



CAUTION: Most recirculating water baths have a safety sensor that will turn off the heater and pump automatically if the water level drops below a critical level. If the water bath turns off because the water level is low, add water to the reservoir and restart the water bath.

5. Check the water level in the reservoir every day. Add water to the reservoir, if needed.

Filling the Perfusion Circuit

1. Make at least 5L of perfusion buffer (Krebs-Henseleit or similar). This is the volume required to fill the perfusion circuit of the system. Fill the clean carboy on the shelf under the left side of the tray top with the fresh buffer.
2. Place the silicone tubing on the inlet of the fill pump under the surface of the buffer in the carboy. Place the tubing on the overflow of the perfusion column in the carboy, but not under the surface of the buffer.
3. Set the desired hydrostatic pressure used to perfuse the heart by moving the overflow tube of the perfusion column up or down. Determine the height that the open end of the overflow tube needs to be above the aorta of the heart to set the desired perfusion pressure.

Calculate the height needed by multiplying the desired perfusion pressure (in mmHg) by the 13.6 conversion factor. For example, to achieve a perfusion pressure of 60mmHg, the end of the overflow tube needs to be 816mm above the level of the aorta of the isolated heart.

NOTE: To adjust the height of the overflow tube, loosen the knurled nut (7 in Fig. 12) and carefully slide the overflow tube to the correct position. If the tube does not slide, loosen the knurled nut a little more. Once the tube is in position, finger-tighten the knurled nut to secure the overflow tube.

4. Start the flow of carbogen to the aeration inlets of the perfusion column and the Neely column. Open the valves on the cylinder of carbogen. Open the regulators on the aeration manifold and the 3-way stopcocks on the aeration inlets of both columns. Begin the flow of carbogen through the aeration lines before the perfusion fluid is added to the system.
5. Open the stopcocks of the H-valve on top of the atrial chamber. This allows air bubbles to escape the atrial chamber as it is filled with buffer. Keep these stopcocks open through the experiment.
6. Use the forceps included with the system to close the atrial inlet tube. This permits the tubing that leaves the atrial chamber to be filled.
7. Turn on the fill pump to move buffer into the perfusion column. The column will continue to fill until the fluid level reaches the top of the overflow tube. Any excess fluid in the perfusion column will flow down the overflow tube and back into the fresh buffer carboy. Keep the pump running while the rest of the system is being filled.
8. Use the constant flow peristaltic pump to fill the Neely column and the atrial chamber. Lower the atrial chamber so that it is at the same level as the middle of the Neely column. Fill the heart chamber with perfusion buffer from a beaker or the perfusion column. To fill the heart chamber from the perfusion column, open the valve tap and buffer will flow into the heart chamber from the aortic cannula. Close the valve tap when the heart chamber is full.
9. Use a beaker or a large syringe to add buffer to the Neely column through the hole in its top. Fill this column until it is about half-full.
10. Turn the stopcock on the drain of the heart chamber so that buffer will flow out of the side port of the drain when the constant flow pump is running. Turn on the constant flow pump to move buffer from the heart chamber to the Neely chamber, and from the Neely column to the atrial chamber. Refill the heart chamber and Neely column with buffer before they go dry. Keeping buffer in the heart chamber and Neely column prevents bubbles from entering the tubing and chambers.

NOTE: The volume of buffer moved by the constant flow pump is determined by the revolutions of the pump and the inside diameter of the tubing used with the pump. Through the use of an algorithm of tubing diameters and speeds in the manual, the correct revolutions for the required flow rate can be determined. Many new pumps have control circuitry that automatically determines and displays the flow rate when the value of the diameter is entered and the speed is set.

11. Keep circulating buffer through the atrial chamber and the Neely column until all the bubbles in the tubing have escaped through the H-valve on top of the atrial chamber (Fig. 33) or the top of the Neely column.

Fig. 33–(Right) The H-valve is on top of the atrial chamber.

12. Open the forceps on the atrial inlet tube to fill that tube with buffer. Clamp the inlet tube with forceps once the tube is filled and free of bubbles.





13. Use the perfusion column to fill the elastic chamber, the flow meter, the H-valve afterload assembly and its tubing with buffer. Lower the H-valve afterload assembly to the lowest point on its support rod and open its stopcocks. Also, open the stopcock on the top of the elastic chamber. Put a small piece of tubing on the end of the aortic cannula and clamp it with a forceps to prevent buffer from flowing out of the cannula.
14. Open the valve tap and buffer will move from the perfusion column through the outlet tube of the heart suspension unit and into the elastic chamber. Once the elastic chamber is filled and free of bubbles, close the stopcock on top of the elastic chamber to drive the buffer out of the elastic chamber and into the flow meter. If the end of the overflow tube in the perfusion column is higher than the afterload assembly, buffer will rise up and over the afterload assembly and into the Neely column. Once the flow meter, the afterload assembly and their tubes are filled and free of bubbles, close the stopcocks on top of the afterload assembly.
15. Fill the elastic chamber with about 2mL of air to dampen the pulsing pressure wave created by the pumping of the left ventricle. Connect a syringe filled with air to 3-way stopcock on the top of the elastic chamber (Fig. 34). If needed, open the stopcock to fill the elastic chamber with buffer. Then, rotate the stopcock until the syringe and the elastic chamber are connected. Push 2mL of air into the elastic chamber by pushing the plunger of the syringe. Close the stopcock on top of the elastic chamber so that the 2mL of air are trapped in the elastic chamber. Keep this stopcock closed throughout the perfusion.

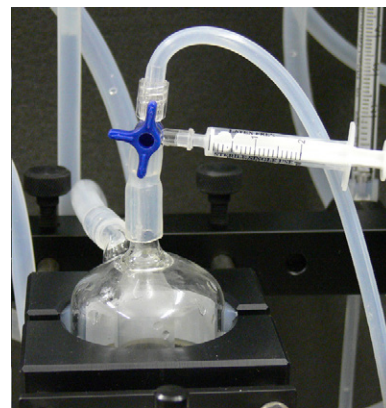


Fig. 34—Connect a syringe to the 3-way stopcock.

NOTE: It is important to maintain the same volume of air in the elastic chamber. This insures the pressure recordings between experimental runs are comparable.

16. Turn on the recirculating water bath and begin warming the system and perfusion buffer. Start warming the system 30 to 60 minutes before starting the isolation of the heart. Also, it takes at least 30 minutes for the PO₂ and PCO₂ values of the buffer to reach equilibrium.

Installing the Sensors

Pressure Sensors

1. The bodies of the pressure transducers are installed in holders on the left and right sides of the valve tap bracket (Fig. 35). Push in the spring loaded plate in the center of the holder and slide the sides of the sensor body under the retaining rails of the holder. Make sure the sensor is oriented so that the cable of sensor is pointed to the rear of the system.



Fig. 35—Install the pressure transducers in the holders on the left and right sides of the valve tap bracket.

2. Install the sensor that measures the perfusion pressure in Langendorff mode, or aortic pressure when in Neely mode, on the left side of the valve tap (A in Fig. 36 and Fig. 37).

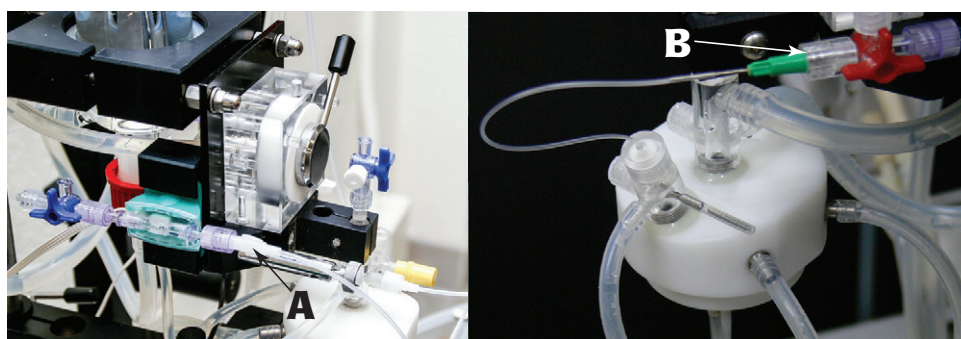


Fig. 36—A show the sensors installed on the left side of the valve tap. B shows the sensor installed on the right side of the valve tap.

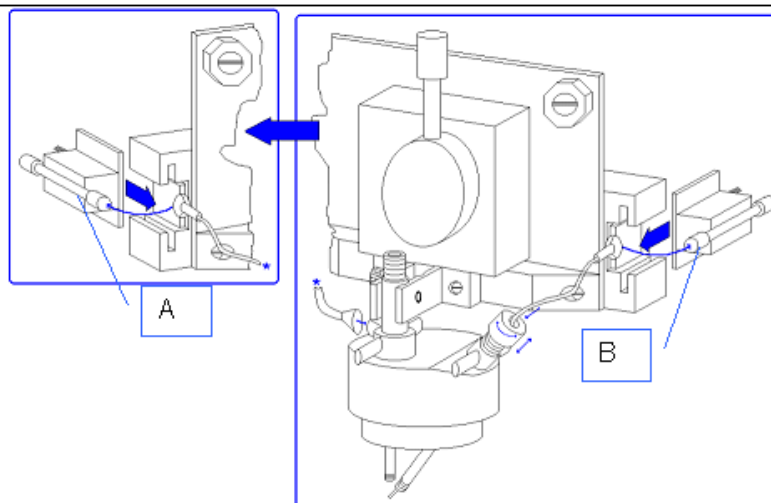


Fig. 37—A show the sensors installed on the left side of the valve tap. B shows the sensor installed on the right side of the valve tap.

- A. Connect the front of the sensor to the Luer fitting on the either the front or the top of the stem of the heart suspension unit with a PVC catheter equipped with Luer fittings.
 - B. Attach a 3-way stopcock with an empty 2mL syringe to the rear port of the pressure sensor to pull buffer into the sensor and its PVC line, and to close off the syringe when measurements are made.
3. If the left ventricular pressure (LVP) is going to be measured, install another sensor on the right side of the valve tap assembly (B in Fig. 36 and Fig. 37).
 - A. Attach your own PVC cannula with matching needle to the front of the sensor.
 - B. Put the other end of the cannula through a small hole in the diaphragm on top of the atrial inlet.
 - C. Make the hole with a needle that is slightly smaller than the diameter of the cannula.
 - D. Loosen the nut that retains the rubber diaphragm over the inlet and allow the diaphragm to stretch.
 - E. Push the PVC cannula through the opening in the diaphragm and down the stainless steel atrial cannula until the tip of the PVC cannula reaches the end of the metal cannula (Fig. 38).
 - F. Tighten the nut on the inlet to compress the diaphragm and seal the hole around the cannula.
 - G. Attach a 3-way stopcock with an empty 2mL syringe to the rear port of the pressure sensor to pull buffer into the sensor and its PVC cannula, and to close off the syringe when measurements are made.
 - H. If the LVP cannula is not going to be used, replace the diaphragm that has a hole with one that does not.

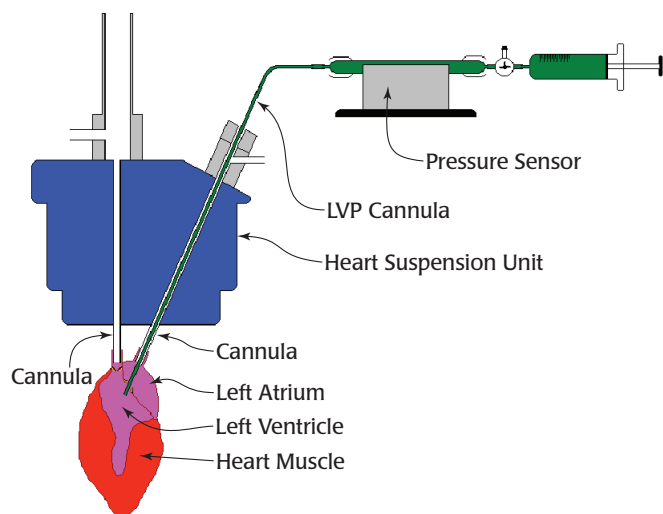


Fig. 38—This diagram shows how the heart is mounted.

Temperature Sensor

1. Connect the plastic tube around the end of the temperature sensor to the stainless steel tube on the back of the atrial inlet (Fig. 39). The end of the temperature sensor should not touch the metal tube on the inlet (Fig. 40).

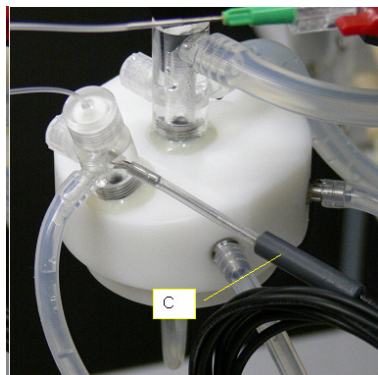


Fig. 39—(Left) Connect the stainless steel tube on the back of the atrial inlet.

Fig. 40—(Right) The temperature sensor does not touch the metal tube.

2. Mount the cable clamp for the lead wire of the temperature sensor on the back of the heart chamber support bracket (Fig. 40) while the heart chamber is lowered.

3. With the heart chamber still lowered, place the cable of the temperature sensor in the slot of the clamp (Fig. 41). Gently tighten the retaining screw to hold the cable in place. Make sure the cable is aligned so that it does not touch the heart, or it is not damaged when the heart chamber is raised.



Fig. 41–The cable from the temperature sensor slides through the slot on the clamp.

Stimulation Electrodes (optional)

1. Screw the mounting post to the bottom of the valve tap assembly. Attach the offset arm that suspends the stimulation electrode from the mounting post (Fig. 42).

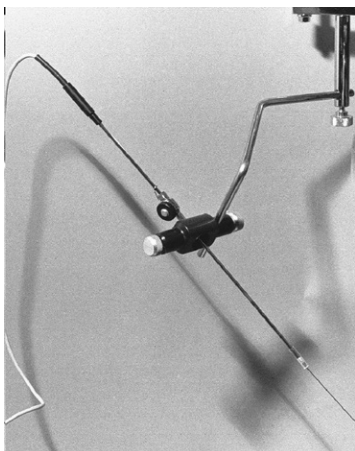


Fig. 42–The offset arm suspends the stimulation electrode.

2. Attach the ball joint bracket of either the monopolar or bipolar stimulation electrode to the end of the offset arm.

MAP (ECG) Electrodes (optional)

The MAP sensor is an optional sensor designed to record monopolar action potentials (MAP) from as many as 12 different locations around the heart (Fig. 43) while the heart is being perfused during Langendorff mode.

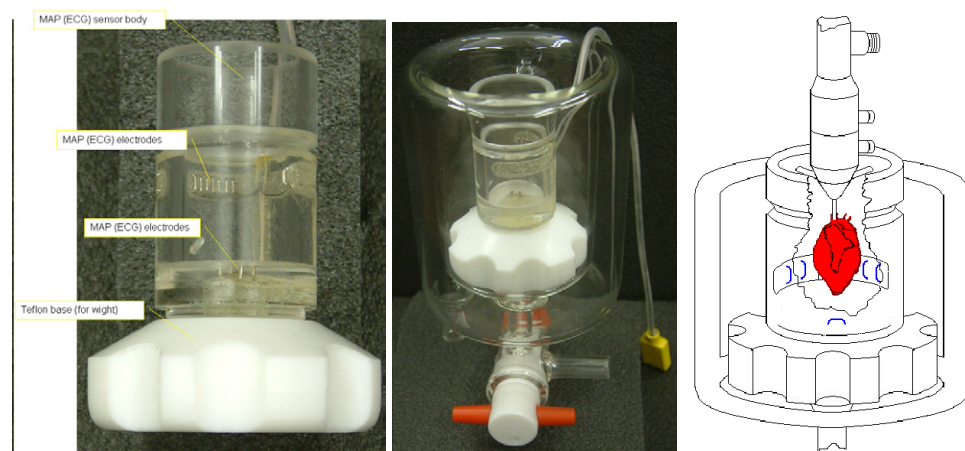


Fig. 43–(Left) The MAP sensor is an option.

Fig. 44–(Center) Place the MAP sensor inside the heart chamber.

Fig. 45–(Right) Position the heart so that it doesn't touch any of the electrodes on the sensor.

1. To use the MAP sensor, place it in the heart chamber that is filled with perfusion buffer (Fig. 44). Raise the heart chamber and sensor around the heart mounted on the aortic cannula. Make sure the heart does not touch any of the 12 electrodes on the sensor or the sides of the sensor (Fig. 45).
2. Connect the lead of the MAP sensor to the extension cable (Fig. 46). Connect the Canon (D-sub) connector on the end of the extension cable to the input of the fourth channel on the amplifier provided with the system. This amplifier is capable of recording signals from 3 different positions around the heart at the same time.

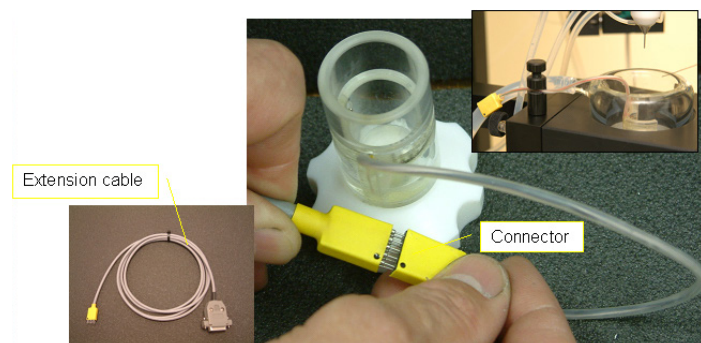


Fig. 46–Connect the MAP sensor to the extension cable.

3. Raise or lower the sensor to adjust the height of the sensor for better alignment of the heart and the electrodes (Fig. 47). Screw or unscrew the base from the sensor to change the height of the sensor as much as 7mm.

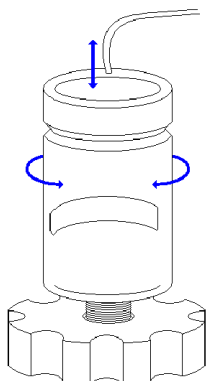


Fig. 47—Rotate the sensor to adjust its height.

4. If different sized hearts are going to be used on this system, or if the electrode array in the sensor is damaged, the sensor can be easily replaced by unscrewing the sensor from the base and attaching a new one (Fig. 48).

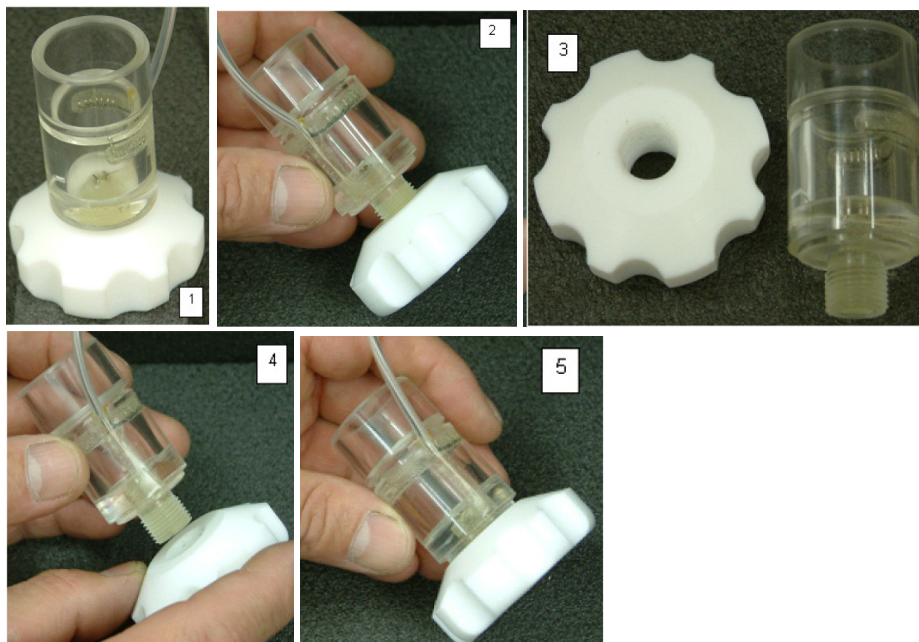


Fig. 48—Steps 1-5 show how to replace the electrode array of the sensor.

OPERATING INSTRUCTIONS

NOTE: A manual that describes the isolation, mounting and perfusion of a heart in a Langendorff or working heart system is available for investigators who are using one of the heart perfusion systems from WPI. Please contact technical support at technicalsupport@wpiinc.com or 866.606.1974 if you need this manual.

For information on the preparation and principles of the isolated heart, refer to the following references:

- Ferdinandy P, Szilvassy Z, Csont T, Csonka C, Nagy E, Koltai M, Dux L. *Nitroglycerin induced direct protection of the ischemic myocardium in isolated working hearts of rats with vascular tolerance to nitroglycerin*. Br J Pharmacol 115:1129-1131 (1995)
- Csonka C, Szilvassy Z, Fülöp F, Páli T, Blasig IE, Tosaki A, Schulz R, Ferdinandy P. *Classic preconditioning decreases the harmful accumulation of nitric oxide during ischemia and reperfusion in rat hearts*. Circulation 100:2260-2266 (1999)

Langendorff Retrograde Perfusion Mode

Helpful hints:

1. Before the heart is isolated, turn the valve tap to a position that allows a slow dripping of the buffer from aortic cannula.
2. Once the heart is removed from the ice cold perfusion buffer, mount it on the aortic cannula as soon as possible. No more than 30 seconds should expire between the time the heart leaves the ice cold buffer and is firmly attached to the cannula and being gently perfused with buffer.
3. Once the isolated heart is fed onto the aortic cannula of the heart suspension unit, tighten the ligature that secures the aorta to the cannula. Tie a second ligature to firmly attach the heart.
4. Once the heart is secured on the cannula, open the valve tap all the way to fully perfuse the heart.
5. If required, rotate the heart around the cannula to place the heart in a suitable position with the left ventricle on the left side suspension unit and the left atrium on the right side of the unit.
6. While the heart is being fully perfused in Langendorff mode, cannulate the left atria with the atrial cannula. To measure the left ventricular pressure, the atrial cannula needs to be inside the left ventricle, not just the pulmonary vein or the left atria. Once the heart is properly positioned, tie off any leaks around the atrial cannula with ligatures.



Neely Working Heart Mode

Helpful hints:

1. To switch from perfusion mode to working mode, close the Langendorff valve tap. Remove the forceps from the atrial inflow and the aortic outflow tubing so that buffer now enters the heart through the atrial cannula and exits through the aortic cannula.
2. Set the preload pressure on the fluid entering the left ventricle by lifting the atrial chamber so the outlet of the atrial chamber is about 180mm above the ventricle. In this case, 180mm H₂O (buffer) is equal to about 13mmHg.
3. Set the afterload pressure on the fluid pumped from the left ventricle by lifting the H-valve afterload assembly about 820mm above the aorta. In this case, 820mmH₂O (buffer) is equal to about 60mmHg.
4. Aortic flow, which is generated by the contractions of the heart, fills the afterload system and gives a constant aortic flow of 30–70mL/min. (rat) as measured by the mechanical flow meter in the perfusion circuit between the elastic chamber and the H-valve afterload assembly.
5. Coronary flow begins with the flow of buffer into the coronary arteries at the base of the aorta. The buffer moves out of the capillary beds at the ends of the coronary arteries and into the heart muscle. The fluid leaving the cardiac tissue, after coronary perfusion, drips off the heart and into the heart chamber. The coronary flow should be 15–30mL/min. (rat) as measured by collecting the volume of buffer dripping off the heart in a known period of time. Coronary flow depends on the size of the heart, the preload pressure and afterload pressure.



CAUTION: If the flow from the coronary arteries is low. Check the position of the cannula in the aorta. If the aortic cannula is too far into the aorta, the cannula blocks the entrances (ostia) of the coronary arteries and the flow of perfusion fluid into the coronary arteries and the cardiac musculature is reduced. If the reduction in coronary flow is significant, the cardiac muscles could suffer from hypoxia and start to die.

MAINTENANCE

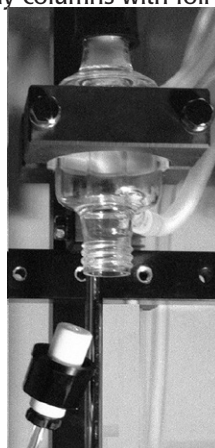
The system should be carefully cleaned every day when the experiment is finished. When used regularly, it is sufficient to rinse the perfusion circuit with deionized water 2 or 3 times, and then empty it. Cover the opening of the perfusion and Neely columns with foil or plastic wrap to keep dust out of the system.

Buffer solution, accumulating under the outlet stub of the perfusion (Langendorff) column, should be drained off by loosening the hollow nut (6 in Fig. 12) of the overflow tube. The inside of the elastic and atrial chambers can be drained or cleaned by loosening the nut and removing the plug at the bottom of each chamber (Fig. 49).

Fig. 49—(Right) Loosen the nut to drain the elastic or atrial chamber.

If there are deposits, like proteins, in the perfusion circuit, fill the circuit with a dilute (2%) solution of hydrogen peroxide. Leave the solution in the circuit for several hours. Then, rinse with 4–5 changes of deionized water.

Change the deionized water in the recirculating water bath every two weeks. It is advisable to put a small amount of germicide, like 0.01% ROCCAL, in the water.



QUICK REFERENCE CHECKLIST

Carefully read the directions in "Setup" on page 5, "Plumbing the System" on page 14, "Filling The System" on page 19, and "Installing the Sensors" on page 22 before using the following quick reference list.

1. Check if all tubes are properly connected.
2. Check the position of all taps and cocks.
3. Check if all pumps and measuring devices are properly attached to the system.
4. Check if electric current supply is proper and switched on.
5. Fill up the perfusion reservoir with approximately 5L of solution. Start the filling pump and fill up the perfusion buffer carboy.
6. Fill up the recirculating water bath with distilled water and set the proper temperature.
7. Start the recirculating water bath to warm up the water jacket.
8. Start bubbling the perfusion fluid with carbogen gas. The intensity of the bubbling should be adjusted by the regulators to be even and continuous.
9. Remove air bubbles from the perfusion system.
10. Switch on all control and recording devices.
11. Check the calibration of the sensors being used.
12. Check the speed of the precision pump.
13. Prepare the heart.
14. Attach the prepared heart to the system.
15. Start the perfusion of the isolated heart by turning the tap valve to the Open position.



APPENDIX A: TUBING INFORMATION

Mark	Name	Mark	Name
A	Perfusion column	J	Heart suspending unit
B	Neely column	K	Heart chamber
C	Elastic chamber	L	Perfusion pump
D	Atrial chamber	M	Filter
E	Afterload	N	Termostate
F	Flow meter	O	Experimetria gasreductor
G	Pump to fill perfusion column	P	Carbogen tank
H	Reservoir	Q	Pressure equilazer of water jacket
I	Two way teflon cock		

No. of tubing	Inner Diameter mm	Length cm	No. of tubing	Inner Diameter mm	Length cm
1	6	150	15	6	8
2	3	135	16	3	19
3	3	35	17	6	125
4	6	70	18	6	30
5	6	150	19	8	250
6	150	6	20	8	80
7	6	20	21	8	65
8	6	20	22	8	150
9	3	15	23	8	10
10	6	25	24	8	30
11	3	55	24a	3	27
11A			24b	3	27
12	6	75	25	8	20
12A			26	8	250
13	6	65			
14	6	100			

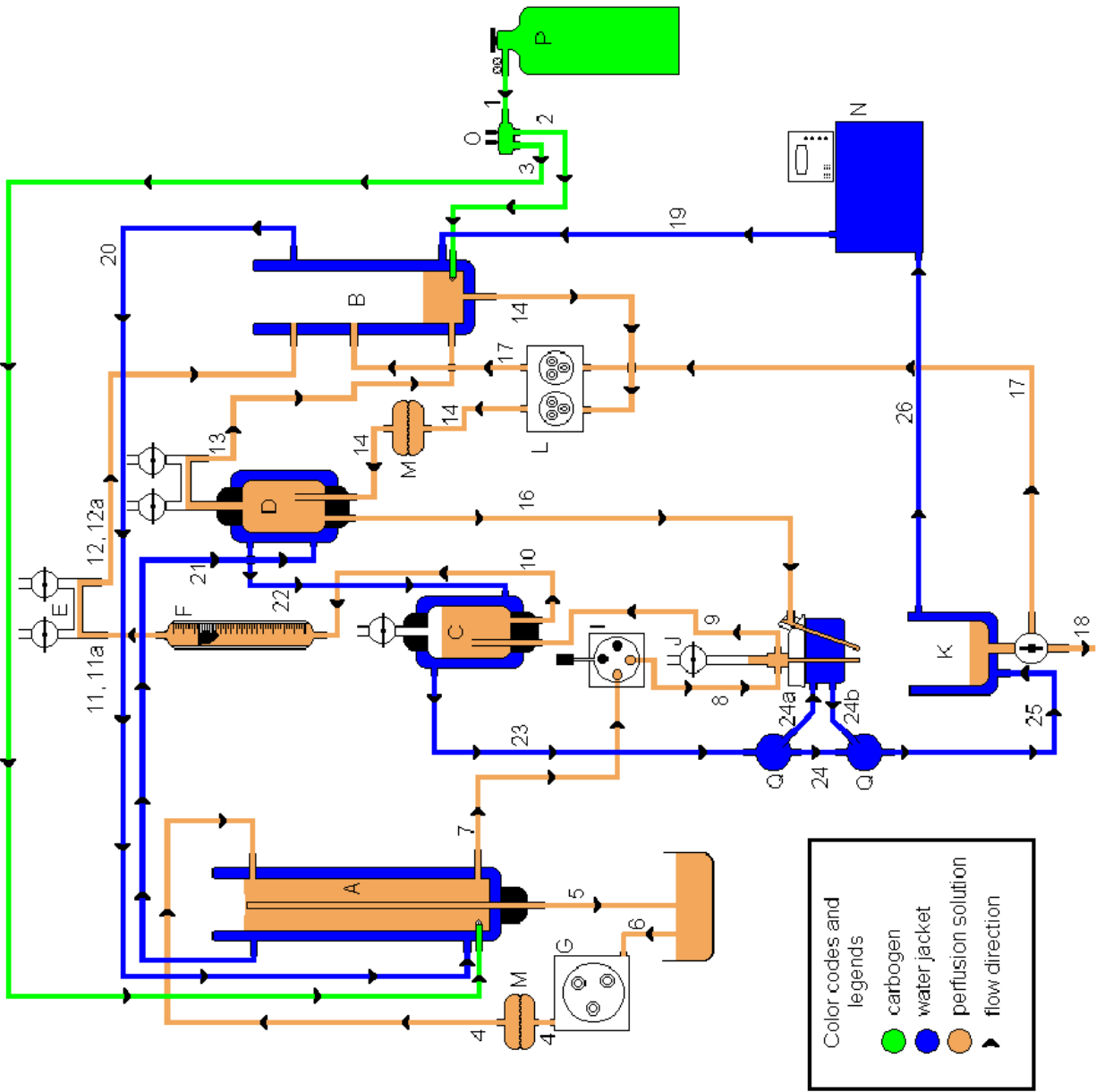


Fig. 50–The letters on this graphic correlate with the tables on the previous page.



APPENDIX B: EPP-01 PERISTALTIC PUMP

General Description

This peristaltic pump (Fig. 51) is used to fill the perfusion column during Langendorff mode. The pump is self-priming and is capable of moving mediums with a wide variety of viscosities from air to heavy slurries. Since the medium being pumped only comes into contact with the tubing, the pump does not become contaminated. The tubing is easily changed so the pump is ideal for use on a variety of jobs requiring the quick and precise movement of diverse materials.

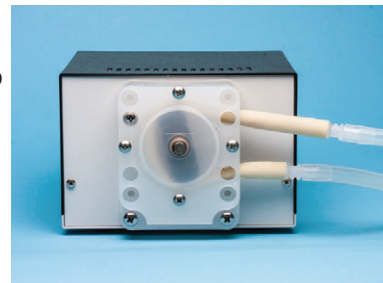


Fig. 51–(Right) Peristaltic pump used to fill the perfusion column.

Specifications

Priming: Self-priming and will hold vacuum when off.

Operation: Pump can run dry without damage

Capacity: From 3mL/min to 987mL/min (see model information chart)

Construction: High strength plastics and long-lasting alloy metals

Maximum Head Pressure: 57 feet of water

Maximum Suction Lift: 28 feet of water

Maximum System Pressure: 20psi continuous, 25psi intermittent.

Certification: All motors are UL listed

Rating: All gear motors are rated for 100% continuous duty

Installation Instructions

Power Requirements: Use power source (voltage & frequency) specified on the unit.

Wiring Connections: All wiring and electrical connections must comply with national electrical codes and local electrical codes.

Placement: Case enclosed model may be placed on any flat surface, assuring space is provided at the back of the case for air circulation through ventilation holes. The pump should be used in a dry location with an adequate supply of cool air. The ambient temperature should not exceed 25°C.



CAUTION: This unit should not be used outdoors or in hazardous locations.

Use the following color coded rollers with the appropriate tubing ID:

Roller Color	Tubing ID	mL per Revolution
Black	1/4"	3.5
Black	3/16"	2.1
Red	1/8"	0.84
Red	1/16"	0.21

Tubing Inspection

Inspect all tubing regularly and replace it if deterioration occurs. Use the following instructions to replace pump tubing.

Tubing Replacement

1. Disconnect the power to the pump.
2. Disconnect the suction and discharge tubes of the system from the tubing between the rollers of the pump.
3. Remove the four screws that hold the pump cover in place. Remove the cover.
4. Remove old pump tubing and discard.
5. Clean roller race, removing any particles that could damage tubing.
6. Position the roller bracket assembly as shown in Fig. 52-1.
7. Push the new tubing into the inlet port anchoring the tubing in grippers (Fig. 52-2) while rotating the roller bracket assembly.
8. Continue to rotate the roller bracket assembly, pushing the tubing into the roller race (Fig. 52-3).
9. Finally, insert the tubing into the outer port (Fig. 52-4), and replace the cover and screws.

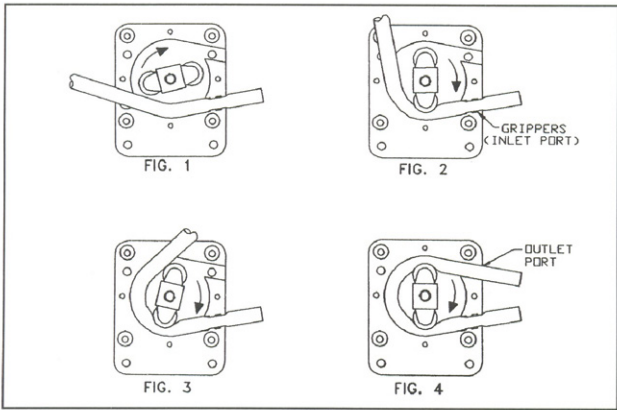


Fig. 52- (Right) Instructions for replacing the tubing.

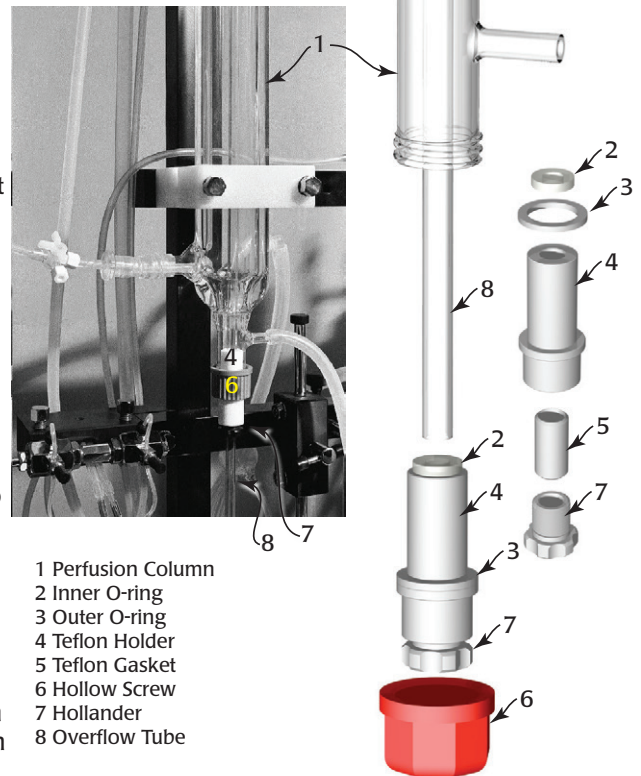
APPENDIX C: ASSEMBLING OPTIONAL SECONDARY PERFUSION COLUMN

Assemble the Perfusion Column and the Overflow Tube

1. Carefully remove the perfusion column and the overflow tube from the packing box. Lay them down on a work table and make sure that they are safe. The overflow tube has already been fitted with parts (1–5, 7 in Fig. 53) that are needed for mounting it in the perfusion column, and a silicone grommet near its top to protect the tube if it touches the inside of the column.

Fig. 53--(Right) This exploded diagram shows the parts of the perfusion column.

2. The perfusion column has already been fitted with a hollow nut (6 in Fig. 53) on the bottom of the column that is used to secure the overflow tube in the column. Remove the hollow nut from the column.
3. Carefully feed the upper end of the overflow tube, with its grommet, through the opening in the bottom of the perfusion column until the shoulder of the Teflon tube support (4 in Fig. 53) touches the bottom of the column. Reinstall the hollow nut over the end of the overflow tube and onto the bottom of the perfusion column. Finger-tighten the hollow nut to secure the overflow tube in the column.



1 Perfusion Column
2 Inner O-ring
3 Outer O-ring
4 Teflon Holder
5 Teflon Gasket
6 Hollow Screw
7 Hollander
8 Overflow Tube



CAUTION: Be sure the inner (2) and outer (3) O-rings and the Teflon gasket (5) are installed on the tube support (4) before assembling the perfusion column.

4. The height of the overflow tube sets the pressure used to perfuse the heart in Langendorff mode. To adjust the height of the overflow tube, loosen the knurled nut (7 in Fig. 53) and carefully slide the overflow tube to the correct position. If the tube does not slide, loosen the knurled nut a little more. Once the tube is in position, finger-tighten the knurled nut to secure the overflow tube.

Mount the Assembled Second Perfusion Column

1. Drain the buffer and water from the perfusion column already mounted to the frame rack (Fig. 54).

Fig. 54–(Right) Mounting the second perfusion column.

2. Locate the extra set of clamps (2), silicone and tape strips, and screws for mounting the second perfusion column.
3. Remove the cap screw from one side of each new clamp (Fig. 55) to open the clamp.



Fig. 55–Remove the cap screw to open the clamp.

4. Find the hole in the center of the back half of each clamp.
5. On the back of the frame rack, locate the single Allen screw on the back of the frame rack that holds the lower clamp of the original perfusion column to the frame (Fig. 54). With the assistance of another person, who is holding the perfusion column, remove this Allen screw.
6. Carefully position the back of the new lower clamp over the hole where the Allen screw used to be (Fig. 56). Use the proper length screw to mount the back of the new column clamp and the lower clamp of the original perfusion column to the frame rack. Tighten the screw to hold the two clamps firmly in place.



Fig. 56–Install the new clamp.

7. Repeat the procedure for the upper clamp, making sure that the original perfusion column is stable while the new clamp is attached.





8. Mount the silicone strips in the front and back halves of both new clamps (Fig. 57). The silicone strip cushions the column within the clamp and prevents it from cracking.

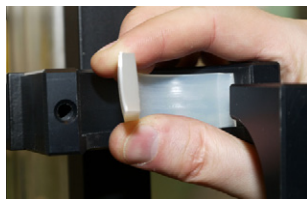


Fig. 57—Mount the silicone strips.

9. Place a strip of Mylar tape over each silicone strip (Fig. 58). The tape prevents the new glass column from sticking to the silicone strip; this permits the position of the column to be adjusted, if needed.

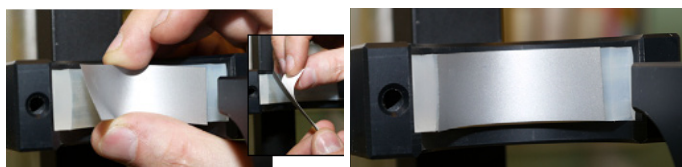


Fig. 58—Place a strip of Mylar tape over the silicone strip.

Fig. 59—(Left) Align the perfusion columns.

10. With the assistance of another person, align the new perfusion column so that it is the same height as the original column (Fig. 59).
11. Hold the new perfusion column in position against the backs of the newly mounted clamps.
12. Place the front half of upper clamp over the column and finger-tighten the cap screws on the front of the clamp (Fig. 60). Do not use a wrench to tighten the screws. Repeat the procedure for the lower clamp on the column.

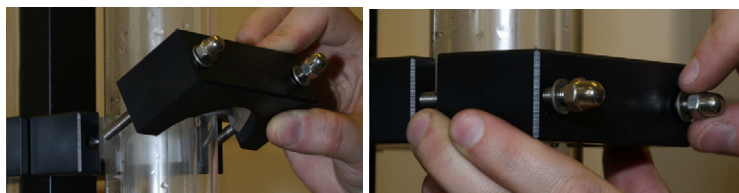


Fig. 60—Install and secure the front half of the clamps.

Replumb the System

Turn off the recirculating water bath, the fill pump and the constant flow pump. Disconnect these pumps and bath from their power sources. Drain the water from the whole temperature regulation circuit and the buffer from whole perfusion buffer circuit. Turn off the carbogen circuit at the regulator on the carbogen cylinder.

Carbogen Circuit

1. Locate the extra piece of silicone aeration tubing with a plastic T-connector already attached. Place the open end of the tubing on the connector and stopcock of the aeration inlet for the second perfusion column.
2. Cut the silicone tubing that runs from the regulator on the aeration manifold to the aeration inlet of the original perfusion column about 15–20cm away from the column (Fig. 61).
3. Connect the tubing on the inlet of the original perfusion column on the open side of the T-connector (Fig. 62). Connect the tubing coming from the regulator on the manifold to the last open end of the T-connector (Fig. 63).

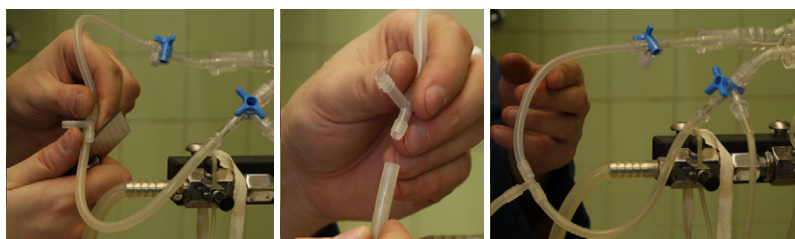


Fig. 61–(Left) Cut the tubing 15–20cm away from the column.

Fig. 62–(Center) Connect the tubing

Fig. 63–(Right) Connect the side barb of the T-connector.

5. The carbogen circuit should now be configured as diagrammed in Figure 65.

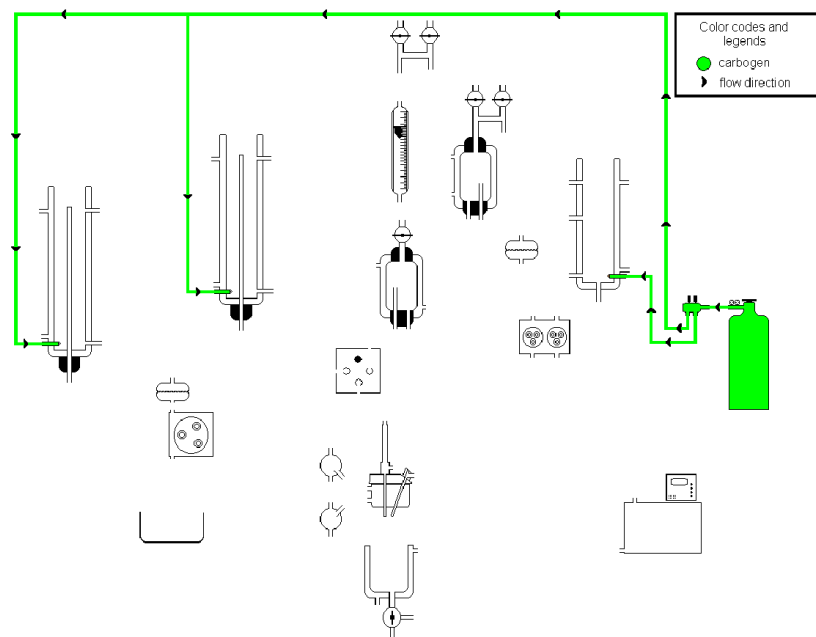


Fig. 64–The new carbogen circuit.

Temperature Regulation Circuit

1. Locate the extra piece of silicone tubing that will connect the water jacket of the second perfusion column to the temperature regulation circuit.

! CAUTION: Do not attempt to pull silicone tubing off any of the inlets and outlets of the columns or chambers. Excessive pressure on any of these ports may cause the port or its vessel to break. It is better to cut the tubing off the port with a sharp blade, and then spilt the remaining piece to release it from the port.

2. Carefully remove the silicone tubing from the water jacket inlet of the atrial chamber (1 on Fig. 65). Use a scalpel or razor blade to cut the tubing off at the inlet. Use the blade to split and remove the piece of tubing remaining on the inlet.
3. Connect the newly opened end of this tube to the water jacket inlet on the bottom of the second perfusion column (4 on Fig. 65). The water jacket outlet of the first perfusion column (2 on Fig. 65) is now connected to the inlet of the second column (4).
4. Connect the extra piece of silicone tubing to the water jacket outlet at the top of the second perfusion column (3 on Fig. 65). Connect the open end of this tubing to the water jacket inlet of the atrial chamber (1 on Fig. 65).
5. Cut the silicone tubing that runs from the regulator on the aeration manifold to the aeration inlet of the original perfusion column about 15–20cm away from the column.

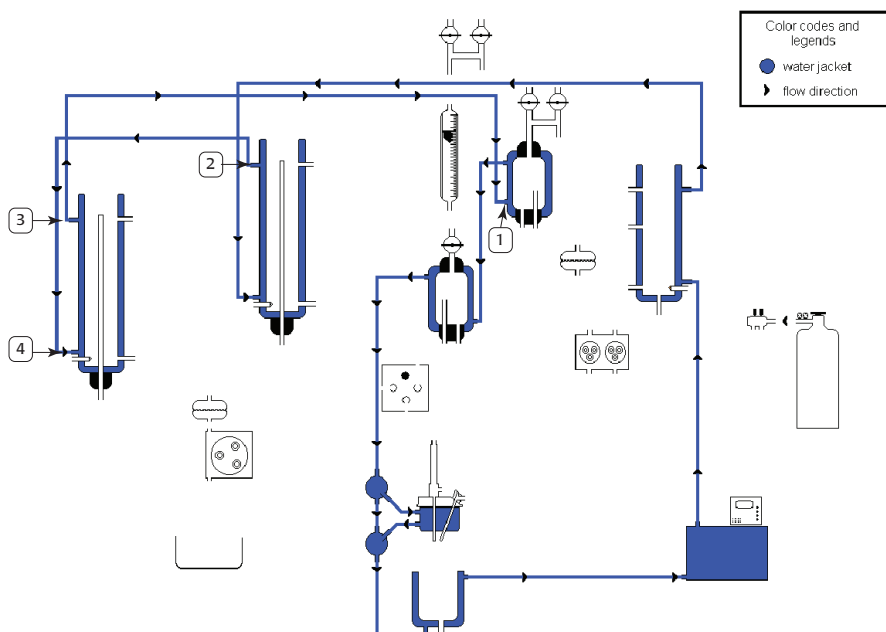


Fig. 65–Water Jacket plumbing diagram.

Perfusion Circuit

1. Locate the large Y- or T-connector and the three extra pieces (1 short, 2 long) of silicone tubing designated for the perfusion circuit.
2. From the longer pieces of silicone tubing, determine the one that fits best between the outlet of the overflow tube in the second perfusion column and the carboy filled with fresh perfusion buffer. This tube should not go below the surface of the buffer when the carboy is filled. Connect the correct tubing (1 on Fig. 71) to the end of the overflow tube. Feed the other end of the tubing through hole in the tray top and into the top of the buffer carboy, just like the overflow tubing from the original perfusion column.
3. Cut the tubing that connects the filter to the buffer inlet of the original perfusion column (2 on Fig. 71) at the level of the buffer inlet of the second column (Fig. 66).
4. Connect the two cut ends of this tubing to two of the openings of the Y- or T-connector. Connect the short piece of silicone tubing between the buffer inlet of the second perfusion column and the remaining open end of the Y- or T-connector.

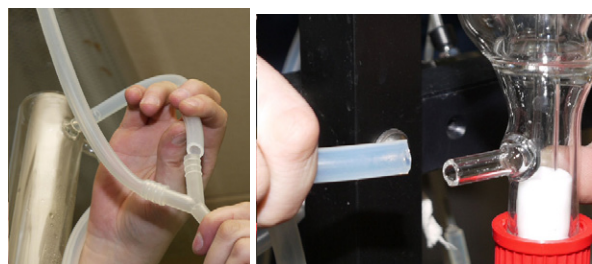


Fig. 66--(Left) Connect the tubing.

Fig. 67--(Right) Connect the buffer outlet of the second perfusion column.

NOTE: Both columns are interconnected and draw buffer from the same source, so the purpose of the second column is to perfuse the heart at a different pressure.

5. Connect the other long piece of silicone tubing to the buffer outlet of the second perfusion column (3 on Fig. 71, Fig. 67). The open end of this tube will be connected to an inlet on the valve tap (4 on Fig. 71)
6. Remove the Teflon plug from the second inlet on the back of the valve tap (4 on Fig. 68). To gain access to the back of the valve tap, it is helpful to loosen and move the holders for the pressure sensors out of the way (Fig. 69). Firmly connect the open end of the outlet tubing from the second perfusion column (3 on Fig. 71) to second inlet of the valve tap (4 on Fig. 68).

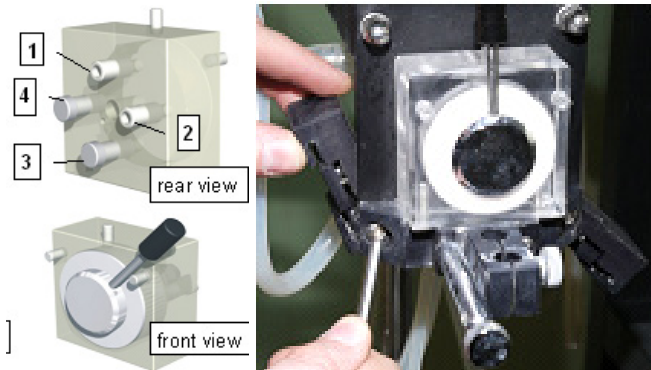


Fig. 68–(Left) Tap configuration.

Fig. 69–(Right) Loosen the holders for the pressure sensors and move it out of the way to more easily access the back of the valve tap.

7. The positions on the valve tap (Fig. 70) can be placed to close flow to the aortic cannula (center), open flow from the first column (left), or open flow from the second column (right). The second column is used primarily to perfuse the heart at a different pressure by setting its overflow tube to a different height.

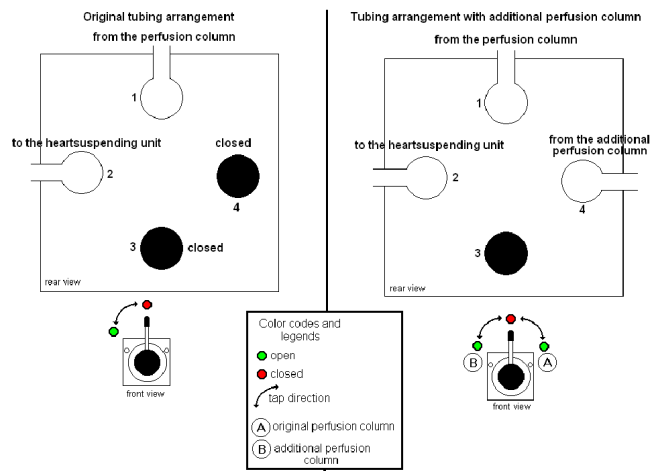


Fig. 70–Positions of the valve tap.

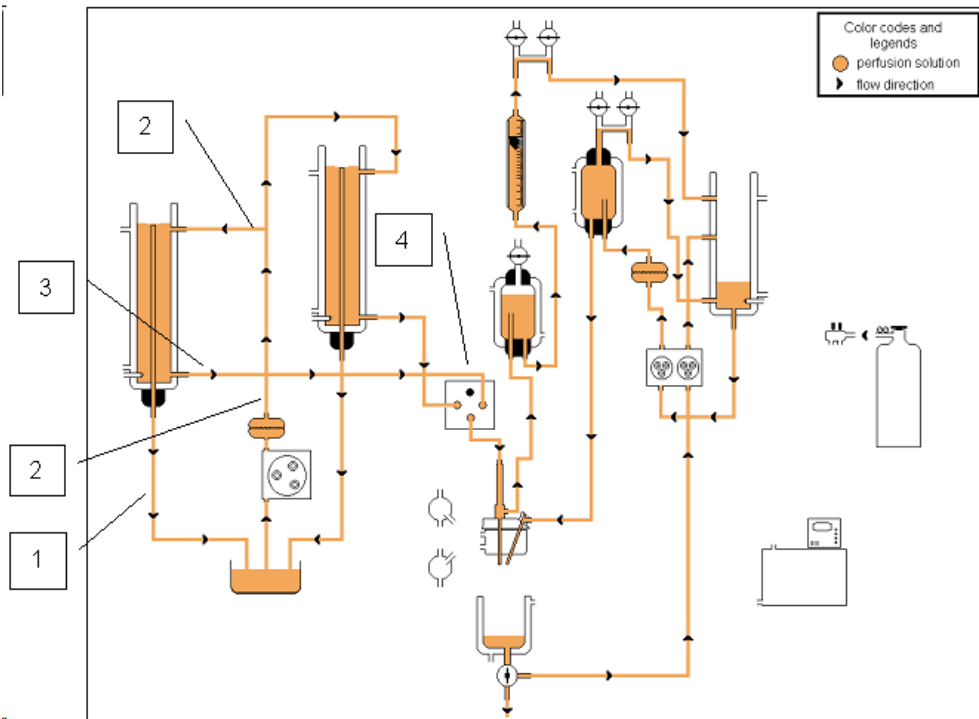


Fig. 71–The new perfusion circuit is shown schematically.

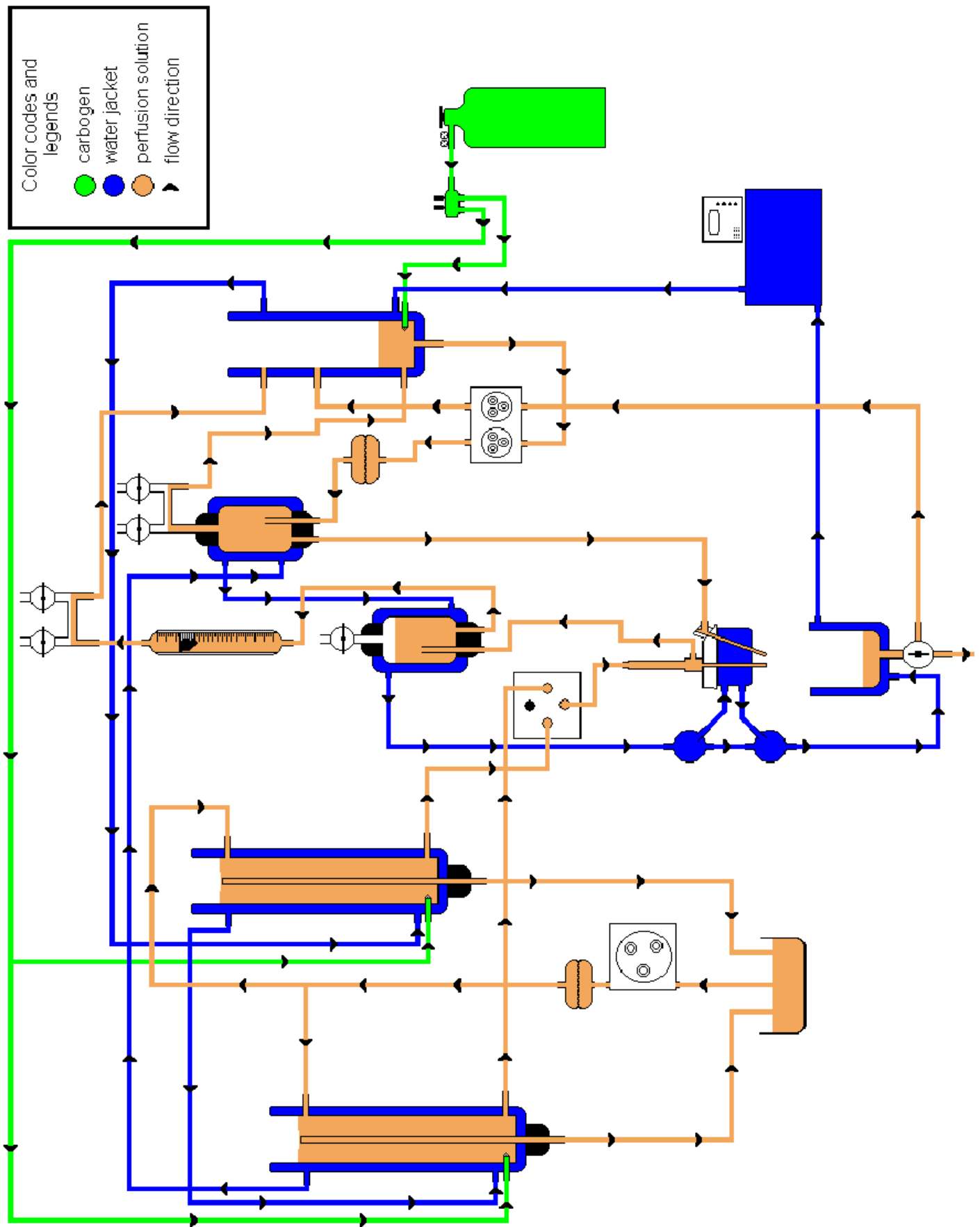


Fig. 72–The new plumbing configuration is shown schematically.

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