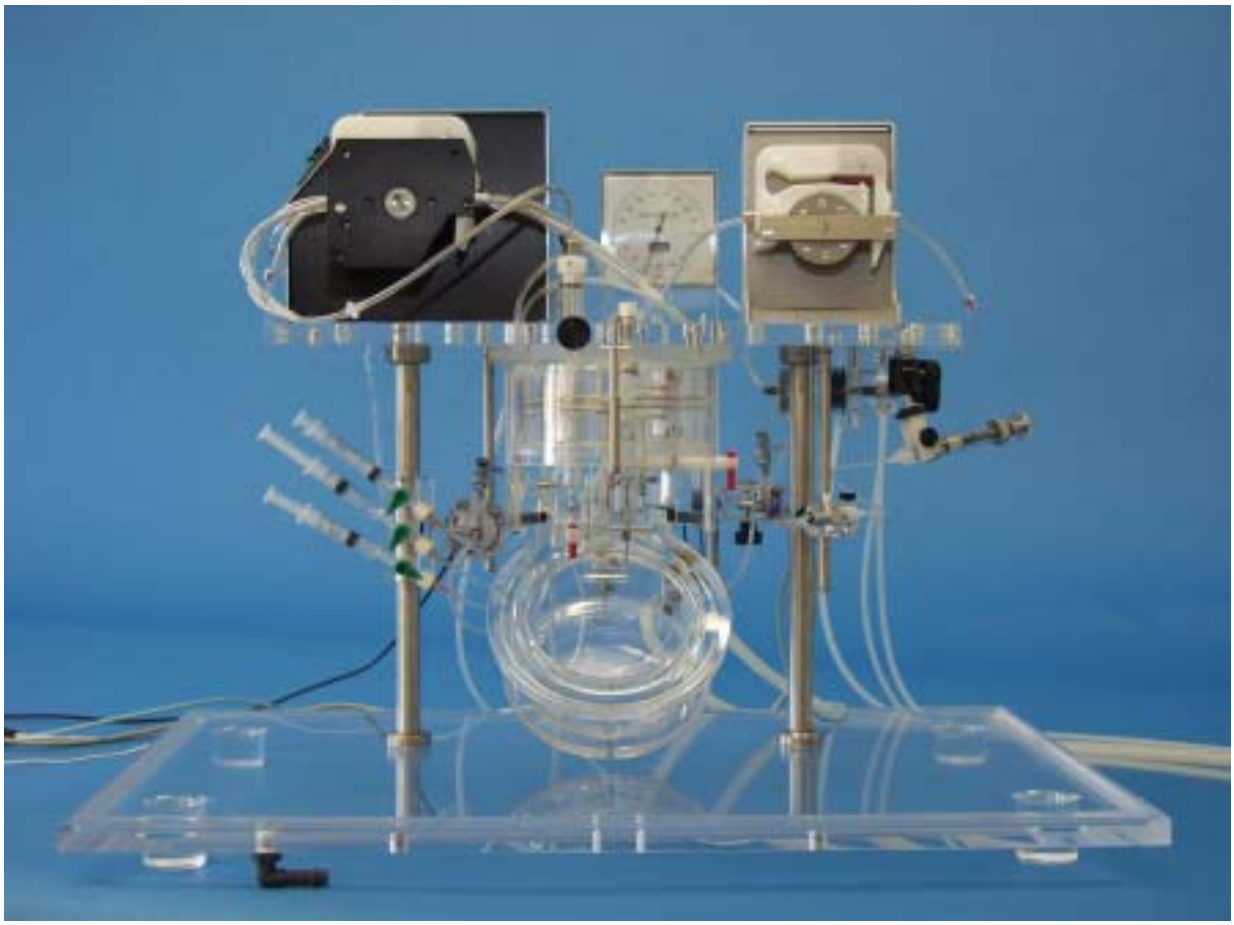
Operating Instructions

for the

ISOLATED HEART FOR SMALL RODENTS
IH-SR, Type 844

Perfusion system for isolated hearts of mice, hamsters, rats or guinea pigs

(Version 2.1 / PB, TB / Printed: April 2010)



NOT FOR HUMAN USE

Contents

1. Manufacturer's details

These operating instructions describe the function of the Isolated Heart for Small Rodents (IH-SR), Type 844. They are a part of the equipment and should be kept closed to it.
All the information in these instruction have been drawn up after careful eximation but do not represent a warranty of product properties. Alterations in line with technical progress are reserved.

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1.1 Copyright

1.2 Reservation

The design of the apparatus Isolated Heart for Small Rodents (IH-SR) is not a fully complete and static construct. It is a modular system and can be expanded. It represents essentially a development of the existing apparatus for larger hearts (IH-5) to adapt it to the conditions for experiments on the isolated heart of mice, hamsters, rats or guinea pigs or small hearts of newborn species which are in a similar size. In order to ensure that the apparatus meets the increasing demands of the user into the future it will in future be necessary to introduce changes and make additions in order to improve its practical application.

It is unfortunately not possible to incorporate every minor change into this manual. It is therefore unavoidable that there will be slight differences between the apparatus and the manual. Where there are extensive modifications to the apparatus there will of course be either suitable corrections to the manual or additional sheets will be provided.

If particular problems can not be resolved by using this manual, please contact the manufacturer HSE either in writing, by fax or by phone (for address see above). **Criticism is always welcome!**

This manual is being written at a time when there has been several years' experience with Working Heart apparatus for rats, guinea-pigs, up to small pigs and mice. The author has attempted to draw on the information available to him and to include the relevant details in this manual. He is however fully aware that he has not dealt with all aspects which are important when working with the apparatus. Other areas, perhaps less important, have been over-emphasized.

In order in the course of time to improve both the apparatus and also this manual we depend on appropriate information and suggestions from you. We therefore ask you, the user, for constructive criticism.

- What is missing?
- What is explained badly or incorrectly?
- What should be described in greater detail?
- What illustrations or drawings should be added?
- What should be omitted?

We shall be grateful for every suggestion and every information. Please tell us what improvements can be made. Thank you.

1.3 Limitations

This manual does not apply to additional equipment required for operating the apparatus. Problems concerning the installation and operation of such equipment have to be resolved using the individual operating instructions.

It is unavoidable that this manual refers to operation and functions of additional equipment. This does not mean that it replaces the information given in the individual operating instructions. In particular it is essential to observe the safety notes in the original operating instructions.

1.4 Safety notes

Do not use flammable or explosive liquids or gases, fire hazard! Combustible substances represent a fire hazard in combination with Carbogen (95% Oxygen, 5%CO2).

Toxic substances:
When using toxic substances (gas, test substances, cleaning agents) it is necessary to observe the appropriate regulations for use in order to avoid health hazards to the user. Proper disposal of toxic substances is also essential !

Use only undamaged and dry mains power cables!

Electric equipment is generally designed to Class 1 protection (metal housing connected to the ground contact of the mains supply). It must only be connected to correctly installed socket outlets with ground contact. In case of doubt, ask a qulified electrician! Damaged socket outlet should be repaired immediately and must not be used. Always observe and adhere to these precautions in order to ensure your own safety.

Protect electrical equipment against moisture:
Never place electrical equipment underneath stored liquids!
Do not position electrical equipment near a water tap (danger of splashing!)!
Electrical equipment should only be operated with dry hands!
Electrical equipment has to be set up and operated in accordance with their Operating Instructions. In setting up the equipment it is particularly important to ensure that no liquid can find its way into the equipment. Therefore do not store any liquids (e.g. perfusate) above such equipment
If any liquid has found its way into the equipment or even if this is only suspected, do not switch on the equipment. Immediately pull out the mains plug and have the equipment checked by an electrician.

Avoid operating the equipment with wet hands. Electrical contacts and perfusion solution are not compatible over longer periods. Additionally the equipment retains its good appearance over longer periods of time. Who likes the looks of a salt-encrusted or dirty front panel ?

2. Unpacking, setting up and installation of the apparatus

2.1 Unpacking

Normally you are only able to read this manual after you have opened the package. If you had found that the package shows externally visible transport damage, it is essential that you record this in accordance with the note “Important information for claims!” which is included with the despatch papers, in order to provide for a possible insurance claim, preferably including the name of a witness.
In the time of digital cameras it is highly recommended to take a photo of the instruments and the packing if you received something damaged.

2.2 Location

The location should already have been selected before you unpack the apparatus.
The apparatus requires a flat horizontal lab table. If you do not have ordered an HSE subframe you require some other flat horizontal surface, at least 150 cm x 60 cm, preferably at normal bench height (75 - 85 cm above floor level). It is highly recommended to have a shelf for the amplifier unit above or beside the apparatus.

2.3 After unpacking

After removal from the packing, place the apparatus on a sufficiently large bench and carefully unravel all tubing and cables. **Do not use any force!** Don´t pull on the tubing or on cables or operating levers. Remember that the apparatus contains delicate and fragile components in addition to strong and rugged ones.

First examine the apparatus visually and check for damaged components. If you find any damage you should prepare a report for a possible insurance claim, preferably in the presence of a witness, and advise the supplier and, if you suspect any transport damage, also the transport organisation.

If you find that something is missing, it is absolutely essential that you first check whether it is in the packing material. Every apparatus is subjected to a trial run by the manufacturer so that it is very unlikely that any ordered part has not been supplied.

2.4 Setting up, details of location

The area provided for the apparatus should not be too small. There must be adequate room around the actual apparatus so that the additional equipment required for operating can be positioned properly. These include for example the following equipment and items:

- Thermocirculator (can be placed at the bottom on the floor or at bench height if no powerful circulating pump is fitted)
- Shelf for the amplifier unit, if possible above the „wet area“
- Electronic measuring and control equipment and recording instrumentation (arrange close at hand and at a suitable level for good visibility)
- Operating table for organ preparation (preferably on the right, next to the Plexiglass stand).

The operation of the apparatus requires a suitable gas supply (usually Carbogen). This may be a connecting point to a central supply system or alternatively a gas cylinder with a suitable pressure regulator or reducing valve. The supplied pressure should be in the 0.5bar to 1bar range.

Also useful is a water tap close to the selected location. But ensure that electrically operated equipment is so set up that it can not be affected by splashing water.

To take the spent perfusate and cleaning solution you require a sufficiently large waste container underneath the apparatus, e.g. a 20 litre plastic can.

2.5 Arrangement and installation

First carefully unravel all the tubing and electrical cable and connect them up to suit the apparatus version in accordance with the appropriate diagram (see 2 and 4). Connect up the thermostating system so that the circulating water flows through the different jacketted vessels from the bottom upwards.

Set up the pumps and measuring instruments required for operation and connect them up in accordance with the appropriate Operating Instructions. Arrange the connecting lines to suit local conditions, at the back around the apparatus so that they do not interfere at the front with the actual experiment later. Mount the pressure transducers on the holders provided.

The connections which are still hanging around, should only be secured when everything has found its final position. Select the fixation points so that if possible no mechanical vibrations can be transmitted to the apparatus through the connections.

Tubing
Figure 1 shows the tubing recommended by HSE.

Normally your apparatus is shipped with all these tubings attached ready for use. The apparatus has been tested prior to shipment to ensure proper function and quality of the apparatus.

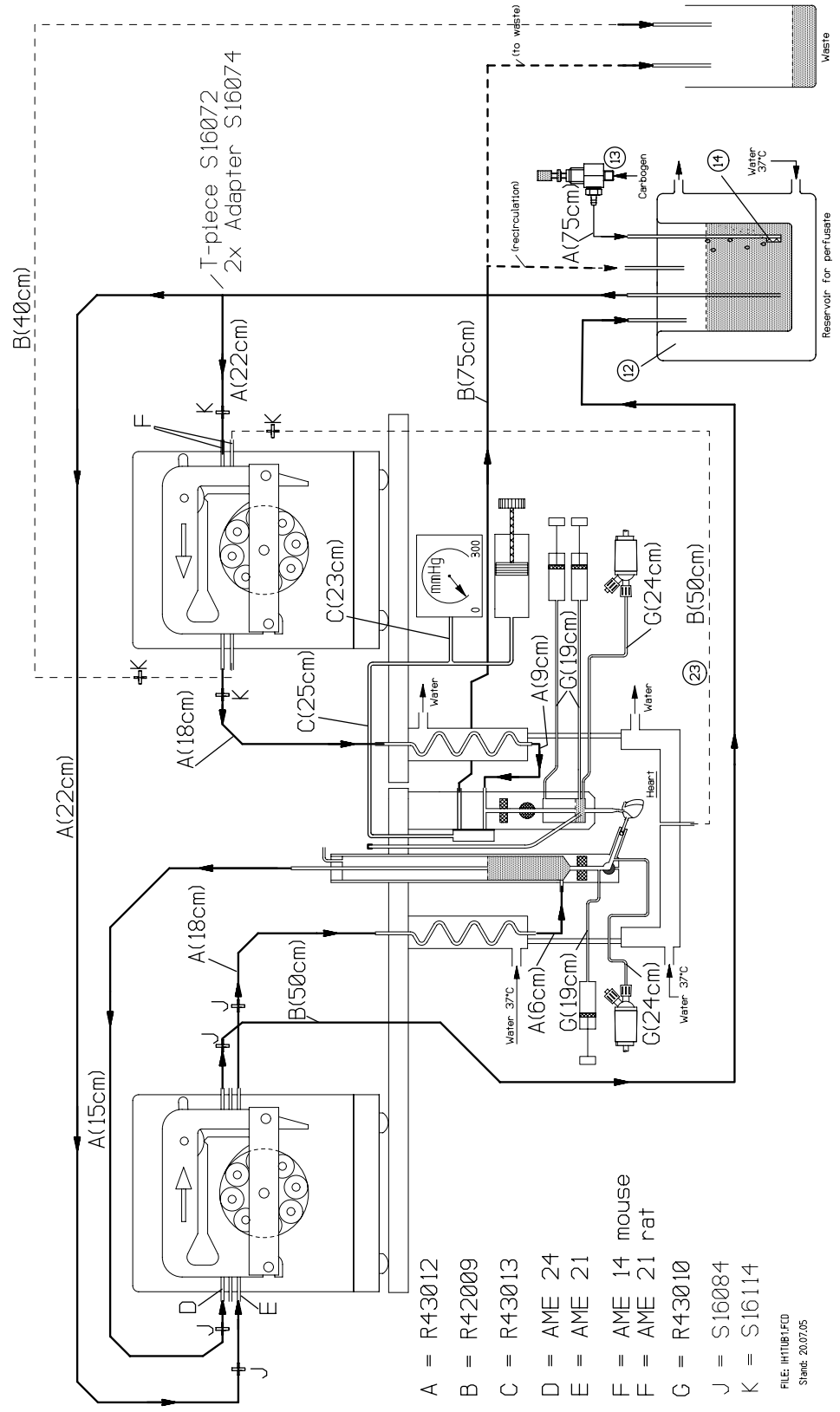


Fig. 1: IH-SR, Recommended tubing. For explanation see next page.

Explanation for the letters in Fig. 1:

	OD (mm)	ID (mm)	Material	HSE-No.	Note
A	3.78	2.06	Tygon	R43012	
B	4.00	2.00	Silicone	R42009	
C	4,8	3,2	Tygon	R43013	
D	4.51	2.79	Tygon	Z92007	pump tubing AME24 (purple-white)
E	3.80	2.10	Tygon	Z92006	pump tubing AME21 (purple-purple)
F	2.90	1.22	Tygon	Z92005	pump tubing AME14 (red-grey)
G	2.86	1.14	Tygon	R43010	
J				S16084	tubing adapter

S16084, Tube to Tube Adapter D=2.5mm / D=2.5mm

K				S16114	tubing adapter

S16114, Tube to Tube Adapter D=2,5 / D=1,5

OD = outer diameter ID = inner diameter

The numbers after the letter indicate the tubing length in cm. The pump tubings (D, E and F) are 40 cm long. The connection to the necessary extension tubing is made with tubing adapters (J, K).

3. Introduction

3.1 Application

This apparatus is intended for work on isolated hearts of small rodents like mice, rats, hamsters or guinea pigs (also hearts of newborn larger species could be attached). By exchanging the cannulae and the pump tubing the IH-SR can easily be adapted to the hearts.
The apparatus is available in two versions: **LH = LANGENDORFF Heart** and **WH = Working Heart**. Both versions allow easy conversion between constant flow and constant pressure conditions. The WH version of the apparatus allows isolated heart experiments in both LH or WH mode. The preparation of a WH is always done in the retrograde LH mode. Each LH configuration can be upgraded easily to a WH configuration later on.

The isolated perfused heart is a widely used method covering a large range of applications in physiological and pharmacological research as well as in safety pharmacology and toxicoligy. Although the method is used mainly with acute acting substances it can of course also be used on hearts of pre-treated animals in conjunction with acute acting substances.

Applications, examples

Investigations of mechanical cardiac parameters
- Inotropic effects [contraction force (p_s, p_d), contractility (dp/dt_{max})]
- Chronotropic effects (heart rate)
- Coronary-musculotropic effects (coronary flow)
- Heart work and efficiency

Investigations of bioelectric parameters
- Dromotropic effects (EG analysis)
- Bathmotropic effects (refractory period)

Investigations of metabolites
- Concentration of energetic phosphate compounds through freeze stop or NMR
- Measurement of O_2 consumption, glucose and other substances
- Concentration (activity) of Na^+, K^+, Ca^{++} in myocardium and perfusate
- Enzyme and/or transmitter release from the myocardium
- Fluorimetric measurement of intracellular free Ca^{++}

3.2 Versions of the apparatus

The apparatus is available in two versions: **LH = LangendorffHeart**, and **WH = Working Heart**. In version WH the heart can be operated in both modes. The changeover from one operating mode to the other is made by a simple manual operation and requires no conversion of the apparatus.

3.2.1 Operating modes

Version LH: only Langendorff mode (retrograde perfusion) under constant pressure or constant flow
Version WH: both Working Heart mode (Working Heart according to Neely) and also Langendorff mode under constant pressure or constant flow.

In the Working Heart mode the heart actually performs pressure-volume work (the heart is ejecting); i. e. the left venticle pumps liquid received from the left atrium through the aortic cannula into the apparatus where a specific membrane system simulates the peripherical afterload. This is different from the Langendorff system where the heart is supplid retrograde with the perfusion solution through the coronaries. The heart does not perform any measureable volume work (ejection) in the Langendorff mode. A balloon in the left ventricle can be used as isovolumetric load. This permits to measure isovolumetric Left Ventricular Pressure LVP and a the first derivision dLVP/dt.

3.3 Nominal data of the apparatus

	mouse	**rat / guinea pig**
Aorta, ID:	0.8 - 1.5 mm	2.5 - 3.5 mm
Pulmonary artery, ID:	0.8 - 1.5 mm	2.5 - 3.5 mm
Aortic flow:	up to 25 ml/min	up to 60 ml/min
Aortic pressure:	up to 300 mmHg	up to 300 mmHg
Atrial pressure:	up to 10 mmHg approx.	up to 10 mmHg approx.

Aterial pressure can be increased with additional hardware (73-0158) up to 30mmHg for high preload studies.

4. Functional description of the apparatus

4.1 General description

The IH-SR is available in a Langendorff (see Fig. 2 and 3) and in a Working Heart configuration (see Fig. 4 and 5). The principle of operation can be seen from the schematic diagrams. The apparatus consists of a basic modular setup which can be flexibly adapted to the requirements and wishes of the user by the addition or omission of components or measuring systems.

The basic setup is very compact. Except for the water circulating thermostat and the measuring instruments, all the parts shown in the two illustrations are mounted on the basic Plexiglass stand. In the design of the apparatus special attention has been paid to maintaining a constant defined temperature of the organ. Critical components in this respect are therefore located inside the thermostated heart chamber so that any cooling through a temperature gradient to ambient temperature is avoided.

The connections to the heart are made through interchangeable aorta and atrium cannulae. Aeration of the perfusate is ensured by introducing gas into the reservoir. When using perfusates which tends to foam, the use of a membrane fiber oxygenator is recommended (see Section ?).

4.2 The IH-SR Langendorff Heart (LH)

In the Langendorff mode the heart beats “empty”, it does not perform any pressure-volume work (no ejection). The coronaries are supplied by retrograde perfusion through the aorta.

Perfusion can be performed at constant pressure and also at constant flow.

The system consists of a roller pump (1), a jacketed and thermostated heart chamber and an aorta block. In addition there is usually a device for measuring the LVP by the so-called isovolumetric balloon method.

The most important component is the adjustable flow resistor on the aorta block which is used to set the required perfusion pressure. The required pressure is selected on the rotary knob (10) of the pressure regulator and indicated on the pressure gauge (2) (set value). The roller pump pumps the perfusate into the aorta block (7) and generates the required perfusion pressure. When the pressure in the aorta block reaches the set value, the flow resistor opens up the return line to the reservoir and regulates the perfusion pressure. By suitable adjustment of the pump output and the flow resistor the heart can be perfused either under constant-pressure or constant-flow conditions. Figure 2 shows an overview of the IS-HR, Langendorff system.

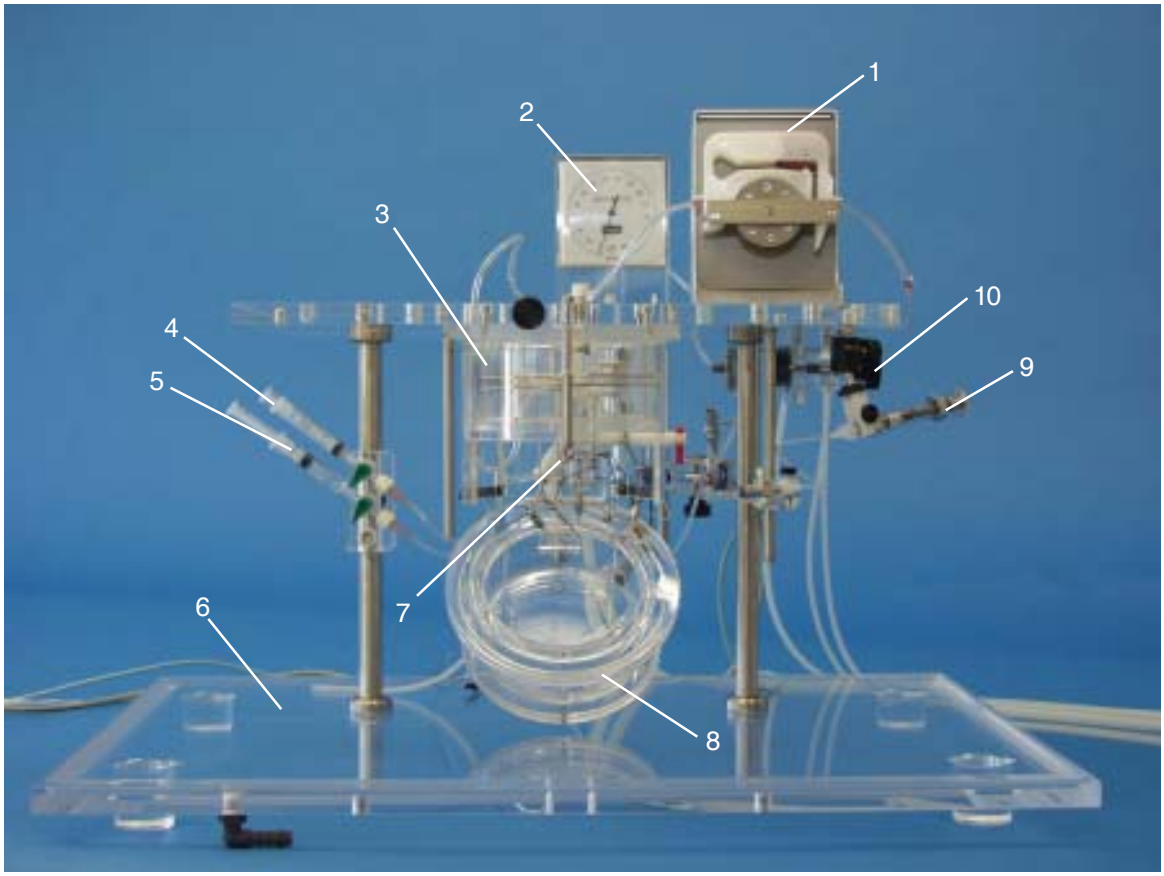


Fig. 2: IH-SR, Langendorff system. Overview.

1 Roller pump used to supply the retrograde flow to the heart.
2 Pressure gauge to indicate the set perfusion pressure.
3 Upper part of the jacketed heart chamber, thermostated. The sliding shutter can be moved up and down.
4 Syringe for adjusting the air vessel (Windkessel and bubble trap).
5 Syringe for bolusinjection or for taking samples.
6 Plexiglass stand.
7 Aorta block. The aorta block includes an air vessel which acts as bubble trap in LH mode and as compliance chamber (Windkessel) in WH.
8 Lower part of the jacketed heart chamber (thermostated). The lower part can be swung away downwards.
9 Spindle syringe with catheter for fine adjustment of diastolic LVP balloon pressure.
10 Rotary knob (large spindle syringe) to adjust afterload in WH. In the LH mode it is used to set the perfusion pressure.

4.2.1 Langendorff heart under constant pressure conditions (LH-CP)

The output of the roller pump must be set larger than the expected perfusion flow through the isolated heart, so that there can be a return flow through the adjustable resistor to the reservoir. Then the required perfusion pressure (e.g. 60 mmHg) is set on the rotary knob. The proportion of the perfusate which at this set pressure does not flow through the isolated heart now flows back to the reservoir through the adjustable resistor. During vascular constriction the perfusion flow through the organ is reduced and the return flow is correspondingly increased. There is no increase in pressure. In the case of vascular dilatation the reverse action takes place. A reduction in perfusion pressure is only observed if the pump delivery has been set too low so that all the pumped perfusate flows through the organ and there is no return flow.

Normal pump settings: mouse 6 - 8 ml/min; rat / guinea pig 16 - 20 ml/min

4.2.2 Langendorff heart under constant flow conditions (LH-CF)

Under constant-flow conditions the entire perfusate pumped by the roller pump flows through the organ. The setting of the adjustable flow resistor has to be selected higher than the highest expected or permitted perfusion pressure e.g. to 120 mmHg. The adjustable flow resistor takes on a protective function for the perfused organ. Any excessive rise in the perfusion pressure acting on the organ, e.g. through faulty operation of the roller pump or through vascular constriction as a result of administering a vasoactive substance, is then limited to the selected setting (e.g. 120 mmHg).

Normal flow settings: mouse 2 - 4 ml/min, rat 10 - 14 ml/min, guinea pig 12 - 15 ml/min
Obtained perfusion pressure should be in the 60 to 100mmHg range, not higher.

Always check and record the perfusion pressure if you do constant flow perfusion!

Figure 3 shows a functional digram of the IH-SR, Langendorff mode.

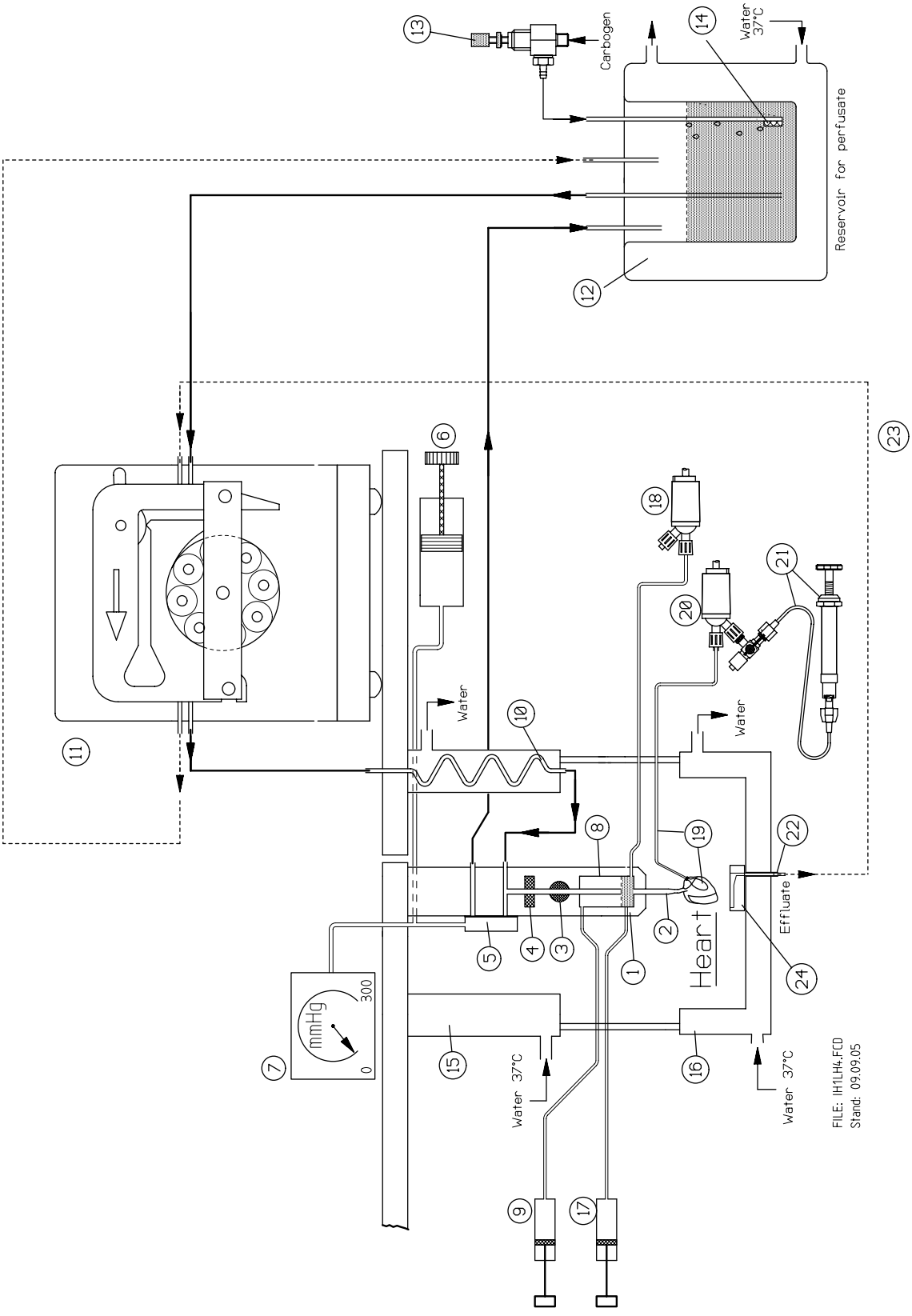


Fig. 3: Functional diagram, Langendorff heart (LH).

Legend for Fig. 3:

1 Aorta block. It serves essentially as an artifical aorta. The block is supplemented by the flow probe adapter (4) and the stopcock block (3), with the required connections and devices in order to meet the various requirements of the possible experiments.

2 Aorta cannula for connecting the aorta. Several cannulae of different size are avaliable.

3 Shut-off stopcock. The stopcock is used to stop the flow to the heart. In the LH mode it can be used e.g. to perform an ischemia.

4 Flow probe. In the LH mode it measures the retrograde perfusion flow which is the coronary flow. In the WH mode it measures the aortic flow. An appropriate flowmeter (TTFM) is required for operating the flow probe.

5 Adjustable membrane flow resistance. In the LH mode it is used to set the perfusion pressure. In the WH mode it determines the afterload (artificial circulation resistance). It consists essentially of an elastic Teflon membrane with clamping cover. The pressure is set on the large spindle syringe (6). The pressure set on the large spindle syringe acts on the rear side of the membrane. If the pressure on the perfusate side of the membrane is larger than the set pressure, the membrane lifts and the perfusate can flow underneath the membrane and pass back to the reservoir.
Note: The membrane thickness is essential for the correct functioning of the adjustable flow resistor. Cover and membrane are matched to each other. Use only original TEFLON membrans with the correct thickness.

6 Large spindle syringe for setting the pressure on the flow resistance (5). Rotation of the knob produces a corresponding movement of the plunger and the pressure is transmitted through the tubing connection to the membrane of the adjustable flow resistor. The pressure setting is indicated on the pressure gauge. (7). By opening a valve on the rear side of this spindle the pressure can be removed quickly and set to zero. This is also the position to move the plunger backwards. Always check the pressure gauge if you move the pluger backwards!

7 Pressure gauge to indicate the pressure set on the flow resistance membrane(5). It does not indicate the actual aortic pressure! Actul aortic pressure is about 8 - 10 mmHg higher due to the hydrostatic pressure of the “water column“ between heart and membrane.

8 Air vessel (Windkessel), air volume for reproducing the compliance of the aorta in WH. It is formed by the upper air volume in the aorta block(1). The volume of the air cushion determines the damping effect of the air vessel. A large air volume produces better damping of the pulsation than a small volume. The air volume is altered with the syringe (9).

9 Syringe for adjusting the air volume in the air vessel (8).

10 Metal heat exchanger, built into the top part of the heart chamber (15).

11 Roller pump, flow range up to approx. 30 ml/min. This pump is used to supply a retrograde flow to the heart in the Langendorff mode (during preparation and recovery phases). For recirculating operation (23) the pump must have at least two channels.

12 Perfusate reservoir, jacketed and thermostated, with suitable inlets and outlets. Thermostated water from the thermocirculator is passed through the reservoir jacket and warms the perfusate. The vessel cover lies closely on the open top and ensures that the aeration gas introcuded through the frit (14) collects above the solution level and does not escape immediately to the surroundings.
Note: If you fill the reservoir with cold (room temperature) solution before the experiment, you will have to wait a longer time (about 20min, depending on the volume) before the solution has warmed to the temperature determined by the thermocirculator. If you have a chance, is advisable to pre-warm the solution to the required temperature e.g. in a heating cupboard, monitored by a thermometer, preferably while bubbling Carbogen gas through it.
It is questionable to refill the reservoir when there is only little perfusate left in the vessel. Thermostating and oxygen saturation of the perfusate would then drop suddenly and would only slowly return to normal levels. Depending on the experiment this could lead to instabilities in the course of the experiment.
Aeration is provided using the gas frit (14). The glass tube of the frit is conected by a short piece of tubing to the shortest metal tube. Aeration operates through small tubing which is connected to one of the two needle valves (13). The intensity of aeration is adjusted on the needle valve.

13 Needle valve, for adjusting the Carbogen flow through the frit (14). Inlet pressure 0.5 - 1 bar.

14 Glass frit for aerating the perfusate.

15 Upper part of the jacketed heart chamber, thermostated by water circulator.

16 Lower part of the jacketed heart chamber, thermostated. The lower part can be swung away downwards to have access to the heart.

17 Syringe for adding an active substance as bolus or alternatively for taking samples. Also a syringe pump could be connected to this port.

18 Pressure transducer (e.g. ISOTEC). In the LH mode it measures the perfusion pressure; in the WH mode it measures the aortic pressure (afterload pressure). A suitable bridge amplifier (e.g. TAM-A) is required for operating the transducer.
Please note: pressure is only measured if stop cock (3) is opened!

19 Intraventricular balloon with connecting catheter for evaluating the isovolumetric left ventricular pressure (LH mode only).

20 Pressure transducer (e.g. ISOTEC) for measuring the isovolumetric left ventricular pressure. A suitable bridge amplifier (e.g.TAM-A) is required for operating the transducer.

21 Small spindle syringe (2 ml) with tubing catheter for fine adjustment of the diastolic balloon pressure to the required value (e.g. 10 mmHg).

22 Discharge tube for discharging the effluate dripping off the heart.

23 Tubing for sucking off the effluate (only required during recirculating operation).

24 Effluate funnel to collect effluate dropping down from the heart.

4.3 The Working Heart (WH) mode according to Neely

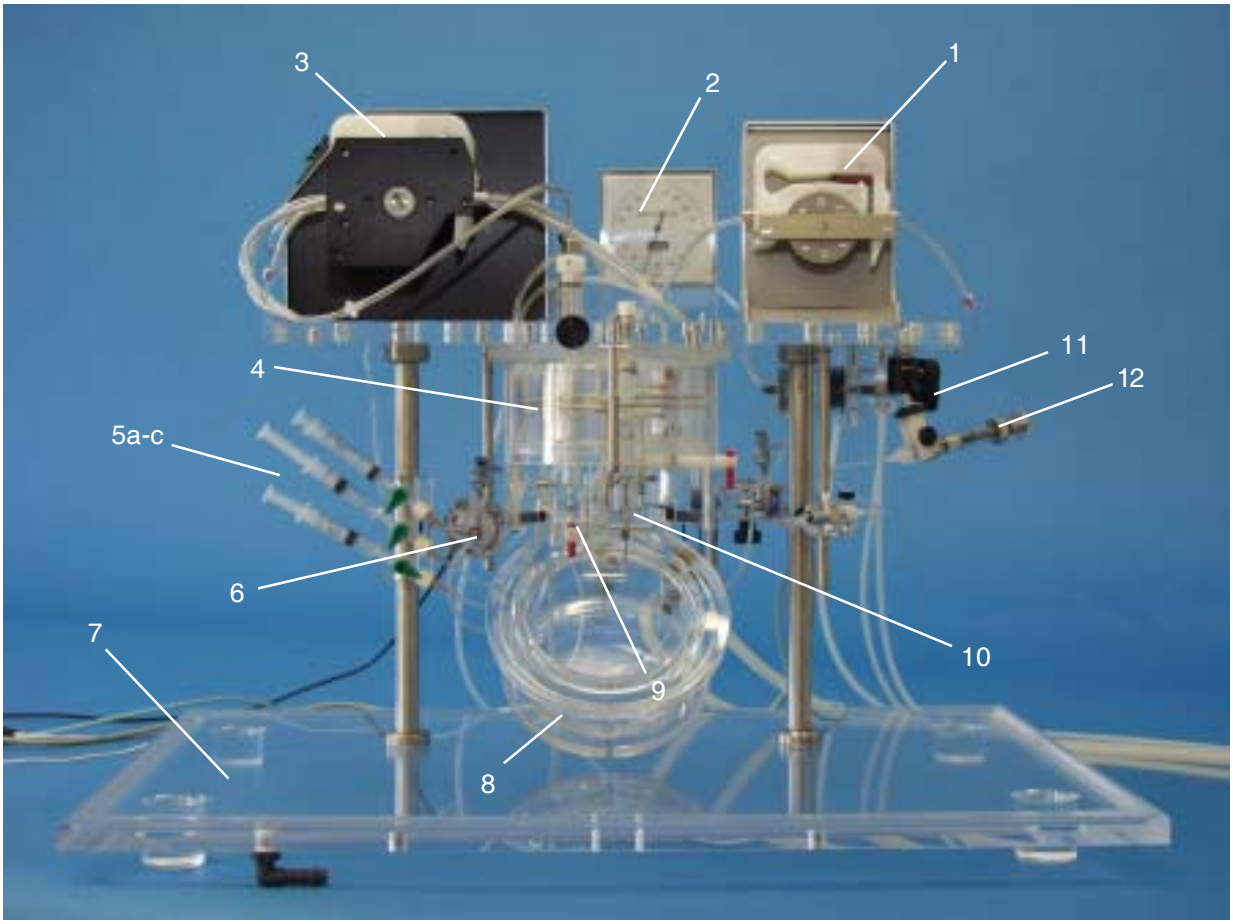


Fig. 4: IH-SR, Working Heart mode according to Neely. Overview.

1 Roller pump. Flow range up to approx. 30ml/min
2 Pressure gauge to indicate the pressure set on the flow resistance.
3 Roller pump, at least 3 channels, flow range up to 60ml/min approx.
4 Upper part of the jacketed heart chamber, thermostated. The sliding shutter can be moved up and down.
5a-c Syringes for bolus injection or taking samples, for air vessel adjustment in the lower aortic block and for removing air bubbles from the preload vessel (9).
6 Pressure transducer for measuring the preload pressure (left atrium pressure). Requires suitable amplifier module for operating.
7 Plexiglass stand
8 Lower lid of the jacketed heart chamber (thermostated). The lower part can be swung away downwards.
9 Atrium reservoir (preload vessel).
10 Aorta block. The aorta block includes an air vessel (Windkessel) for simulating the compliance of the aorta (in WH), the main stop cock, a flow probe and an adjustable flow resistance for afterload settings.
11 Large spindle syringe to adjust afterload pressure in WH or perfusion pressure in LH mode.
12 Small spindle syringe for balloon pressure adjustment in Langendorff experiments.

In the WH mode the heart performs both, pressure-volume work and acceleration work. Figure 5 shows the functional diagram. First the heart is perfused retrograde in the LH mode. The heart is attached to the aortic cannula and perfused to hold the ischemic time as short as possible. This allows now to cannulate the left atrium with the left atrium cannula (29). The heart is supplied with perfusate through the preload reservoir (26). The roller pump (24) pumps more perfusate into the preload reservoir (26) than flows into the atrium. The solution not required is pumped back into the main reservoir (12) through the suction tube (26a) by one of the pump channels. The preload can easily be adjusted by moving the suction tube up and down (higher column = higher preload pressure) .

The perfusate ejected by the left ventricle passes into the air vessel (Windkessel) (8) of the aorta block (1). From there it flows through the main stop cock (3) and the flow probe (4) to the adjustable flow resistance (afterload) (5) which represents an artificial circulation resistance. The aortic pressure (= afterload) can be continuously adjusted by varying the flow resistance [turning the large spindle syringe (6), always check pressure indication (7)].

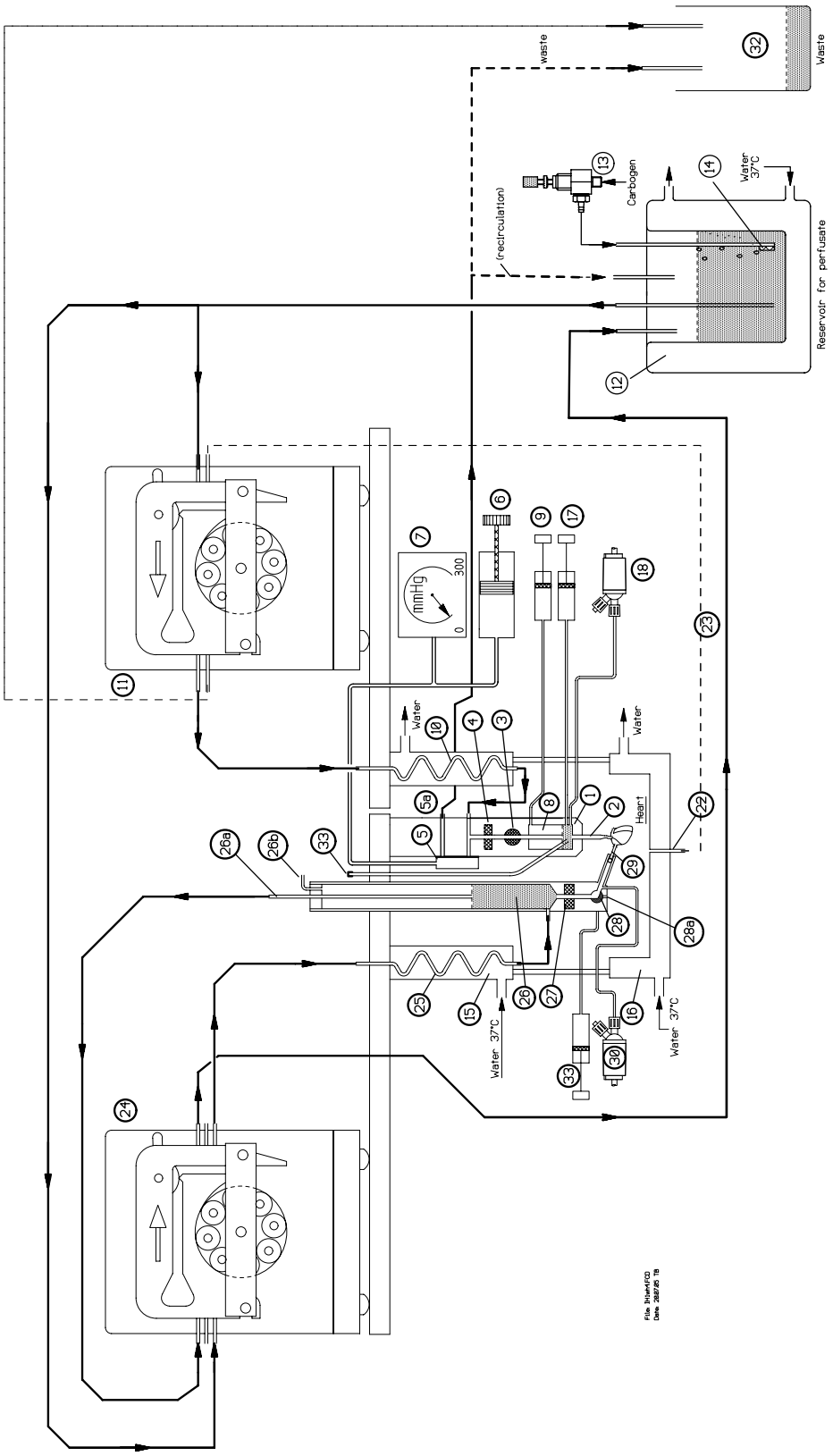


Fig. 5: Functional diagram Working Heart (WH).

Legend for Fig. 5:

1 Aorta block. It serves essentially as an artifical aorta. The block is supplemented by the flow probe adapter (4) and the stopcock block (3), with the required connections and devices in order to meet the various requirements of the possible experiments.

2 Aorta cannula for cannulating the aorta. Several cannulae of different dimensions are available.

3 Main shut-off stopcock. The stopcock is used to close the outflow to the heart or from the heart in the WH mode. In the LH mode it can be used to e.g. to perform an ischemia.

4 Flow probe. In the LH mode it measures the perfusion flow which corresponds to the coronary flow. In the WH mode it measures the aortic flow. An appropriate flowmeter (TTFM) is required for operating the flow probe.

5 Adjustable membrane flow resistance. In the LH mode it is used to set the perfusion pressure, in the WH mode it determines the afterload (artificial circulation resistance). It consists essentially of a Teflon membrane with clamping cover. The pressure is set on the large spindle syringe (6). The pressure set on the spindle syringe acts on the rear side of the membrane. If the pressure on the perfusate side of the membrane is larger than the set pressure, the membrane lifts and the perfusate can flow underneath the membrane and passes back to the reservoir. The membrane holds the pressure constant.
Note: The membrane thickness is essential for the correct functioning of the adjustable flow resistor. Cover and membrane are matched to each other. Use only Teflon membranes with the correct thickness (ordering number T33019).

6 Large spindle syringe for setting of perfusion (LH mode) or afterload pressure (WH mode) together with the flow resistance (5). Rotation of the knob produces a corresponding movement of the plunger and the pressure is transmitted through the tubing connection to the membrane of the adjustable flow resistor. The pressure setting is indicated on the pressure gauge. (7). By opening a valve on the rear of the large spindle syringe the pressure can be removed quickly and set to zero. This is also necessary to move the plunger back.

7 Pressure gauge to indicate the pressure set on the flow resistance (5). It does not indicate the actual aortic pressure! Real aortic pressure is 8 - 10 mmHg higher due to hydrostatic column between heart and membrane system.

8 Air vessel (Windkessel), air volume for reproducing the compliance of the aorta. The volume of the air cushion determines the damping effect of the air vessel. A large air volume produces better damping of the pulsation than a small volume. The air volume is altered with the syringe (9).

9 Syringe for adjusting the air volume in the air vessel (8).

10 Metal heat exchanger, built into the top part of the heart chamber (15).

11 Roller pump, flow range up to approx. 30 ml/min per channel. This pump is used to supply the retrograde flow to the heart in the LH mode (during preparation and recovery phases). For recirculating operation the pump must have two channels.

12 Main perfusate reservoir, jacketed and thermostated, with suitable inlets and outlets. Thermostated water from the thermocirculator (required for the operation) is passed through the reservoir jacket and warms the perfusate. The vessel cover lies closely on the open top and ensures that the aeration gas introduced through the frit (14) collects above the solution level and does not escape immediately to the surroundings.
Note: If you fill the reservoir with cold (room temperature) solution before the experiment, you will have to wait a longer time (about 20min, depending on the volume) before the solution has warmed to the temperature determined by the thermocirculator. It is advisable to pre-warm the solution to the required temperature e.g. in a heating cupboard, monitored by a thermometer, preferably while bubbling Carbogen gas through it.
It is questionable to refill the reservoir when there is only little perfusate left in the vessel. Thermostating and oxygen saturation of the perfusate would then drop suddenly and would only slowly return to normal levels. Depending on the experiment this would lead to instabilities in the course of the experiment.
Aeration is provided using the gas frit (14). The glass tube of the frit is connected by a short piece of tubing to the shortest metal tube. Aeration operates through small tubing which is connected to one of the two needle valves (13). The intensity of aeration is adjusted on the needle valve.

13 Needle valve, for adjusting the Carbogen flow through the frit (14).

14 Glass frit for aerating the perfusate.

15 Upper part of the jacketted heart chamber, thermostated.

16 Lower lid of the jacketted heart chamber, thermostated. The lower part can be swung away downwards.

17 Syringe for adding an active substance as bolus or alternatively for taking samples (LH only).

18 Pressure transducer (e.g. ISOTEC). In the LH mode it measures the perfusion pressure; in the WH mode it measures the aortic pressure (afterload). A suitable bridge amplifier (e.g. TAM-A) is required for operating the transducer. The transducer is mounted on the right side on the vertical column on a holder with height adjustment. The connection tubing is connected to the metal tube above the aortic casnnula.
Note: For correct pressure measurement both the tubing and the transducer dome must be filled bubblefree and the transducer must be adjusted to the level of the aorta.

22 Discharge tube for discharging the effluate dripping off the heart.

23 Tubing for sucking off the effluate (only required during recirculating operation).

24 Roller pump, at least 3 channels, flow range up to 30 ml/min per channel approx.

25 Heat exchanger for heating solution going to the preload reservoir, built into the top part of the heart chamber (15).

26 Atrium reservoir (preload vessel).

26a Metal suction tube to maintain a constant perfusate level in the preload vessel. The required level is set by vertical movement of the suction tube (higher column = higher preload pressure).

26b Vent tube, must remain open during operation.

27 Flow probe for determining the inflow to the left atrium. The measurement represents the cardiac output (CO). A suitable flowmeter (e.g. TTFM) is required for operating the probe.

28 Changeover stopcock. To open preload vessel to left atrium (WH).

28a Atrium relief bore. Important when the heart is being operated in the LH mode and the atrium is cannulated.

29 Atrium cannula with ball adapter for the atrium connector.

30 Pressure transducer (e.g. ISOTEC or P75) for measuring the preload pressure (left atrium pressure). A suitable bridge amplifier (e.g. TAM-A) is required for operating the transducer. The pressure transducer is mounted on a holder with vertical adjustment. The connection tubing is connected to the longer, inwards projecting tube of the atrium head.
Note: For correct pressure measurement both the tubing and the transducer dome must be filled bubblefree and the transducer must be adjusted to the level of the atrium. Pressure is only measured if changeover stop cock (28) is open!

32 Waste beaker to take the used perfusate.

33 Access port for introducing a miniature tip catheter pressure transducer to measure the intracardial left ventricular pressure (LVP).

5. Starting up

The procedure suggested below has been proved satisfactory in practical use. Depending on the type of experiment proposed it may be useful to proceed other than described. The actual details of the experimment must therefore be left to the decision of the experimentator. Note however that the introduction of the heart into the apparatus must be carried out as rapidly and as gently as possible in order to avoid ischemia. If this is not done in the right way the heart may be is not fully functional. Carry out a check before the start of the experiment by using a functional test (e.g. response on a step by step perfusion pressure change or a afterload change in WH, Frank-Starling Test).

5.1 Preliminary notes for Working Heart (WH)

At the start of an experiment in the Working Heart mode the heart is initially being operated in the Langendorff mode and only switched over to the WH mode when it has recovered from the stress of the preparation and the atrium has been cannulated. The flow through the coronaries is thus initially retrograde in the LH mode and supplies the heart muscle without it having to perform any work. Pump in conjunction with the adjustable flow resistance produces the necessary aortic perfusion pressure.

Later, after the recovery phase and when the atrium cannula has been bound in correctly, the atrial flow is opened up at the stopcock of the preload vessel, the left atrium is filled, the left ventricle is filled and due to the contraction the heart really ejects into the aortic block and works against the artificial resistance. The LH pump is then switched off. The afterload pressure (aortic pressure) can be adjusted at the pressure regulator (large spindle syringe (6) as exactly as before the perfusion pressure. The pressure gauge indicates the pressure setting. Real mean aortic pressure is the pressure on the manometer plus the hydrostatic pressure. So your measured pressure will be around 8 to 10mmHg higher than the pressure on the pressure gauge. The aortic flow is measured with the flow probe in the aortic block.

5.2 Preparation

5.2.1 Preparing and filling the thermostatic circuit

Arrange the connecting tubing of the thermocirculator so that the warm water passes through the various jacketted vessels from the bottom upwards. Fill the thermostat tank with distilled water to which you add an additive for suppressing algal growth (e.g. Thermoklar).
Please note: Check that your solution for suppressing algal growth does not contain any alcohol, as alcohol damages plexiglass!

Now switch on the thermocirculator and ensure that all the flow passages are filled completely. Note the reduction in the liquid level in the thermocirculator tank and refill in good time. Check the complete flow system for leakage.

In a first step to get an overview of the function of the apparatus it is highly recommended to perform a test run with the apparatus to understand the function and the flow directions.

5.2.2 Preparation for the experiment

Before you start the experiment you should carry out the following preliminary steps:
- Prepare the perfusion solution
- Prepare the drug dilutions
- Start up and fill the apparatus with buffer solution
- Switch on the measurement systems and after a warm period (approx. 15 min.) perform zero adjustment and calibration of all measured signals
- Adjust the perfusion pressure e.g. to 60mmHg
- Prepare the accessories such as aorta cannulae, operating table, surgical instruments, anaesthesia syringe etc.

5.2.3 Preparing the perfusion solution

The perfusate used with isolated hearts is usually a saline solution according to Krebs-Henseleit (KH) or a modified KH solution. The solution is always aerated with Carbogen (95% O2, 5% CO2) to get a pH of 7.4
For further details of the most widely used Krebs-Henseleit solution see page xx. It is essential that the made-up solution is filtered. This is important because the used chemicals often contain insoluble particles which for obvious reasons must not pass into the vascular system of the heart.
Always warm the perfusion solution to the required temperature (e.g. 37°C) in the heated buffer reservoir before starting the perfusion.

5.2.4 Preparing the drug dilutions

Suitable dilutions have to be prepared in accordance with the administration of the drug (as bolus or in the perfusate).

5.2.5 Filling and starting up the apparatus

If the apparatus is new or has not been used for a longer period, it has to be cleaned thoroughly before the first experiment. During normal daily use the apparatus should be filled with with distilled water. Once a week the use of a cleaning solution (RBS50 or MUCASOL) is recomended. Do not forget to to rinse bubble trap, side branches and catheters to remove all solution residues. After draining the cleaning solution and thorough flushing with distilled water the apparatus has to be emptied completely.

Switch on the thermocirculator (37°C), allow the apparatus to warm up to the operating temperature.

Fill the apparatus with perfusate (the item numbers refer to Fig. 3 and Fig. 5:
- Place perfusate into the reservoir (12) and turn on the Carbogen aeration by adjusting the needle valve (13).
- Close the main stopcock (3) on the aorta block; in the WH mode also close the preload stopcock (28) in the atrium block.
- Switch on the pump (11) for Langendorff operation and in the WH mode also the pump (24) (set the flow rate to e.g. 20 ml/min on the LD pump). Ensure the correct direction of rotation for both pumps!
- On the adjustable flow resistance close the vent valve; using the knob of the large spindle syringe to set the afterload pressure to about 60 mmHg. The pressure is indicated on the pressure gauge.

Filling the aorta block:

Switch on pump (11) for LH operation; ensure correct direction of rotation! Close the pump cassette.
Note the information in the operating instructions of the pump. Set a low output, e.g. 8 - 10 ml/min for mice hearts or 15 - 20 ml/min for rat heats. Recommended tubing AME 14 for mice heart and AME 24 for rat hearts.
Close the vent valve on the pressure regulator (large spindle syringe) using the black knob of the pressure regulator (6) set the afterload pressure to about 60 mmHg. The pressure is indicated on the pressure gauge (7).
If you are using a Millar micro tip catheter pressure transducer (MTPT) (only possibe in WH mode) for later measurement of the LVP, release squeeze seal (36) on the access port (33) and insert the catheter. Advance it so far that it is located in the connection tube of the aorta cannula (2) so that it is initially in a protected location. If you are not using a tip catheter, close the Luer taper (33) with a Luer blind stopper.
Using the syringe (9) and its shut-off stopcock, allow the air vessel (8) to fill about half full with perfusate.
Filling the air vessel can also be achieved by a different method:
Remove syringe (9) and open the corresponding stopcock. Then press on pump (11) the "MAX" key and at the same time prevent flow through the preparation tubing by squeezing it. The liquid level in the air vessel now rises rapidly. When the required level is reached (half full), close the open stopcock (9) again, release the squeeze on the preparation tubing and release the "MAX" key. The previously set lower flow now flows again through the preparation tubing back to the reservoir. The pressure in the air vessel is then 0 mmHg.

Filling the atrium vessel (Working Heart Mode):

Close stopcock (28)!

Switch on pump (24) for the atrium supply; note direction of rotation! Close the pump cassette. Set the flow rate higher than the expected atrial flow, e.g. 10 ml/min for mice or 60 ml/min for rat/guinea-pig hearts. The atrium reservoir (= preload vessel (26)) fills itself up to the level of the draw-off tube (26a). If the pump tubing diameters have been chosen correctly (outflow tubing must be larger than the inflow tubing) the liquid level does not rise any higher. Check this condition before the vessel overflows!

Now vent the liquid system to the atrium head and the corresponding connecting tubing to remove all air from it. Carefully open the stopcock (28) and wash out all air bubbles. Then set the stopcock (28) so that there is a small perfusate flow out of the atrium cannula. To fill the tubing to the pressure transducer (30) and the dome itself free from air bubbles you close off the atrium cannula with the finger and open the venting one way stopcock on the dome.

Close the stopcock again!

Adjust the aeration in the main buffer reservoir (12) with the corresponding needle valve (13).

5.3 Test run, a "dry run"

After you have completed the preparations up to this point it is useful during the first start-up to carry out a few "dry runs" without an organ, in order to become familiar with the behaviour and function of the apparatus with its connected instrumentation and to practice its operation. In order to simulate the Working Heart you require a pump with a suitable output. If necessary you can use pump (11). The test run in the Langendorff mode should be carried out first since this involves the use of pump (11) in the apparatus. If you then carry out the test run in the Working Heart mode you can use pump (11) as heart replacement.

5.3.1 Test run in the LH mode

Place any aorta cannula on the aorta connection and close the outlet with a short piece of tubing (coronary replacement) which you partly close off with a hose clip during the test in order to simulate a low coronary flow.

Check that the return tubing (5a) is not kinked and that the return flow is not impeded.

Switch on pump (11) and set a flow rate of approx. 10 ml/min.

Set the pressure regulator (6) connected to the adjustable flow resistor (5) to 60 mmHg [vent valve must be closed; the pressure setting is read on the pressure gauge (7)].

The pressure in the air vessel (8) should now rise to about 68 - 70 mmHg. The rise in pressure is measured by the pressure transducer (18) and can be monitored on the pressure amplifier and the associated recording device.

Check all connections for leakage. Rectify any leakage before continuing.

Now change alternatively the pressure [by turning the pressure regulator knob (6)] and the output of pump (11) and note the corresponding changes in flow and pressure.

Now check the effect of the air vessel (8). Set the afterload pressure with the control of (6) to about 50 mmHg, the pump (11) to a medium flow rate (e.g. 10 ml/min) and alter the filling level in the air vessel [use syringe (9) and the corresponding stopcock, remember the minimum filling level (= top edge of outlet bore!)]. On the recording device or daq system you can see the variation of the pressure pulsations caused by the pump. The pulsation amplitude which depends very much on the type of pump used, becomes larger as the air cushion is reduced, and vice versa. During the subsequent experiment you will observe the same effect. You have to select the air volume in the air vessel so that the recorded pressure curve has the most “physiological“ shape; then you have found the optimum air vessel adjustment.

5.3.2 Test run in the WH mode with roller pump as heart replacement

The description below assumes that the apparatus has been prepared according to Section 7.2. As mentioned earlier (Section 7.3) you require for this test run an additional pump as heart replacement.

Connect the additional pump as heart replacement into the apparatus between atrium cannula (29) and aorta cannula (2).

Ensure that all the pressure tubing is being filled free from bubbles. Any bubbles adhering to the tubing wall can usually be released by gently tapping the tubing and can then be flushed out.

- In the WH mode the preload vessel fills up to the suction tube (26a). If the correct size tubing has been fitted to the roller pump (24) the level does not rise any further. (Suction tubing must always be larger than filling tube)
- The perfusate supplied by the pump (11) flows through the adjustable flow resistance (5) back to the reservoir (12),
- Connect a short piece of tubing to the aorta block in place of the aorta cannula (2) and close it with a tubing clamp. Then open the main stopcock (3) in the aorta block. Now release the tubing clamp slightly so that the air vessel (8) and the corresponding connections fill with perfusate and perfusate drips off from the end of the tube. Filling is quicker if you do not leave the pressure on the flow resistance on zero. Adjust it to a value of about 60 mmHg.
- Fill the connecting tubing to the pressure transducer (18) and also its dome so that they are free from bubbles.
- The connecting tubing of the pressure transducer (30) and its dome are best filled through opening the one way stop cock on the pressure transducer to air. This stopcock is located on the 45° port of the ISOTEC pressure transducer.
- Adjust the pressure transducers (18) and (30) to be at the correct level (see Section 0).
- Switch on all measuring instruments and calibrate each complete instrument chain in accordance with the instructions for the transducers and instruments.

Switch on pump (11) and set an output of approx. 20ml/min.

Open the stopcocks (3 and 28) and switch on the heart replacement pump. Initially set a pump output of about 10ml/min. The pump output is measured with the flow probe (4) or (27) and can be read on the corresponding flowmeter.

If you now set an afterload pressure of e.g. 50 mmHg on the pressure regulator (6) [can be read on the pressure gauge (7)], the aortic pressure should also increase due to the pump output.

On your recording device you can see the various measurement signals:

Aortic pressure [pressure transducer (18)]. Because of the additional hydrostatic pressure of the liquid column (air vessel - aortic block) the recorded pressure is slightly higher (about 8 - 10 mmHg) than the value read on the pressure gauge (7). Pulsation is more or less pronounced depending on the air cushion set in air vessel (8).

Atrial pressure [pressure transducer (30)]. In this test the recorded atrial pressure may exhibit pronounced negative peaks, due to the (non-physiological) suction effect of the heart replacement pump used. Evaluation and comparison with the later experiment (with the heart bound in) is therefore possible only to a limited extent.

Aortic flow [flow probe (4)]. The associated flowmeter should indicate the flow set on the heart replacement pump. With pulsatile recording a strong pulsation can be seen, due to the action of the mechanical pump.

Atrial flow [flow probe in (27)]. The associated flowmeter should indicate the flow set on the heart replacement pump. With pulsatile recording a strong pulsation can be seen, due to the action of the mechanical pump.

The indications for atrial flow and aortic flow should differ very little (<1%). If you find larger differences, then this may be due to different magnitudes of the flow pulsations at the two measuring points. The only remedy is the use of a heart replacement pump with less pulsation or a different pump tubing on the pump.

Now vary the output of the heart replacement pump (stay in the range of the flow probe and in the range of your species flow) and observe the behaviour of the different recorder traces. Aortic pressure increases slightly with an increase in flow, but not proportional (see characteristic curves of the adjustable flow resistor [afterload], page 40).

During the variation check for the maximum possible atrial flow. If you exceed the flow rate set on pump (24), the reservoir (12) will obviously run dry. If you later use a heart with a large ejection capacity you have to set the output of pump (24) correspondingly large enough so that there is always sufficient perfusate available in the reservoir. You should however not set an excessively large flow rate in order to avoid unnecessary wear on the pump tubing. Stay 20% higher than max. flow in WH mode.

If you have carried out the test procedures up to this point and have tried out the different settings of the apparatus, you should restore the settings as indicated below (before the start of the test runs) before you proceed to the next stage.

settings for mice:	LH pump (11):	6 - 8 ml/min
	WH pump (24):	10 ml/min
settings for rats:	LH pump (11):	20 ml/min
	WH pump (24):	60 - 70 ml/min

The apparatus is now in a suitable condition so that you can carry out the heart preparation and mount the heart on the apparatus.

5.4 Heart preparation and mounting the heart in the LH mode

In order to avoid irreversible damage the preparation of the heart has to be carried out as quickly as possible. The unavoidable ischemia time has to be kept as short as possible.

Remove the heart from the animal and tie in the aorta cannula. You must not insert the cannula too deeply in order to ensure that the coronary ostia remain unobstructed. Open the shut-off stopcock (3) in the aorta block a little so that perfusate drips off slowly. Then position the aorta cannula (with the heart attached to it) below the connection nipple and allow the perfusate to flow into the cannula so that no air remains in the connection part of the cannula. Then carefully (not too quickly) place the cannula on the connecting nipple of the aorta block. Ensure that no air is trapped.

Now open the shut-off stopcock (3) completely and select the required aortic pressure (e.g. 50 mmHg) on the control (6). Check for leaks, if your pressure is not coming up to 60mmHg you probably have a leak in the aorta. Check coronary flow, on rat hearts it should be between 10 and 14ml/min on mice heartsbetwenn 2 and 4ml/min
Check all measured values (pressure, flow, heart rate ...) and rectify any measurement errors or faults in accordance with the appropriate Operating Instructions. Any data acquisition system or recorder connected to the apparatus should now record plausible signals without any disturbances.

Wait until the heart function has stabilised (10 - 15 minutes) before you connect the left atrium (**WH mode only**) or define initial reference values and start the actual experiment by administering test substances. The heart should be beating regularly and rhythmically, any extrasystoles should have died down, the coronary flow should have stabilised at a reasonable value (about 5 ml/min).

5.5 Preparation and mounting of the heart in the WH mode

The heart can now be prepared and placed into the apparatus. An extensive description of the actual preparation of the heart (for rats and guinea-pigs) will be found in the publication Biomesstechnik V: “The isolated perfused heart after Langendorff“, Biomesstechnik-Verlag March GmbH (available through HSE, see literature reference).

Before the start of the actual organ preparation the apparatus should be prepared as described in detail in Section 5.22. Summarising briefly, the apparatus should be in the following condition:

- The reservoir is filled with fresh perfusate and is at the correct temperature (pre-warmed).
- Thermocirculator is switched on, all parts of the apparatus are at the correct temperature.
- WH Pump (24) is switched on, the required output is set as listed above.
- Aeration of perfusate is adjusted.
- Atrium reservoir and all tubing and transducer domes are free of air bubbles.
- Changeover stopcock (28) is closed.
- LD Pump (11) is switched on, the required initial output is set to a low flow (1ml/min).
- Air vessel (8) is half full with perfusate
- Afterload pressure is set on (6) to 50 mmHg as read on pressure gauge (7)
- Preparation tubing is placed on aorta connector and filled free of air bubbles; the small perfusate flow (1ml/min) provided by pump (11) drips off the free end of the preparation tubing and can initially drip into a container at the operating table
- Measuring instruments and recording device switched on, calibrations and zero adjustments have been completed
- Tubing to the two pressure transducers (18) and (30) and the two domes are filled free from air bubbles

Now start the organ preparation (here the in-situ reparation is described. It is also possible to remove the heart and to put it into ice cold KH solution and to attach it to the cannula.):
- Anaesthetise the animal, perform tracheotomy and ventilate. Open the thorax.
- Transfer the organ into the apparatus in two steps:

Spray the heart with cold perfusate (spray bottle) to cool it, place a suitable aorta cannula on the end of the in situ preparation tubing, clamp off the vena cava, open the pulmonary artery, bind the cannula into the aorta and immediately raise the output of pump (11) (e.g. to 20ml/min on rats or guinea pig hearts, to 10ml/min on mice hearts) so that an aortic pressure of about 60mmHg is obtained. (Blood is washed out, heart muscle colour changes from red to light pink). Check for leaks if you don´t reach the 60mmHg pressure.

Since the organ is now being supplied with oxygen and perfusate, the remaining preparation stages can be carried out less urgently. Take the heart out of the animal as it hangs from the preparation tubing, and remove any disturbing adhering tissue.

Remove the aorta cannula with the attached heart from the preparation tubing and mount it as rapidly as possible directly on the aorta connector (2) in place of the preparation tubing. Ensure that no air bubbles enter the aorta cannula during this operation. Position the cannula so that the left atrium points to the left.

- Allow the organ to stabilise and recover (15 - 30 min).

- The flow sensed by the flow probe (4) corresponds to the coronary flow (in view of the flow direction the reading is negative!). You can reverse the direction on the flowmeter (TTFM).

- Perform a test on the organ for perfect function [Bayliss test (rapid perfusion pressure change], or Frank-Starling test [increase perfusion pressure in steps of 10mmHg and check the autoregulation of the heart], administer test substance, or similar).

- If you do a WH preparation place a suitable atrium cannula (29) on the atrium head and fill it with fresh solution free from air bubbles [open stopcock (28) a little so that perfusate drips off the cannula (29)].

- Bind the atrium cannula into the left atrium. Take care that as much of the atrial function as possible is retained in order to ensure good left atrium filling.

- Bind off open vessel stumps on the atrium. Now fully open stopcock (28) and allow atrial flow. Check the atrium for any open vessel stumps (leaks) and bind these off. Don´t bind off the pulmoanry artery. This is the vessel where the coronary outflow exits.

The heart should now beat regularly and rhythmically and perform pressure-volume work. Perfusate is ejected through the aorta. The actual aortic flow is measured by the flow probe (4) and can be read on the associated flowmeter. The polarity of the flow indication changes from negative to positive according to the flow direction in the aorta cannula. Now switch off LD pump (11) and monitor the aortic pressure. If the heart ejection is too small so that no positive aortic flow is produced, the LD pump (11) must initially remain switched on to ensure an adequate supply to the heart. The problem must be rectified before switching off the pump.

A few possible errors related to the organ:

- Atrium cannula is badly positioned and impedes the function of the mitral valve.
  Remedy: place the atrium head into a better position, if necessary you may have to withdraw the cannula slightly from the atrium.

- The heart is not hanging freely but is pulled sidewise or twisted, interfering with the valve function.
  Remedy: slightly rotate the heart with the aorta cannula and/or place the atrium head into a better position.

- Aorta cannula impedes the ejection through the aortic valve.
  Remedy: the aorta cannula is probably bound in too deeply and must be pulled back slightly.

- The organ has been severely damaged during preparation. This can already be recognised by frequent extrasystoles and because it is not beating regularly and rhythmically.
  Remedy: If the damage is not severe, the organ should recover after an additional waiting period (15 - 30 min). If there has been no improvement after this time, you should not continue working with this heart. It is preferable to discard it.

Other possible errors not due to the organ:

- Perfusate not saturated well with oxygen.
  Remedy: check aeration, perhaps you have forgotten to switch on the “bubbling“. Check gas composition! (has the correct cylinder been connected up?). Check pH of your solution it should be 7.4

- Perfusate composition is not correct.
  Remedy: make up a fresh perfusion solution.

- pH of perfusate differs from its proper value (Krebs-Henseleit solution: pH = 7.3 - 7.5).
  Remedy: correct the relationship between CO_2 content of the gas and the individual perfusion solution components (see literature, note literature reference!).

Note: Less than 5% CO_2 leads to a pH higher than 7.4, more than 5% leads to a pH lower than 7.4 !

General hints:
Please note: the aortic pressure must not drop appreciably below 50 mmHg to ensure the supply to the heart muscle via the coronaries.

Adjust the control of the pressure regulator (6) to set the aortic pressure to the value you require and allow the organ to stabilise under the new conditions before starting the actual experiment.

If you are working with recirculation, remember that the test substances (drugs) remain in the perfusion circuit and accumulate. When (or before) administering the test substance it may be useful not to return the perfusate discharge to the reservoir but to collect it in some other container.

If you are using a Millar Micro Tip Catheter pressure transducer (MTC) for measuring the LVP in the WH mode you can now advance it into the left ventricle. The MTC is a sensitive and, as you must know, a very expensive item. Handle it with care and never use force when handling it. If it is damaged it is impossible to have it repaired. You must then purchase a new one or abandon this excellent and precise method of measuring LVP (note Fig. 21, page 46).

To insert the MTC, release the pressure piece of the squeeze seal (Tip cather port) slightly until the MTC can be moved easily, and carefully move it forward. If the catheter is difficult to move in the squeeze seal you should apply a little vaseline to it or check the seal in the Tip cather port.

Now advance it further until you can feel the movement of the aortic valve in your finger tips. Monitor the pressure trace on the appropriate recording channel. First you see an arterial pressure curve (Tip is in the aorta). If you come near the valves you see an incision in the AP signal. Then quickly slide the catheter tip into the left ventricle when the aortic valve is open during systole. The characteristic changes in the pressure curve show up immediately that the catheter tip with its pressure sensor is in the ventricle. With a little practice you will find that inserting the MTC is a very simple and safe procedure.

The preparations for the experiment are now completed. Adjust the filling level of the air vessel (8) (Windkessel) so that the shape of the aortic pressure traces are as nearly physiological as possible. Do remember, however, that the rigid pipe system of the apparatus can never completely replace the elastic vascular walls the heart sees in in situ. The traces of the pressure and flow signals obtained on the apparatus can therefore never show an exactly “physiological“ form but you will see the curves look very similar.

Now perform the experiment as planned: administer test substance as bolus or continuously with a syringe pump. It also can be directly mixed into the perfusate (two buffer reservoirs recommended). You also can use different aeration gases or alter the temperature or whatever is your application.

5.6 Further hints for heart preparation and mounting in the WH mode

As soon you have the heart on the aorta in the Langendorff mode rotate the heart with the aorta cannula so that the left atrium points to the left towards the atrium cannula.
Always carefully insert the cannula into the left atrium, but not too deeply so that the distal cannula aperture remains open and is not in contact with the heart tissue or the mitral valves. Ensure that no air has been trapped and tie in the cannula carefully.

As soon the changeover stopcock on the atrium block is completely opened, check the atrium for any leakage (small fountains!). Any points of leakage must be sealed by fine ligatures.
Don´t bind off the pulmonary artery! This is the vessel, where the coronary flow exits!

If the heart is not ejecting (you see this in a change in flow direction in the aortic flow, in Langendorff you have retrograde flow, as soon the heart ejects the flow is no longer retrograde)
Wait again until the heart function has stabilised, if it does not eject well and the heart is not able to provide the set pressure (e.g. 60mmHg), switch back to Langendorff mode and check for leaks. When the heart is working sufficiently well the aortic flow measured with the flow probe (4) in increases.

Typical values mentioned in WH publications are:

	coronary flow	**aortic flow**
mice	2 - 4 ml/min	4 - 8 ml/min
rats	10 - 14 ml/min	30 - 60 ml/min

The previously indicated coronary flow changes to the aortic flow as soon the heart starts ejecting.

If you observe the reversal of flow direction in the aortic cannula as mentioned above, you can switch off the LH pump (11). Then monitor the aortic pressure. If it falls clearly below 55 mmHg, the ejection capacity of the heart is not sufficient to maintain the set aortic pressure; in that case switch on the LD pump (11) again and give the heart a further recovery time before you try again to switch off the pump (11).

Start the experiment only when there are stable conditions. If you are working with recirculation you should remember that any active substance administered remains in the entire perfusate volume if you do not switch to “non-circulation” for a time so that the effluate ejected by the heart and that pumped back by the connection (22) is diverted to waste (32). It also may happen that toxins can be created by the heart which may damage the heart in recirculation mode.

After the experiment has been completed you should immediately discard the heart and clean the apparatus. The procedure is explained in Section 8.

5.7 Changeover stopcock in atrium head, explanation of function

The changeover stopcock (28) in the atrium reservoir (26) has three relevant positions (A, B, C) which are shown in Fig. 6. Please note the explanations below for the item numbers in the illustration.

Position A: closed position.
In this position the flow to the atrium as well as the relief bore (28a) are blocked. If a heart is being operated in the LH mode, it is possible with a leaking heart valve for pressure to build up in the atrium which is dangerous for the atrium. A transducer (30) connected here would measure the pressure.

Position B: normal operating position in the WH mode (= open position).
In this position perfusate flows from the preload vessel through the atrium cannula into the heart. The relief bore is blocked off. The pressure transducer measures the atrial pressure.

Position C: operating position when the atrium is cannulated and the heart is being operated in the LH mode.
In this position the inflow from the preload vessel is blocked; the relief bore (28a) is free. This stopcock position should be selected if the atrium is cannulated and the heart is being operated in the LH mode. The relief bore ensures that no undesirably high pressure can build up in the atrium if the heart valves are not completely leaktight.

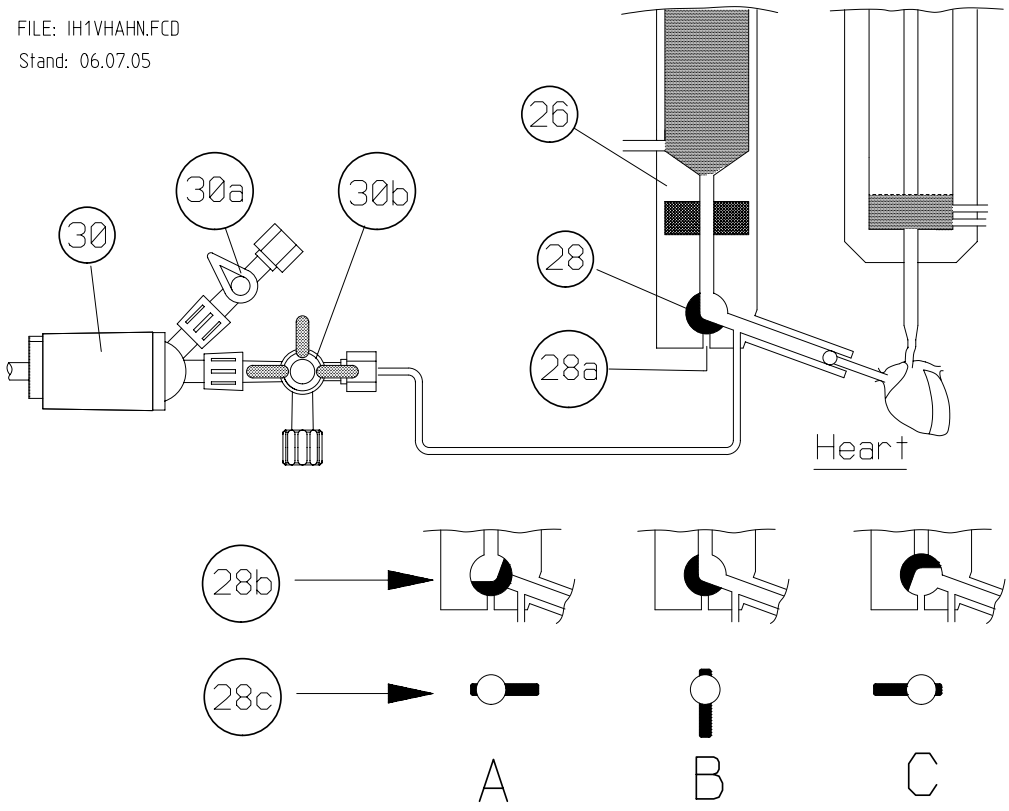


Fig. 6: Changeover stopcock with atrium head.

26 Atrium reservoir (preload vessel).
28 Changeover stopcock.
28a Relief bore.
28b Positions of the stopcock (28) depending on the lever positions (28c).
28c Lever positions of the changeover stopcock (28).
30 Transducer for measuring the atrial pressure, with shut-off stopcock (30a) and 3-way stopcock (30b). NOTE: remember the height adjustment of the pressure transducer (see page 42).

5.8 Operating notes and special features during operation

5.8.1 Use of foaming perfusate

General notes on fiber membrane oxygenators

When using perfusate which tends to foam, or with certain perfusate additions (albumin) there may be problems with aerating the reservoir (12) by bubbling gas through the frit (14). In that case it is better to aerate by using a fiber membrane oxygenator.

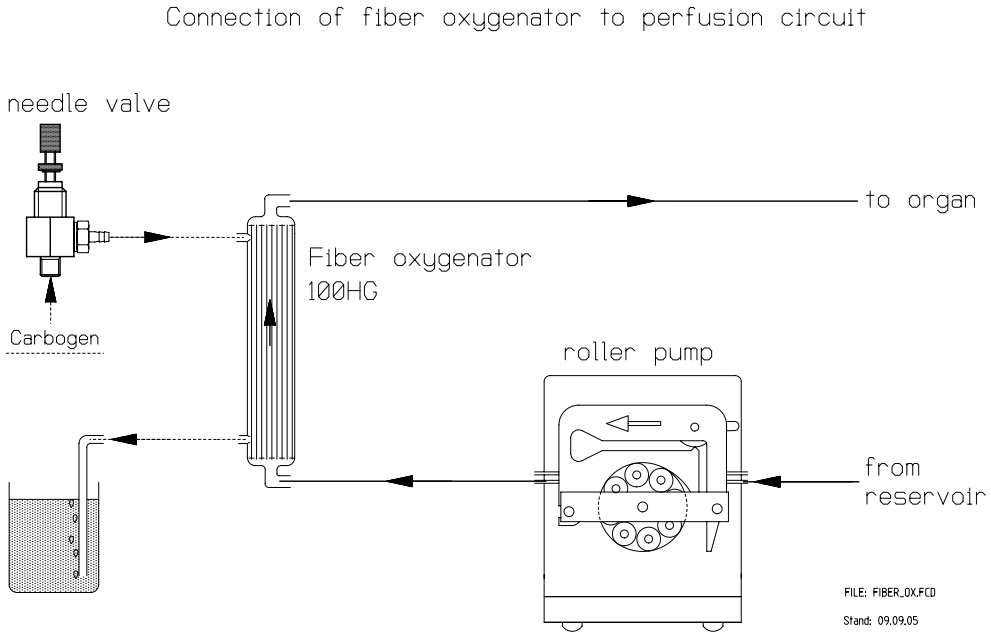


Fig. 7: Connection of the fiber membrane oxygenator to the perfusion circuit.

A fiber membrane oxygenator is a flow through oxygenator (working like a dialyzer) for aerating buffer solutions or blood. The perfusate is aerated according to the counter-current principle. The oxygenator must be mounted vertically in a stand with a holder. The perfusate has to pass through the fiber membrane oxygenator from the bottom to the top, the gas from top downwards. As the perfusate passes through the oxygenator it is satureated with the gas introced (e.g. Carbogen). The gas flow is adjusted using the needle valve. Note that dialysis units have a certain leakage rate; it is therefore useful to connect a piece of tubing at the gas outlet, with its end immersed in a beaker half full with water. The rising gas bubbles can then be used to monitor the gas flow setting.

User notes for using the fiber membrane oxygenator
When using the the fiber membrane oxygenator to oxygenate the perfusate it must always be installed on the pressure side of the corresponding roller pump (see Fig. 7) Do not suck the perfusate through the oxygenator unit!

IMPORTANT: flush through fiber oxygenator before use!
The membrane of the dialysis unit is treated with glycerol for stability. Ensure that no glycerol passes into the liquid system of your apparatus. It is therefore important that a new oxygenator unit is thoroughly flushed with perfusate before using it. The perfusate washings have to be discarded.

NOTE: dialysis units have a certain leakage rate so that a small amount of perfusate flows out of the gas outlet connection. The leakage rate is very low in most cases and does not interfere with the experiment except that a little more perfusate is required. Typical value: 3 ml/h * pressure (mmHg). Depending on the manufacturing batch there may be a higher leakage.

Re-use

A dialysis unit handled with care and flushwd carefully can be used up to 10 times (depends on the used bufer solution).

Cleaning after use

- Flush the liquid side with about 1 litre of ordinary tap water. Use the appropriate roller pump for this purpose.
- Then dry the inner side. Blow dry and oil-free air or gas through the dialysis unit from the top downwards. The pressure produced must not exceed about 0.1 bar. At higher pressures the dialysis membranes may burst.

Replacing the dialysis unit:

When you replace the dialysis unit by a new one, remove all connecting elements and use them to connect up the new unit!
Replacement connecting kits e.g. for the oxigenator 100HG or the D150 are arvailable. These kits consist of 5 sets of tubing connectors for perfusate and gas to the oxygenator.

Fixation of the fiber membrane oxygenator

Figure 8 shows the fixation of the fiber membrane oxygenator using a special holder.

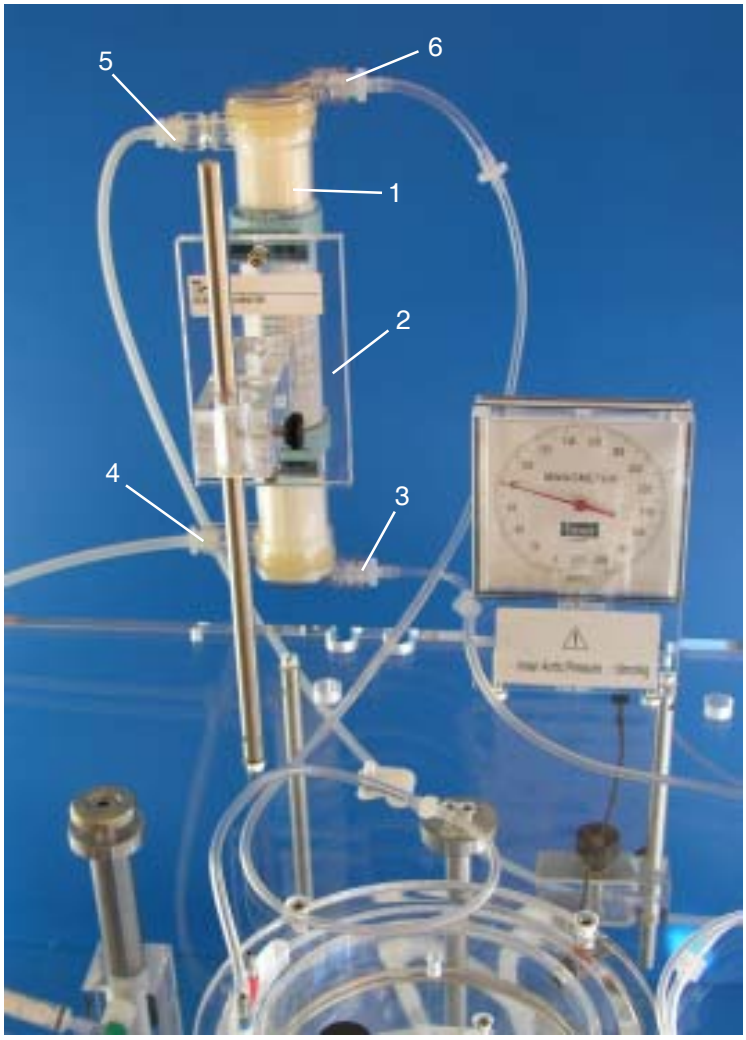


Fig. 8: Fixation of the fiber membrane oxygenator on top of IH-SR system.

1 Fiber membrane oxygenator.
2 Holder for fiber membrane oxygenator. The holder can be mounted on any vertical 8mm rod.
3 Inlet perfusate, from roller pump. Caution: Do not suck the perfusate through the oxygenator!
4 Outlet gas (Carbogen).
5 Inlet gas (Carbogen).
6 Outlet oxygenated perfusate.

5.8.2 Fiber membrane oxygenators in the LH mode and in the WH mode

Figures 9 and 10 show schematically the use of a membrane oxygenator (34) in the two operating modes.

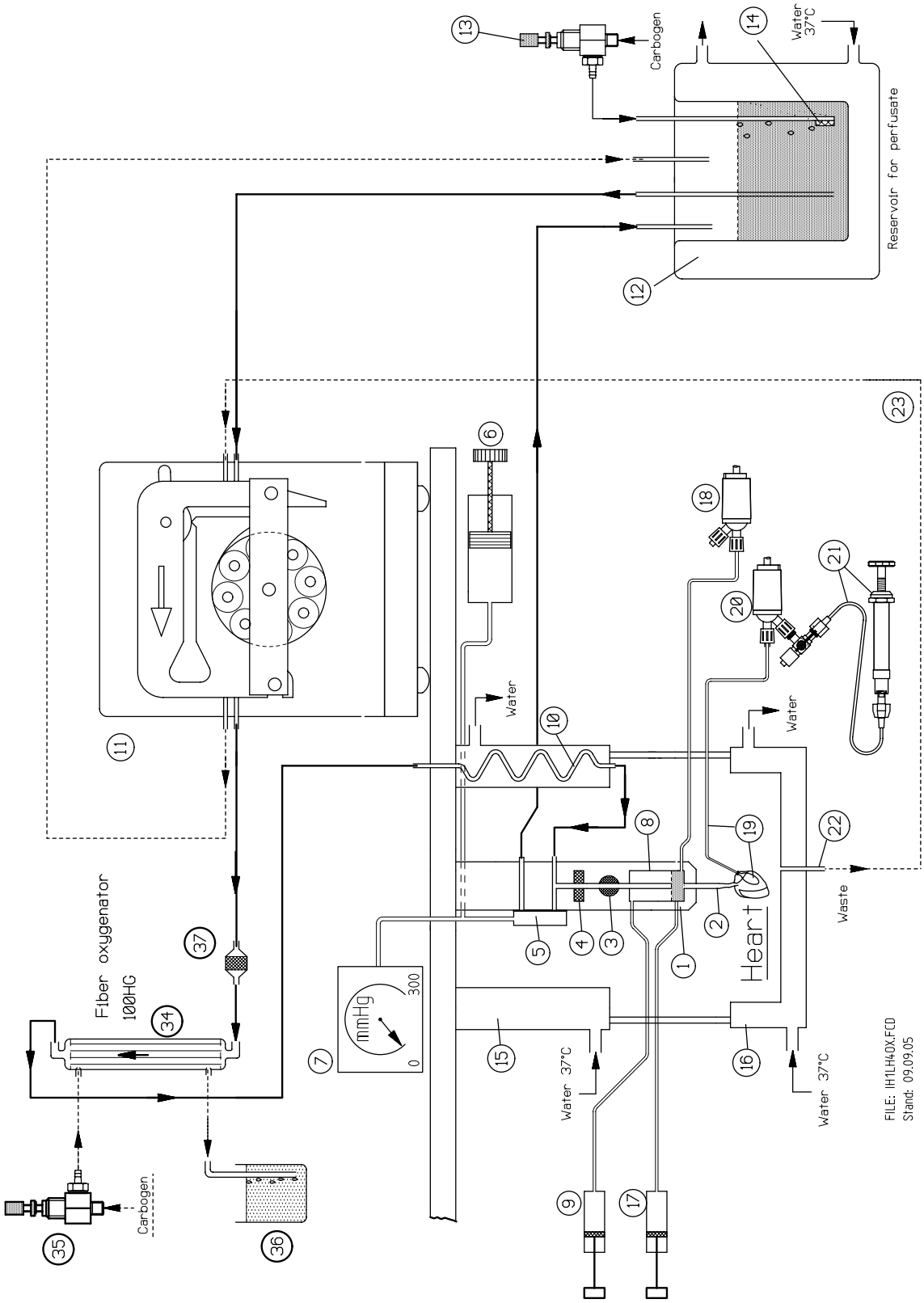


Fig. 9: Fiber membrane oxygenator in LH mode.

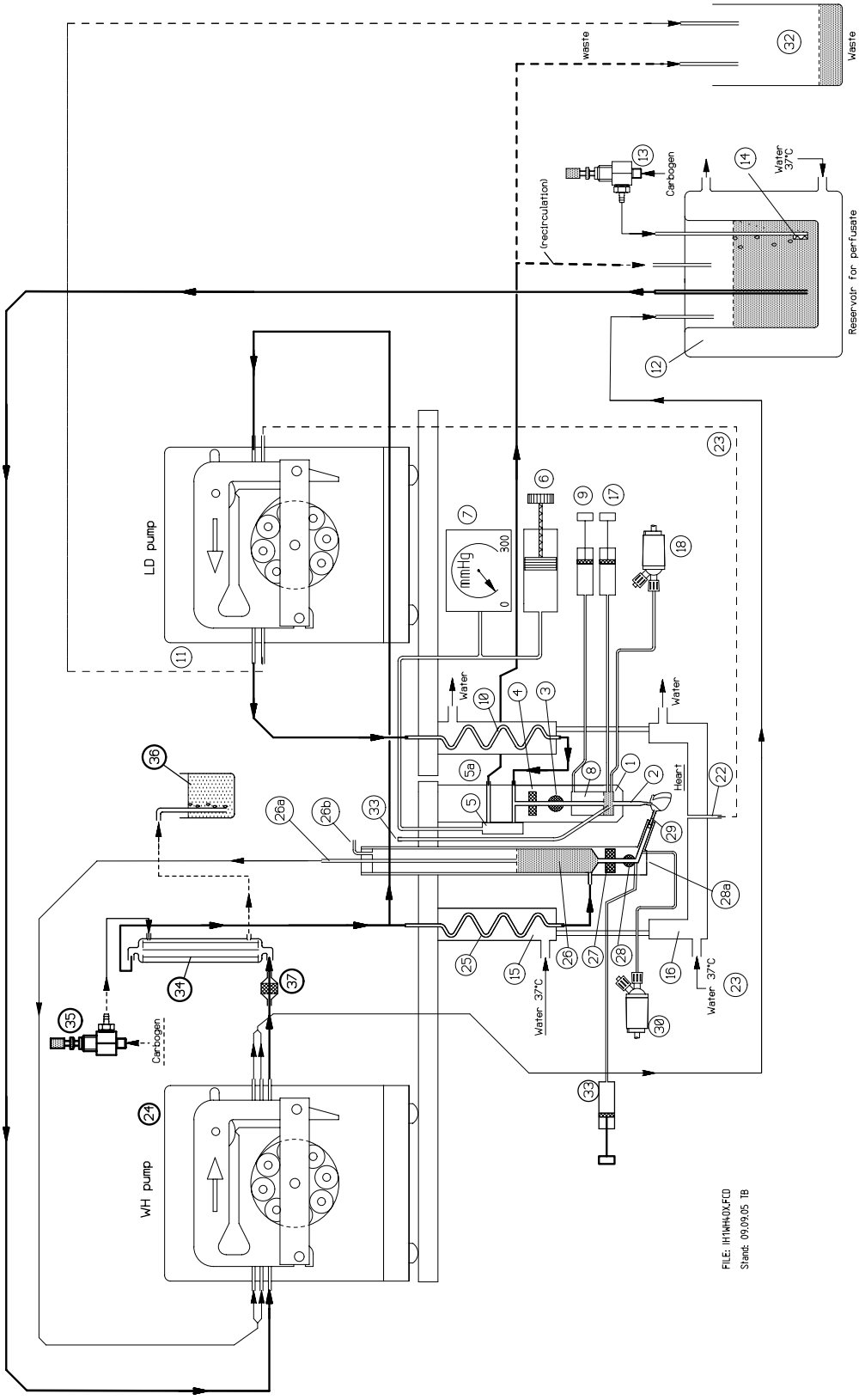


Fig. 10: Fiber membrane oxygenator in the WH mode.

5.8.3 Filtering during recirculation

There is a danger (especially during recirculating operation) that small particles pass into the coronary vasculature and produce varying degrees of embolism. It is therefore advisable in that case to filter the perfusate pumped to the heart. A suitable filter (37) is shown in Fig. 11 and 12. At this low flow rate a 25 mm diameter filter is sufficient. The filter housing is made up of two parts which are screwed together. The actual filter consists of an interchangeable thin filter disc. The recommended pore size is in the range 45 to 80 µm.

IMPORTANT: note the flow direction during installation!
The filter housing has to be installed so that the liquid flow presses the filter disc against the internal flat support grid.

Pressure side: Luer lock, female (1) Outlet side: Luer, male (2)

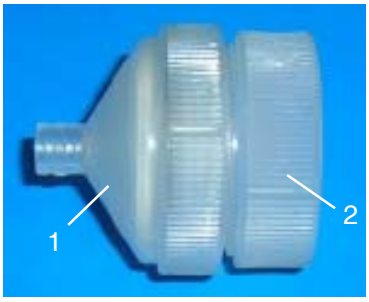


Fig. 11: Inline particle filter.

1 Inlet side (pressure side). Luer lock female.
2 Outlet side. Luer male.

Mounting the complete filter
Hold the components in the following order from the bottom upwards:
- Filter housing bottom, outlet side, the flat grid points upwards;
- Filter disc (pore size 45 or 80 µm);
- Sealing ring (ext. dia. 26 mm);
- Filter housing top, inlet side, the female Luer lock connection points upwards.

Carefully screw the upper part on to the lower part. Ensure that the thread is fitted correctly and is not damaged. Do not use force.

When screwing up the filter housing, ensure that filter disc and sealing ring are fitted correctly in order to produce a reliable filtering action and to avoid any leakage from the filter housing during use.

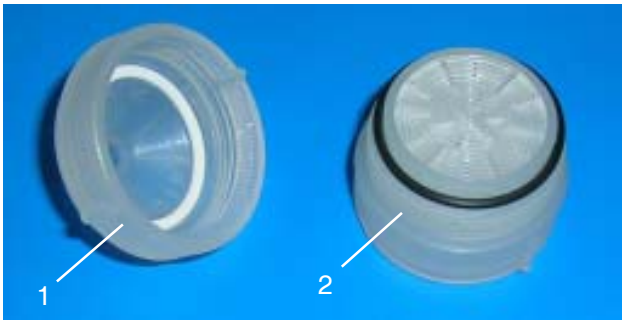


Fig. 12: Inline particle filter. Dismantled.

1 Inlet side (pressure side) with white seal ring. Connection Luer lock female.
2 Outlet side with seal ring and flat grid for filter disc. Luer male.

5.8.4 Increased preload in the WH mode

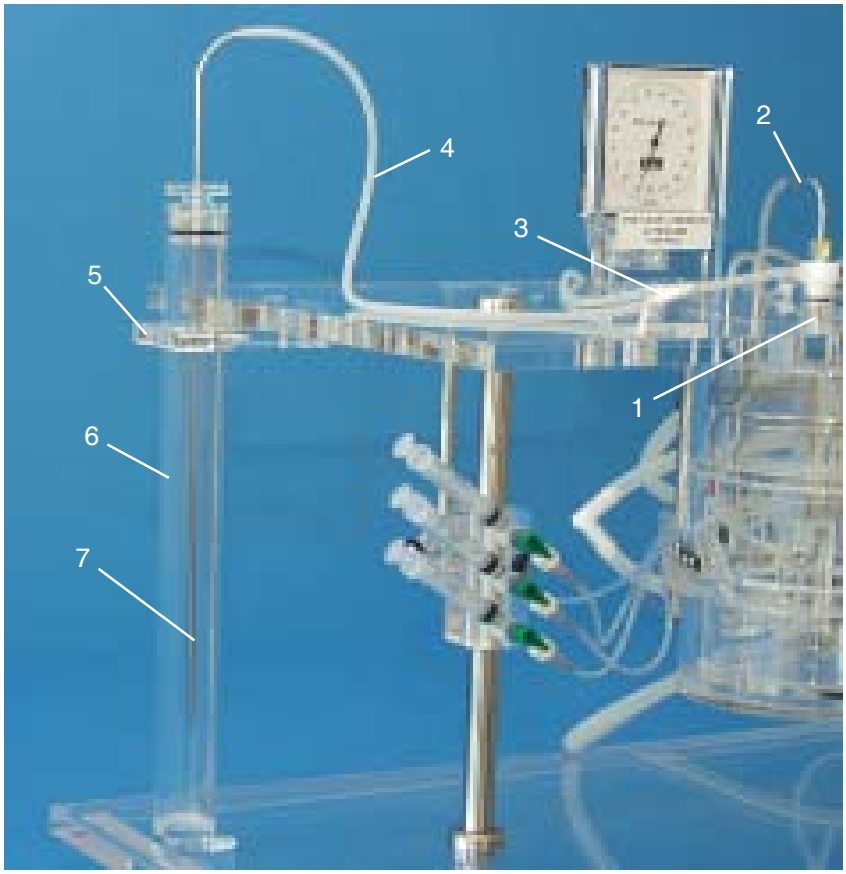


Fig. 13: Gottlieb valve (73-0158) to increase preload on IH-SR WH mode.

1 Atrium reservoir (preload vessel).
2 Metal suction tube of atrium reservoir (preload vessel). The tube is used to adjust the water column (preload) in the atrium reservoir (26a in Fig. 14). Horizontal male LUER port is connected to a piece of tubing going to T-piece (3).
3 T piece (39 in Fig. 14). By using one of the needle valves Carbogen is blown in here to increase the pressure in the atrium reservoir by using the Gottlieb valve.
4 Connecting tubing from atrium reservoir (2) to Gottlieb valve steel tube (7).
5 Holder for Gottlieb valve (5).
6 Gottlieb valve.
7 Tube to adjust water column in Gottlieb valve (6).

The standard preload reservoir has provision for varying the preload in the range from 4 to 11 mmHg by suitable adjustment of the suction tube (26a) and the driving water column. If the maximum pressure is not high enough to ensure satisfactory filling of the ventricle during the experiment or if you need unphysiologic high pressures to simulate a disease, it is possible to extend the pressure range by the method shown in Fig. 13 and Fig. 14. In this case the pressure is adjusted with a Gottlieb valve (40). Using the needle valve (38) a small flow of Carbogen (or air) is blown into the tubing T piece (39). The pressure in the air cushion of the preload vessel (26) then increases until the liquid in the immersion tube of the Gottlieb valve is displaced and gas/air bubbles rise through the liquid. The effective preload (p_V) is then the sum of the hydrostatic pressure in the preload vessel (p_H) and the immersion depth (T) in the Gottlieb valve (40).

$p_V = p_H + p_G$
p_V = acting preload pressure
p_H = hydrostatic pressure in the preload vessel
p_G = hydrostatic pressure in the Gottlieb valve (= immersion depth T)

It is advisable to measure and monitor the actual preload pressure with a pressure transducer (30), e.g. a venous pressure transducer P75.

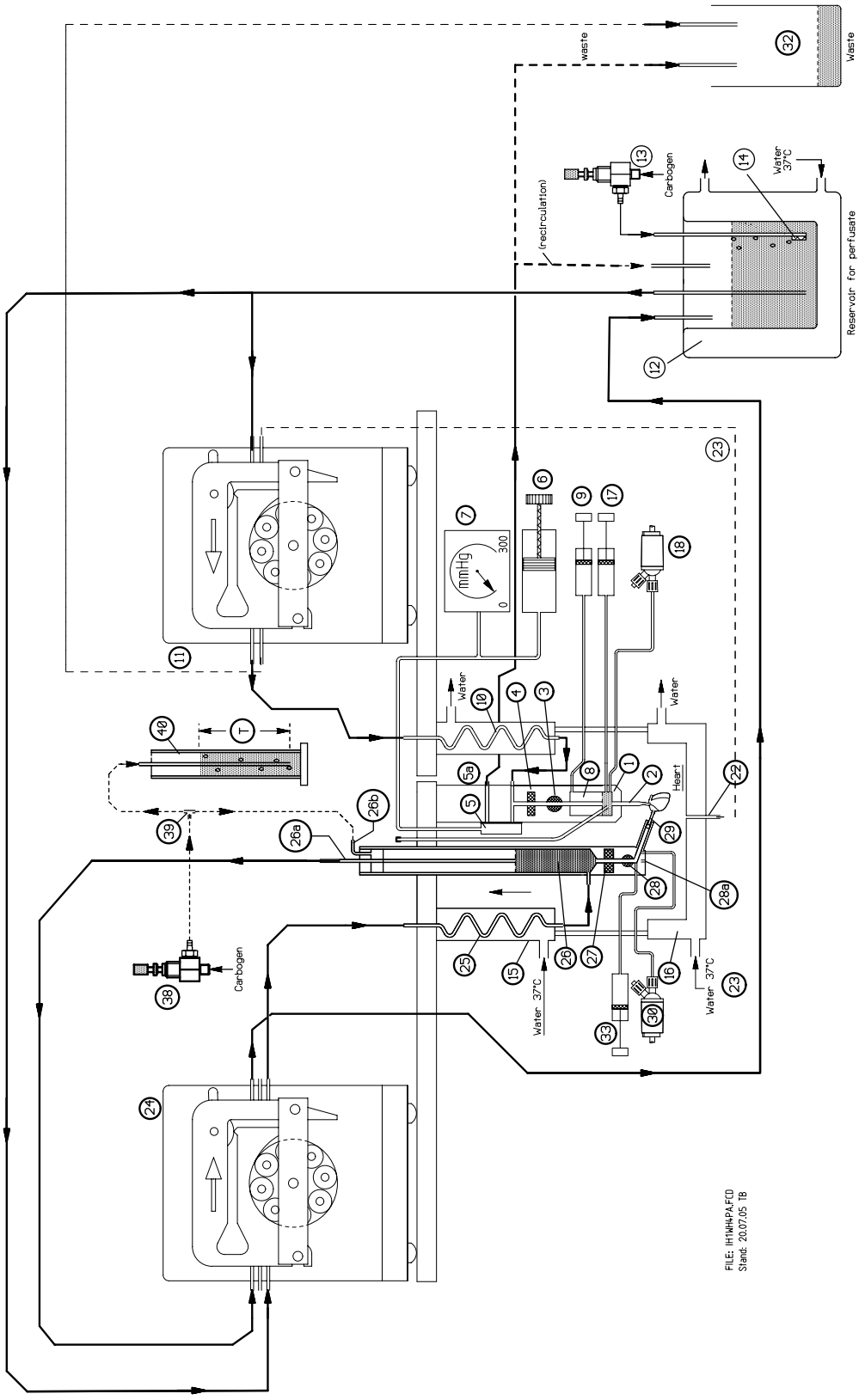


Fig. 14: Arrangement for increased preload setting (Gottlieb valve).

5.8.5 Characteristics of the adjustable flow resistance

The essential component which determines the pressure regulation during perfusion under constant pressure in the LH mode, as well as the afterload in the WH mode, is the adjustable membrane flow resistance (5) which is fitted on the side of the aorta block (1). The required back pressure is selected on the control of the large spindle syringe (6) and is indicated on the pressure gauge (7) (set value). When the pressure reaches the set value during perfusion, the flow resistance membrane (5) opens the return line to the reservoir (12). By suitable adjustment of the pump output (pump must deliver always a little more than the heart needs) and of the flow resistance setting it is possible to perfuse the heart either at constant flow or at constant pressure. This device also acts as afterload in the WH mode. If the heart has sufficient pumping capacity the pressure in the air vessel (8) increases until it exceeds the pressure set on the flow resistance (5). The outlet line (5a) is used to pass the aortic flow.

Figures 15 and 16 show typical characteristics in the two operating modes. Pressure settings are 0 mmHg, 20 mmHg, 40 mmHg, 60 mmHg, 80 mmHg and 100 mmHg.

Langendorff mode

Fig. 15 shows the characteristic for perfusion in the LH mode under constant pressure. Plotted on the X-axis is the flow through the flow resistance [= flow rate of pump (11) less coronary flow], and on the Y-axis the resulting pressure measured with the pressure transducer (18). The parameter is the pressure set on the flow resistance. Note the characteristic line for a pressure setting of 0 mmHg. This characteristic is valid only if the distal tubing end (5a) is set accurately at the level of the heart, and is therefore shown dotted. For other values of the pressure parameter this influence is not so pronounced.
It also should be mentioned here that it is necessary to measure the real aortic pressure, as this is always around 6 to 8mmHg higher as the set pressure, due to the water column in the aortic block.

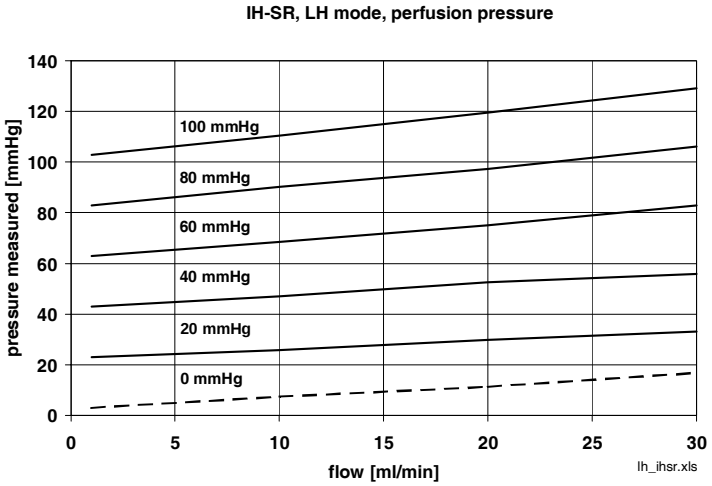


Fig. 15: Characteristic of the flow resistance in the LH mode.

Working Heart mode

Fig. 16 shows the characteristic of the afterload in the WH mode. The aortic flow is plotted along the X-axis, and along the Y-axis the resulting aortic pressure as measured by the pressure transducer (18). The parameter is the pressure set on the flow resistance. Note again the characteristic line for a pressure setting of 0 mmHg. This characteristic is only valid if the distal tubing end (5a) is set accurately at the level of the heart and is therefore shown dotted. Also here it should be mentioned that it is necessary to measure the real aortic pressure, as this is always around 6 to 8mmHg higher as the set pressure, due to the water column above the heart in the aortic block.

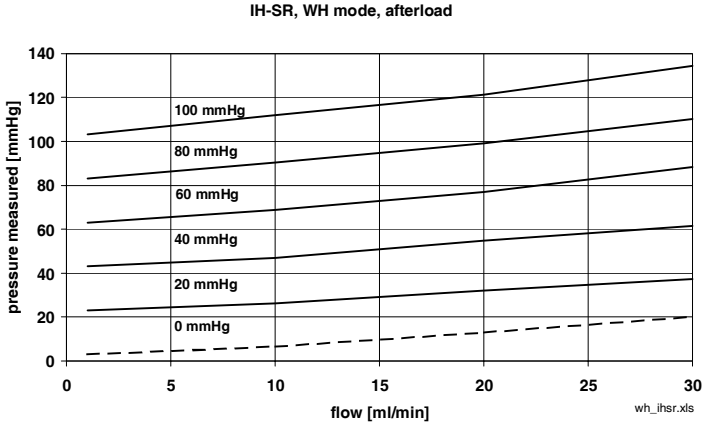


Fig. 16: Characteristic of the flow resistance in the WH mode.

6. Measuring system - electrical stimulation - special features

6.1 Pressure measurement

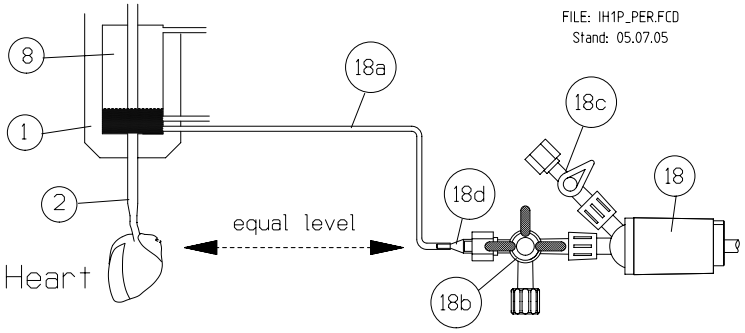


Fig. 17: Pressure measurement.

The perfusion pressure (LH mode) or the aortic pressure (WH mode) and the atrial pressure require two pressure transducers, e.g. ISOTEC pressure transducers or P75 for strial pressure, as well as suitable amplifiers, e.g. the HSE PLUGSYS module "Transducer Amplifier Module TAM-A". Details on use and operation of these instruments can be found in the appropriate operating instructions.

Two holders fitted on two 8 mm rods are provided for mounting the pressure transducers; the rods are screwed into the upper Plexiglass plate from the lower side. These holders are normally suitable for taking ISOTEC pressure transducers or a P75 for the preload pressure. Alternatively different holders can be supplied suitable for other transducer types.

Figure 17 shows schematically as an example the arrangement for measuring the perfusion pressure. At the pressure transducer dome the side connection takes a shut-off stopcock (18c) and the central connection a 3-way stopcock (18b). In order to assist with the necessary filling free from bubbles, it is convenient to mount the pressure transducer so that the 45° side connection of the dome points upwards. The pressure transducer is connected up with a tubing catheter carrying at one end a female Luer taper (Tygon tubing, ID = 1.14 mm, shortened). The catheter end with the Luer taper is connected to the 3-way stopcock, the other end to the thin metal tube at the the rear of the aortic block.

Pressure measurement is only correct and free from errors if certain preconditions are fulfilled:

1. Tubing and dome of the pressure transducer must be filled with liquid free from bubbles. Especially when the evaluation involves analysing the trace (e.g. recognising the notch in the trace of a WH in order to measure the heart ejection time) it is important that even the smallest bubble is removed so that steep parts of the trace can be measured accurately!

3. The pressure measuring system must be calibrated (see operating instructions for pressure transducer,Transducer Amplifier Module TAM-A).

4. Pressure transducers and connecting tubing must not move during measurement! Movement of tubing or transducer produces movement artifacts which are superimposed on the measured pressure signal.

5. The pressure transducer must be mounted at the level of the aorta or atrium.

6. Perform zero adjustment correctly and in the correct order. Open stop cock 18b to air and adjust zero at your amplifier.

6.1.1 Isovolumetric LVP measurement in the LH mode (balloon method)

Preparing and filling the balloon

In the LH mode LVP measurement is performed using the so called balloon method. Latex balloons in the required size are available for rat and guinea pig hearts. Some customer prefer self made ballons made of condoms. For mouse hearts Latex ballons cannot be used because they would not be able to follow the high heart rate. Because of the regular pear shape and the internal stresses in the material it is virtually impossible to maintain the diastolic pressure constant over longer periods at the required value (e.g. 10 mmHg). In addition the latex material produces a damping effect. At higher heart rates this results in an appreciable error in the differentiated pressure signal (dp/dt).
More suitable in this application is a balloon produced from thin domestic film (ceran wrap) which is formed by hand into a sack-like shape and is tied on to the balloon cannula. For this purpose we recommend the HSE "Mouse Ventricular Balloon Kit" (73-2787). This kit consists of a stand with two holders and crocodile clamps, hex screwdriver modified for balloon forming, syringe, scissor, ceran or kling wrap and PE tubing.

Before starting to produce a balloon it is necessary to fill the complete measuring system up to the tip of the balloon cannula (19b) with degased destilled water free from bubbles. A mixture of alcohol (20%) and distilled water has been proved very satisfactory as filling liquid. The simplest procedure for filling free from bubbles is as follows:

- Take a beaker with the filling liquid (20% alcohol, 80% distilled water).
- Hold the balloon cannula with its distal end into the liquid.
- Suck up liquid by rotating the spindle syringe and fill the entire system (balloon cannula, catheter tubing, Isotec transducer, 3-way stopcock, spindle syringe) free from bubbles.

The manufacture of a balloon is relatively simple and after some practice takes only a little time.
Cut off a sufficiently large piece of ceran wrap and place its centre on a round pin of about 3 - 4 mm diameter whose end is well rounded. The resulting sack-like shape is then taken off the pin and loosely tied on to the balloon cannula. The balloon cannula is then held vertical with the balloon downwards so that air can be driven out of the balloon from the bottom upwards between film and cannula as the balloon is filled with liquid from the spindle syringe. The balloon is then carefully tapped and squeezed to ensure that no air bubbles remain inside it. When the balloon has the required size and is filled free from bubbles it can finally be tied firmly to the cannula. To get it thight is is highly recommended to use a little piece of silicone on the used catheter

Now check that the balloon is leaktight. Using the spindle syringe (see Fig. 18 next page, item 21a) carefully add sufficient liquid so that a pressure of about 100 mmHg is produced. Then close the 3-way stopcock (see Fig. 18 next page, item 20a) and monitor the pressure. It should not decrease appreciably over a period of one minute.

Assembling the balloon pressure measurement system

The measurement system consists of the following components: arterial blood pressure transducer (e.g. Isotec) with catheter and balloon, 3-way stopcock, and 2 ml spindle syringe. Refer to Fig. 18 and the corresponding legend during assembly.

The balloon is positioned in the left ventricle of the heart using the balloon cannula (19b). The pressure transducer (20) is located outside the warm chamber space and is secured at the heart level with the holder on the right front support rod. A length of thin flexible tubing is used to transmit the pressure from the balloon catheter (19c) to the pressure transducer (20).

The spindle syringe (21a) which is mounted with a ball joint holder on the upper apparatus plate, is linked to the pressure transducer (20) through a tubing catheter (21b) and the 3-way stopcock (20a). The plunger of the syringe carries a thread. Rotation of the plunger allows the balloon to be filled accurately to the required diastolic pressure of e.g. 10 mmHg. The 3-way stopcock serves for filling balloon, catheter and dome free from bubbles and later to ensure pressure-tight separation of the filled measuring system from the spindle syringe.

It is essential to note the arrangement of the 3-way stopcock [13, (20a)]. No additional stopcock must be placed between dome and balloon catheter. Any air bubble trapped in this region would greatly worsen the pressure measurement response so that the differentiated pressure signal dLVP/dt would be severely faulty.

To fill the measuring system it is preferable to use a mixture of alcohol and distilled water (mixing ratio approx. 20:80). This mixture has good wetting properties so that filling free from bubbles is relatively easy. In addition, only little air is dissolved in this mixture, preventing the formation of interfering bubbles later during the measurement. It is additionally recommended to use degassed water.

The pressure transducer is connected with its cable to the appropriate amplifier.

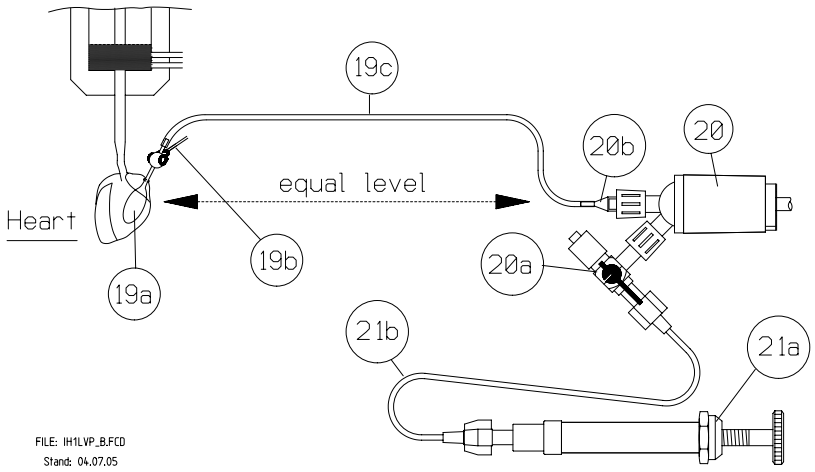


Fig. 18: Isovolumetric pressure measurement.

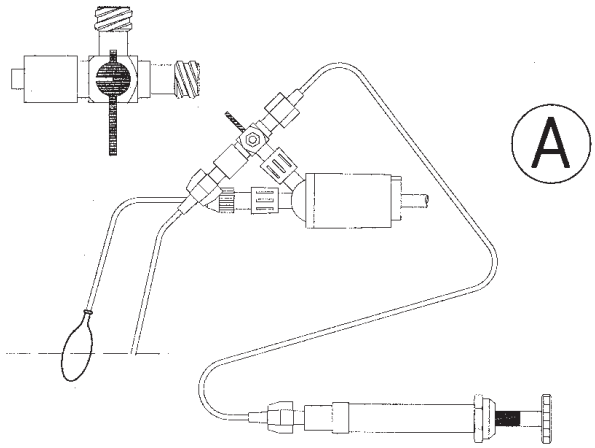
19a Balloon, size to suit heart dimensions.
19b Balloon cannula with mounting clip to mini ball joint holder.
19c Tubing catheter, keep it short! NOTE: do not fit a stopcock at the connection to the pressure transducer (20)!
20 Pressure transducer (e.g. ISOTEC) with a very low volume displacement.
20b Luer cannula (shortend) to fix tubing catheter (T45054).
20a 3-way stopcock.
21a Spindle syringe, 2ml (73-0320).
21b Connecting tubing catheter (Tygon, ID = 1.14 mm, 50 cm; R43010) for setting the preload pressure with the spindle syringe.

Figure 19 shows schematically the arrangement for measuring the isovolumetric internal LV pressure using the so-called balloon method. Note in particular the positions of the 3-way stopcock in the digrams A-C.

Please note:
Always ensure first that the measuring system is filled free from bubbles!

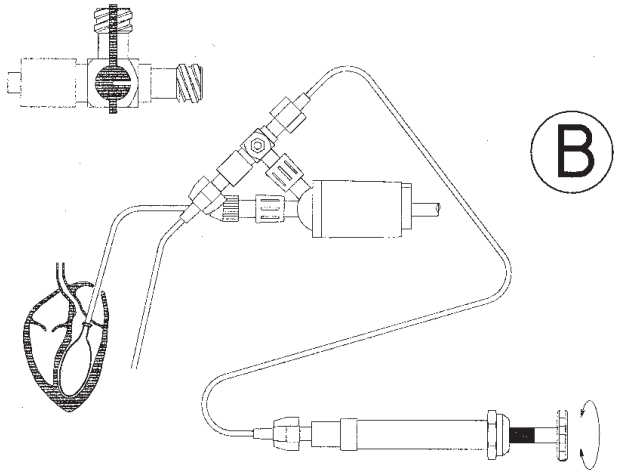
A) Perform zero adjustment of the ISOTEC pressure transducer

Zero adjustment is performed. Dome opened to atmosphere. Move the 3-way stopcock into the position shown. To compensate the hydrostatic pressure (the liquid column between balloon and pressure dome) the distal end of the short length of tubing must end at the level of the balloon (note the horizontal line drawn across the balloon!).
This piece of tubing must be completely filled with liquid (bubble free!)!
It is also possible to move up the ballon to the level of the ISOTEC.



B) Set the balloon pressure (end diastolic pressure!)

After the balloon has been inserted into the left ventricle the required end diastolic pre-load is set. Move the 3-way stopcock to the position shown and adjust the required pressure on the spindle syringe. For accurate adjustment of the pressure it is essential to have a computer data acquisition system or an oscilloscope connected to the pressure amplifier so that the dynamic balloon signal (LVP) and the enddiastolic pressure can be monitored.



C) Normal operation with measurement of isovolumetric LVP

After the pre-load has been set (B) the dome is closed by changing over the 3-way stopcock into this position. The pre-set diastolic pre-load should remain unchanged after switching over the stopcock. If this is not the case, a leak in the measuring system must be suspected.

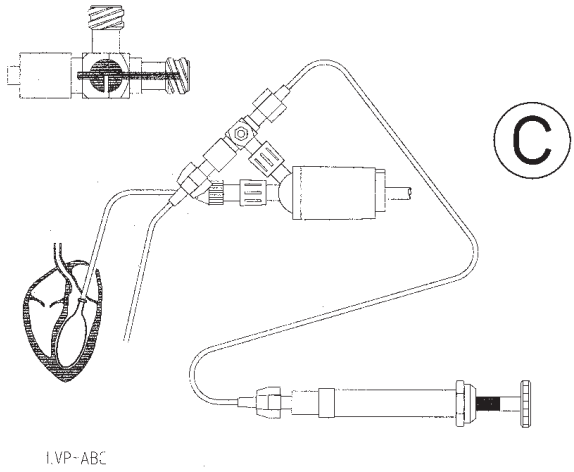


Fig. 19: Isovolumetric LVP measurement. Note legends A) to C).

6.1.2 LVP measurement in the WH mode

In the Working Heart (WH) mode it is important to measure the intracardial pressure in the left ventricle, the LVP. This is not quite simple in this small heart. Very small micro tip catheter transducers with a tip diameter of 1.4 F (D = 0.46 mm) are available which are however quite expensive. In spite of their small size there remains a possibility that the valve action is affected by the catheter passing through it. Another possibility of obtaining the LVP signal consists of measuring it with a tubing catheter and a conventional pressure transducer. This arrangement is much less expensive. A disadvantage is the method, by no means uncontroversial, of piercing the heart apex. A technical disadvantage compared with the measurement by tip catheter is the limited frequency response (see below) especially if air bubbles are in the catheter.

6.1.2.1 LVP measurement using a tubing catheter

A method in practical use is reported in the literature and illustrated in Fig. 20.

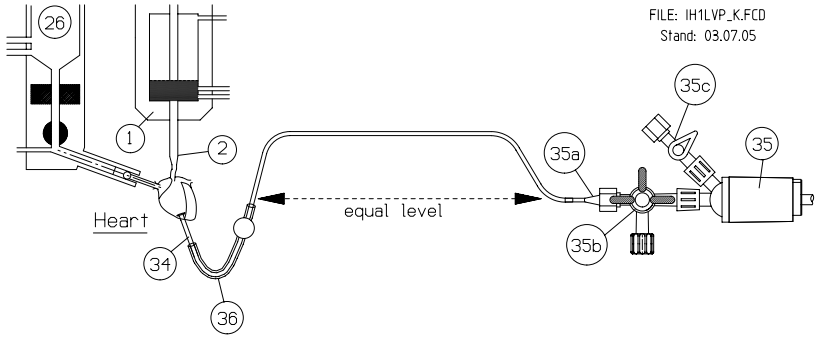


Fig. 20: LVP measurement with tubing catheter.

A thin tubing catheter (34) is pointed at the end and is passed through the left atrium and through the mitral valve. From the inside of the heart it is then made to pierce the heart apex and is taken to the outside where it is connected to a conventional pressure transducer (e.g ISOTEC). In order to prevent it slipping out through the heart apex the rear end of the catheter should before introduction be given a small funnel-shaped bulge by heating it (trumpet form). A J-holder (36) (73-0179) maintains the PE-catheter (34) forced through the left ventricle apex. In combination with mini ball joint holders a precise fixation without stress load from PE-catheter is possible. The thin tubing catheter (PE-50, D = 0.58 mm) is connected through the Luer taper of a syringe cannula (35a) (21G, 0.8 mm dia., shortened to about 10 mm) to the pressure transducer (35). Stopcocks (35b) and (35c) are fitted to permit filling free from bubbles.

In order to ensure that the steepness (slope) of the LVP signal is captured accurately it is essential that the complete pressure transmission system (tubing catheter, dome and especially the hidden corners of the stopcocks) are absolutely bubble free. When using a pressure transducer other than the suggested ISOTEC transducer it is important that the type used has extreme low volume displacement under pressure. With unsuitable pressure transducers or with bubbles in the measuring system the measured pressure curve is falsified, leading to correspondingly large errors in the measured pressure and also the dp/dt signal.

A LVP measuring kit (73-0167) consisting of a J-holder for PE-cathers, Luer adapter, 2 mini ball joints, 1 ball with thread and 10 PE-catheters is available.

6.1.2.2 LVP measurement with MILLAR Tip catheter

Optimum results in accurate curve reproduction and in the differentiated dLVP/dt signal are obtained by using a micro tip catheter pressure transducer, e.g. Millar Miniature Tip Catheter Model SPR-835 (tip catheter) for mouse hearts or Millar Miniature Tip Catheter Model SPR-407 for rat hearts. With these transducers the pressure is measured directly at the catheters tip so that there is no need for pressure transmission from the ventricle interior to the outside. This prevents falsification of the trace such as occurs in measurements with catheter tubing. The result is a frequency response up to the kilo-Hertz range. The distal region of the catheter is only 1.4 F (0.46 mm dia.) thick over a length of 15 cm and is therefore extremely thin. The introduction of the catheter has little effect on the function of the aortic valve.

Please NOTE:
The introduction device (Tip cather port, 73-0231 for SPR-407, 73-0727 for SPR-835) for the tip catheter must be purchased separately. It is not fitted as standard on every apparatus.

In addition to the tip catheters SPR-835 and SPR-407, an adapter box TC 510 and a pressure amplifier, e.g. the HSE PLUGSYS module “Transducer Amplifier Module TAM-A“ are required. The SPR-835 also requires additionally a special adapter cable TEC-10D (73-0940).
Handling and operation of these instruments are described in the appropriate operating instructions. It is essential to carry out zeroing and calibration of the entire instrument chain before it is used.

IMPORTANT NOTES:
Remember that the Tip catheter represents an extremely delicate and also very expensive component. Handle it very carefully and keep it in good condition. The catheter tip in particular must not be exposed to mechanical stresses. Special care is required when pulling out the catheter tip transducer. During this operation ensure that the catheter tip does not flip to the side and hits a hard object. Pay full attention to its operating instructions.

In most cases the catheter gets damaged because users forget to remove it from the heart (pull it back into the aortic block) before cutting the heart from the cannula.

It also should be mentioned here that Tip catheters should be ZERO adjusted at the temperature they are used. This means the ZERO adjustment should be made by holding the Tip into 37°C warm water (don´t immerse it more than a few millimeters).
If you record at any time negative end diastolic pressures you have forgotten to calibrate the TIP at the correct temperature.

Figure 21 shows schematically the measuring arrangement using a micro tip catheter (MTC); the item numbers below refer to this illustration. The tip catheter (37) is introduced into the left ventricle through a small metal tube (33c) which protrudes at an angle to the front and upwards from the air vessel of the aorta block (1). From here the tube is extended upwards through semi-stiff catheter tubing (33c) to the metal tube (33a) of the Luer taper (33) which is used to hold the introduction adapter (36; Tip cather port). The normal Luer cover is replaced by the introduction adapters 73-0231 for SPR-407, 73-0727 for SPR-835). After releasing the clamping screw (metal hexagon) of the introduction adapter the tip catheter slides in easily.

The difference between the two tip catheter ports is the seal.

T24014 Silicone Seal D=3/1.5 L=4
T24012 Silicone Seal D=3/1.0 L=4
(dimensions in mm, D=diameter, L=length)

During the preparation phase it is advisable that the tip catheter is already inserted so far that the tip is in the windkessel of the IH-SR system a few millimeters below the metal guide tube (33c). Here it is protected and the introduction device (tip catheter port) can be sealed by carefully tightening the clamping screw on the introduction adapter (36).

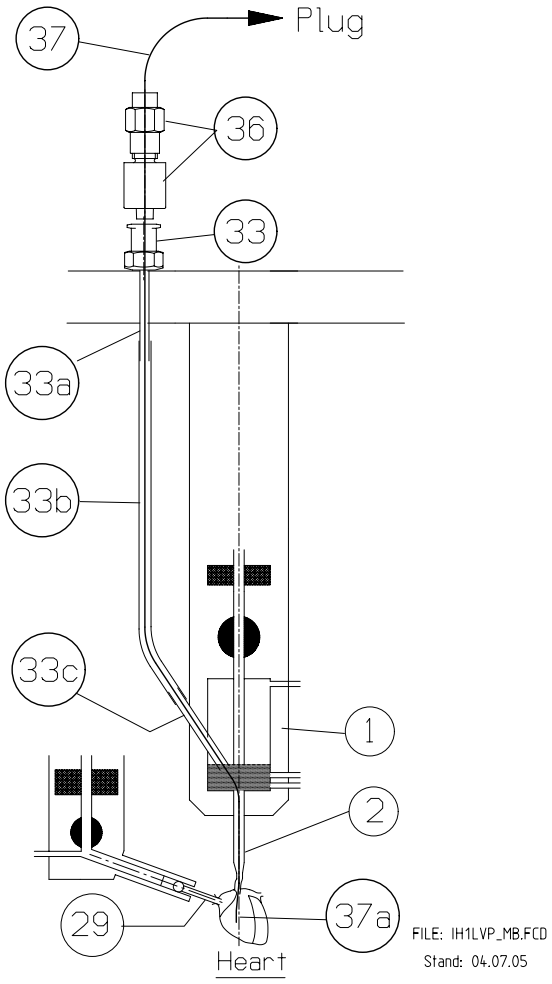


Fig. 21: LVP measurement with MTC. For explanation see text.

The tip catheter is moved forward into the ventricle when the heart has recovered from the preparation phase and from the procedure of mounting it in the apparatus, and performs ejection work. To slide the catheter forward the clamping screw of the introduction adapter (tip cather port) is released slightly.

Sliding the tip catheter forward must be done with care and under full control. Special care must be taken to avoid excessive mechanical stresses on the catheter tip (37a). When sliding it forward it may have to be twisted slightly so that it immediately passes into the hole in the air vessel bottom which leads down to the aorta cannula. It must never be kinked, especially in the tip region. This could immediately cause irreparable damage the tip catheter.

The correct position in the ventricle can be judged by the shape of the recorded pressure trace. If the traces have unusual spikes superposed on them this indicates that the catheter tip (37a) hits the inner wall of the ventricle. The catheter tip is in an unsuitable position and should be moved slightly. This fault can usually be rectified easily by careful displacement and rotation of the catheter while monitoring the pressure curve.

Finally, a piece of advice which should not be overlooked: after the end of the experiment and before taking all the other necessary steps, withdraw the distal end of the tip catheter into the protective air chamber if the IH-SR system (see above). You would not be first one who on removing the heart from the apparatus cuts through the catheter with scissors and thereby destroys it.

6.2 Flow measurement

6.2.1 Coronary flow measurement in the Langendorff mode (LH)

If the isolated heart is being operated in the Langendorff mode, the coronary flow is measured at the inflow to the aorta cannula. The other method used in experiments on the heart of larger species, to cannulate the pulmonary artery and to measure directly the effluate of the coronary flow, is not possible on the murine hearts.

The flowprobe (4) is fitted in a special perspex holder mounted into in the aorta block above the air vessel (8) and the main stopcock. The holder can be removed completely with the glued-in flowprobe after releasing the fixing screws.

6.2.2 Atrial flow measurement in the Working Heart mode (WH)

For measuring the atrial flow a flowprobe (27) is fitted in a special holder in the lower part of the preload vessel (26). The holder complete with the fitted flowprobe can be removed after releasing the fixing screws.

Build-in flowprobe to measure aortic flow in Working heart and coronary flow in Langendorff applications

Build-in flowprobe to measure atrium flow (cardiac output) in Working heart

Flow probe with holder

Figures 22 and 23 show the mounting of the flow probe in its holder (perspex block in preload chamber).

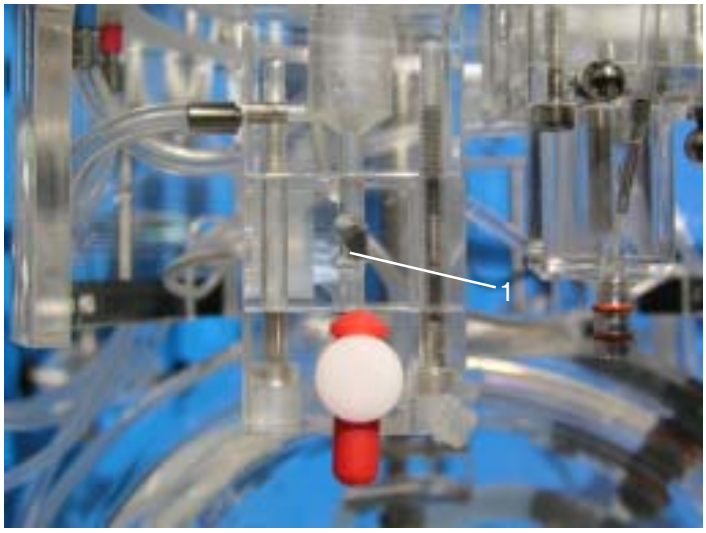


Fig. 22: Flow probe with holde in aterial block.

1 Flow probe with connecting cable to flow meter. The flow probe is glued into a perspex block and mounted in the preload reservoir above the change over stopcock. This flowprobe measures the flow into the atrium. For experiments on mouse hearts a flow probe 1 RB is required, for experiments on rat hearts a flow probe 2.5 RB is required at this position.

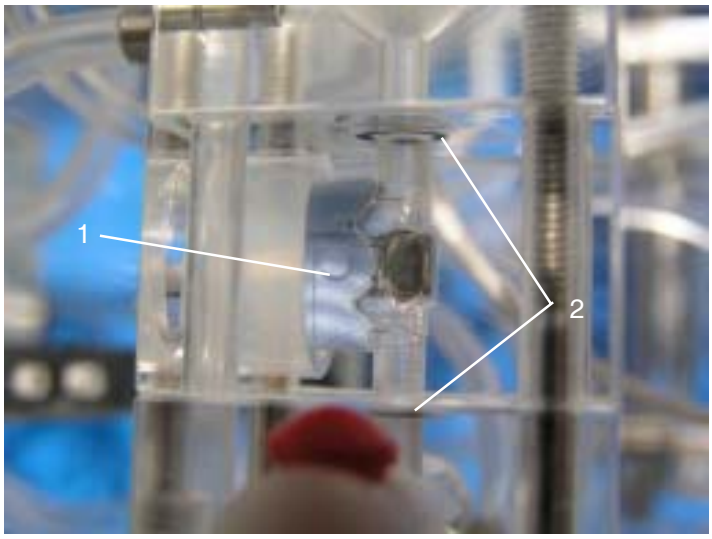


Fig. 23: Flow probe with holder (detail view).

1 Build-in flow probe with connecting cable to flow meter.
2 Two seals (O-rings).

6.2.3 General notes on ultrasonic flow measurement

The following information should give some more information about the measurement technic with the ultrasonic Transit Time Flow Meter TTFM Type 700 together with the appropriate flow probes. The first source of information for working with this instrument should be the Operating Instructions for the TTFM which are supplied with the module. You find this Operating Instructions in the white folder coming with each PLUGSYS housing.

Basic details: do you remember?

Ultrasonic transit time flow measurement depends on measuring the transit time of ultrasound through the liquid. By measuring it in two opposite directions the movement of the liquid produces a displacement of the transit time which is converted by an integrating procedure into a flow in ml/min or l/min.
Flow measurement by this principle is very accurate and almost free from interference.

The only requirement for obtaining good results is to fill the flow probe free from bubbles, also the perfusate must be bubble free.

Any stray voltages wich often cause problems in electromagnetic flow measurement do not influence the ultra sound transit time measurement. The flow probes are pre-calibrated. The zero point can be corrected on the flowmeter.

In general, the following points have to be observed:

- Allow the measuring head lumen to dry out only after it has been thoroughly rinsed with distilled water! This applies especially when working with whole blood or solutions containing erythrocytes. It is generally recommended to leave the probes filled with destilled water over night.

- When starting up a dry measuring head, fill the lumen with perfusate free from bubbles and allow it to stand (“soak“) for about ½ hour before starting the measurement.

The points mentioned in the introduction lead to the following additional requirements:

- Plugs of flow probe and extension cable must be kept dry! Wet or moist plugs cause electrical shunting, leading to measuring errors or even to complete failure of the flow measurement. Salt can not be removed from the connector if it comes inside! This can also case a damage of the connected flowmeter

- Never arrange flowprobe and/or extension cables parallel to a mains supply cable! The mains voltage and spikes superimposed on the mains voltage can influence the measurement.

Connecting the flow probe to the TTFM flowmeter results in automatic identification of the probe type and calibration values.

The serial number of the flow probe is engraved on the connector so that it can be uniquely identified at all times.

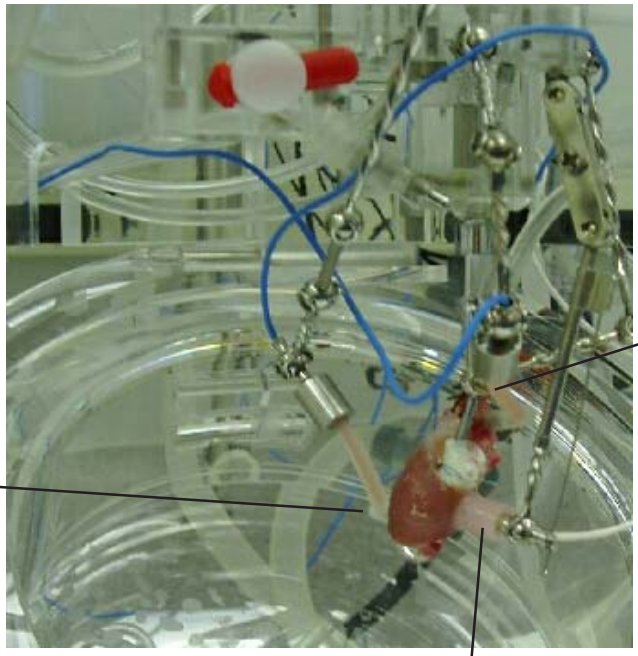
The manufacturer’s calibration has been determined based on 0.9% saline solution at 37° C. Calibration for whole blood is available to special order.

Probes can be recalibrated by HSE in the factory in Germany.

6.3 ECG recording

In order to achieve a large R-wave it is advisable to select apico-basal ECG recording on the isolated heart. This corresponds to Einthoven lead II in man since the line connecting the right arm and the left leg is also roughly parallel to the anatomical heart axis. Since such leads do not correspond to the standard lead schemes (e.g. Einthoven) they are also referred to as “electrogram” (EG).

EG recordings are made essentially with three leads: one is taken from the neutral zero electrode and the other two are linked to the recording electrodes A and B. Electrode A is placed on the base of the heart, e.g. on the right heart side next the left atrium, while electrode B is located on the heart apex. The zero electrode is a metal tube close above the heart which is connected to the heart through the electrically conducting perfusate.



6.3.1 Electrical stimulation

For certain experiments it is necessary to maintain a constant heart rate by electrical stimulation of the heart. If the heart rate required conforms to the in-situ values it is necessary to exclude the sinus node. This can be achieved by large-scale excision of the right atrium. If a slightly higher heart rate (10 to 20 beats/min) does not disturb the experimental protocol, it is possible to overcome the sinus node activity through electrical stimulation and selecting a suitably increased stimulation frequency.
If it is intended that the heart beats below the natural frequency the sinus node must be removed or damaged.

The stimulation electrode is arranged as a coaxial electrode (see Fig. 24). It consists of a central pin inside a metal tube (D1d). Both parts are electrically isolated from each other. Stimulation takes place concentrically between centre pin and tube. This largely avoids spreading the stimulation artifact into the tissue.

Generally stimulation does not introduce any difficulties if the electrode is applied at the sinus node. Other stimulation points may be troublesome since the stimulus spread then follows other non-physiological paths. In that case there may be disturbance of the regular course of the contraction wave over the heart, thus detracting from the heart force.
The stimulation amplitude is set between 2 and 4 Volt, the stimulus width to 1 and 2msec. The stimulus amplitude should not appreciably exceed the indicated value of 4 Volt, otherwise noradrenalin is released from the vesicles of the sympathetic nerve fibres of the heart, thus producing a positive inotropic effect. This is particularly pronounced if the stimulation electrode is applied close to or directly on the ventricle. The rate depends on your application e.g. a basic rhythm of 250ms or 4Hz results in 240 beats per minute.

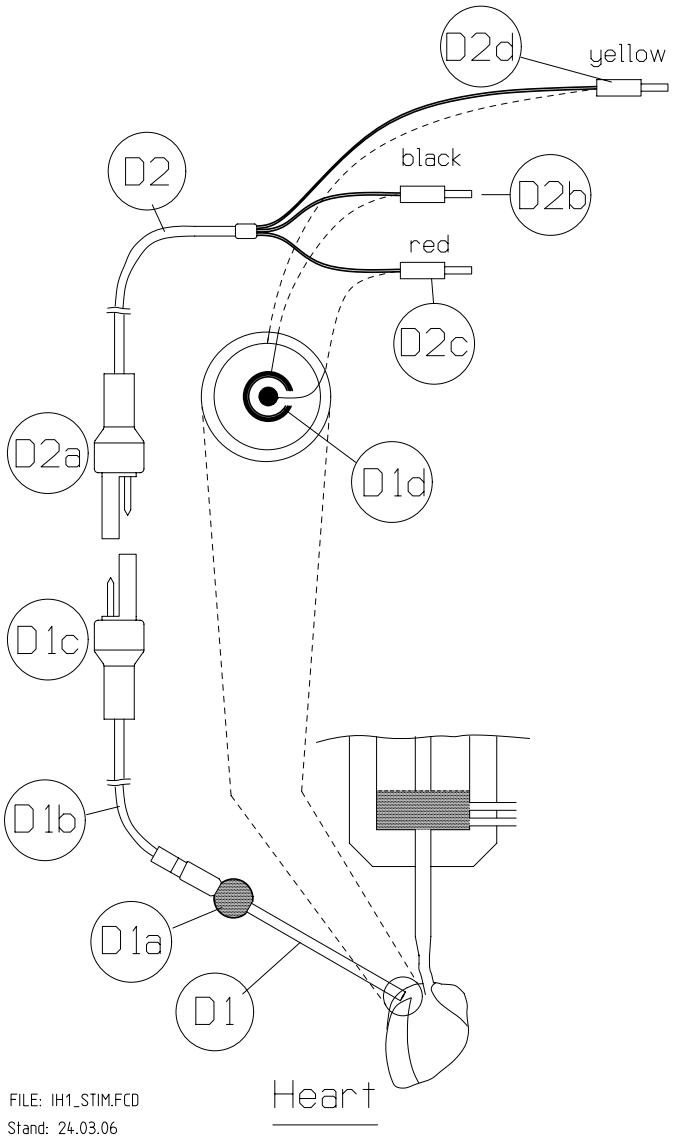


Fig. 24: Electrical stimulation.

D1 Shaft of the coaxial stimulation electrode (not electrically insulated).
D1a 5 mm ball, suitable to HSE mini ball joint holders. Note that the ball is electrically connected to the electrode shaft (D1) and to the outer ring of the coaxial electrode (see D1d).
D1b Thin flexible connecting cable, 40 cm long.
D1c Miniature plug, 2-way.
D1d Enlarged view of the distal end of the coaxial stimulation electrode. The central metal area is connected to the red plug (D2c), the outer ring to the black plug (D2b) of the delivered adapter cable.
D2 Connecting cable to stimulator, approx. 1.5 m long.
D2a Miniature plug, 2-way.
D2b Black banana plug (safety connector) for connection to the stimulator output. This plug is connected to the outer ring area of the coaxial electrode (see D1d).
D2c Red banana plug (safety connector) for connection to the stimulator output. This plug is connected to the central electrode area of the coaxial electrode (see D1d).
D2d Shielding cable, connected to stimulator housing or ground socket.

6.4 Recording of epicardial Monophasic Action Potentials (MAP)

Recording of MAP can be performed either epicardial or endocardial (not on mice hearts).

6.4.1 Epicardial MAP recording

Epicardial MAP recordings require the following items:
73-0150 MAP-TIP ELECTRODE

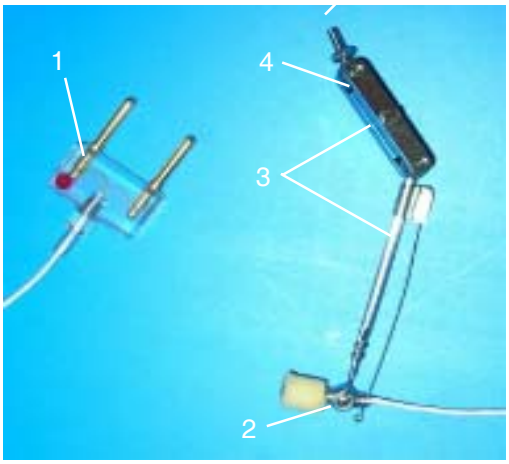


Fig. 25: MAP Electrode with mounted sponge and holder.

1 Plug for connection the MAP Electrode to MAP Input box or direct to MAP amplifier (BPA).
2 MAP-Electrode (D=2.5mm, l=6mm) with mounted sponge. The electrode is connected to the MAP input box or to an adapter cable for the BPA module.
3 Spring loaded holder for MAP electrode including link for higher loading (4). The holder is equipped with ball joints to position the MAP electrode.
4 Link for higher loading. The clamping force is adjustable.
5. Ball with thread

6.4.1 Endocardial MAP recording

intracardial MAP recording requires the following MAP electrode:
73-3715 MAP ELECTRODE FOR INTRACARDIAL MAP, STRAIGHT

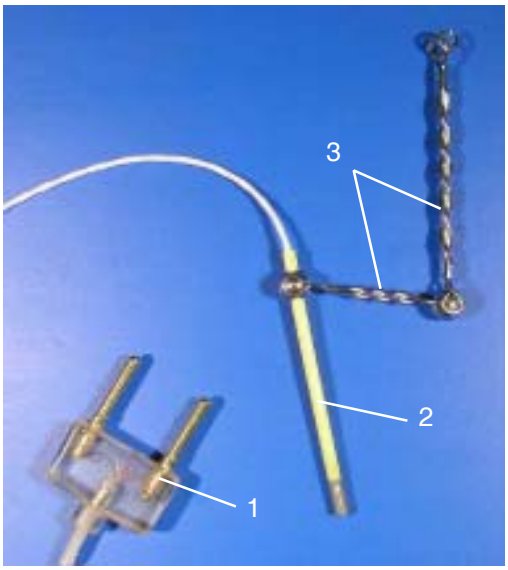


Fig. 25: MAP Electrode with included mini ball jointg holders.

1 Plug for connection the MAP Electrode to MAP Input box or adapter cable.
2 Intracardial MAP-Electrode. Here no sponge required
3 Mini ball joint holders to hold the MAP electrode in position.

1. Preconditioning of MAP Electrodes

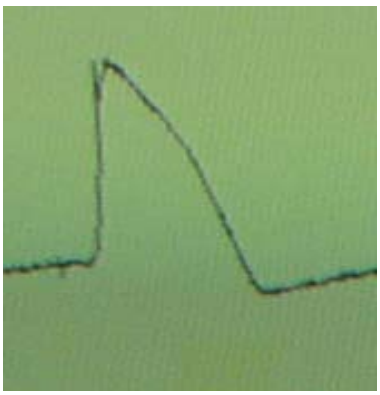
If you start working with a new dry electrode (just unpacked) you may get a MAP signal with a lot of noise. To get a good signal it is necessary to immerse the MAP electrode a few hours or over night in destilled water.

Note for handling:
Never touch the tip of the electrode or the reference electrode (on the side of the electrode body) with bare fingers. Never leave saline solution dry out on the electrode. Salt layers can not be removed.
Always flush the electrode and the sponge (if in use) after use carefully with destilled water.

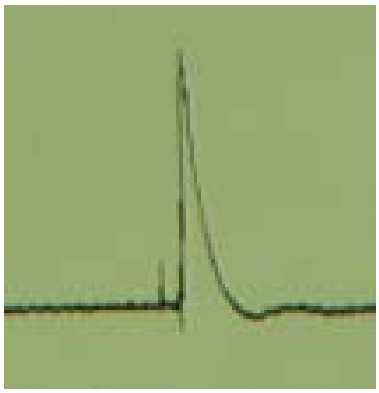
2. Using of Sponges
If you don´t immerse your heart in saline solution you must use the delivered little sponge on the electrode. The function of the sponge is to contact the reference electrode, which is build into the MAP electrodes body on the side. If you use sponges they must be kept wet with saline solution to get a good electrical contact between the heart and the reference electrode.

3. Getting a good MAP signal shape
The MAP signal depends a lot on the correct position and the pressure you give to the tissue under the electrode. All MAP electrodes have to be build into a spring loaded holder.
You get the best MAP signal shape if the electrode is attached in a 90 degree angle to the heart surface. If the angle is not correct you will not get an optimal signal.
If you record only an ECG instead of a MAP the electrodes pressure to the heart tissue is not high enough. Try to give a little more pressure. The electrode should move in its spring holder with the contrqaction. You also have to wait sometimes 20 - 30 seconds until the MAP signal gets better in shape. If this does not help change the position of the electrode so that you see the spring loaded electrode moving with the beating heart.

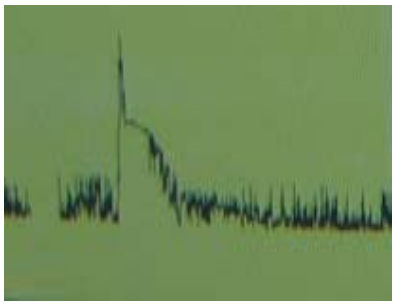
4. Position of electrode on the heart
If you are measuring epicardial MAP, check that your MAP electrode is not attached on the main coronary vessels (right or left LAD). The pressure on the MAP electrode would block the LAD which results in ischemic areas below the LAD obstruction.



good epicardial MAP



Ischemic epicardial MAP



MAP with 50/60Hz hum

Sponge use and care
Always immerse the sponge in saline solutione before using the MAP electrode. If the sponge does not take up the saline solution press it between the fingers or attach a syringe filled with saline solution to make it wet. Only with a wet sponge you get good MAP recordings. Never let the wetted sponge dry out. Salt layers destroy the electrode and the measurements.

7. Perfusion solutions and gas mixtures, typical consumption values

7.1 Perfusate

It is usual to use Krebs-Henseleit solution (original recipe and variations).

Composition (variant):

Salts (without water of crystallisation)	g/l	mmol/l
NaCl	6.9	118.0
KCl	0.35	4.7
$CaCl_2$	0.28	2.52
$MgSO_4$	0.20	1.64
$NaHCO_3$	2.09	24.88
KH_2PO_4	0.16	1.18
Glucose	1.09	5.55
Na-pyruvate	0.22	2.0

Krebs-Henseleit solution should be made up fresh each day and should then be filtered before use. The pore size of the filter used is usually 0.5 µm for the fine filter and 5 µm for the pre-filter. If no compressed air filter or suction filter is available, a suitably powerful roller pump can be useful. For filtration you require then only a disc filter holder (approx. 50 mm dia.) and suitable filter disc in the appropriate pore sizes (suppliers e.g.: Sartorius, Millipore).

Filtration should not be omitted. Even high-grade starting chemicals contain insoluble constituents which lead to micro embolisms in the heart if the solution is not filtered.

7.1.1 Consumption of perfusate

The perfusate consumption depends on the operating mode of the apparatus.

Mouse hearts
With retrograde perfusion of a mouse heart in the LH mode the expected consumption is about 4 to 7 ml/min. This does however apply only to a heart with an intact and undamaged coronary system. With a damaged vasculature the consumption increases to more than 10 ml/min.

In experiments with the apparatus in the WH mode a slightly higher consumption up to about 5 - 10 ml/min has to be expected.

Rat hearts

LH mode	10 - 14 ml/min (coronary flow)
WH mode	up to 60 ml/min (full cardiac output, aortic flow + coronary flow)

7.1.2 Aeration

In general Carbogen gas (5% CO_2, 95% O_2 v/v) is used for equilibrating the perfusion solution. This percentage composition should be adhered to as accurately as possible. Otherwise a wrong pH is obtained after equilibrating the perfusate. With constant aeration, Krebs-Henseleit perfusate maintains a pH of 7.4.

Ensure that you use only Carbogen gas of the composition mentioned above. When making up Carbogen or other mixtures yourself you have to ensure that you use only gas of the highest purity. In particular, technical nitrogen contains large amounts of oil.

If the amount of CO2 is larger than 5% your pH will be below 7.4,
If the amount of CO2 is lower than 5% your pH will be above pH7.4

Carbogen gas is normally provided in high-pressure gas cylinders. Before the gas is introduced into the apparatus its pressure should be reduced to an inlet pressure of 0.5 to 1 bar by means of a finely adjustable pressure reducing valve. If higher pressures are applied the tubing may burst or may slip off from the connection nipples.

The pressure regulator used should be provided with a pressure gauge with a suitable scale. The pressure setting must be indicated reliably and be read easily.

7.1.3 Consumption of Carbogen

The gas consumption depends greatly on the setting of the aeration rate. When the apparatus is used daily and the needle valve is adjusted to a reasonable setting the consumption is about 20 - 25L/min which results in 1 - 2 gas cylinders (10 litre size) per month.

8. Maintenance

8.1 Maintenance of the perfusate circuit

Before first start-up and after the end of the experiment the apparatus has to be cleaned thoroughly. Especially the perfusion circuit and all parts coming into contact with the perfusate have to be kept absolutely clean.

Daily cleaning is important particularly because of bacteria.
Apart from other effects, bacteria in the apparatus lead to early fail of the heart!

8.1.1 General cleaning recommendations

In general it is sufficient to flush the apparatus carefully and thoroughly with distilled water after the end of the experiment. Distilled water should be left in the apparatus overnight.

Thorough cleaning using one of the recommended cleaning solutions (see below) is required:
- Weekly, preferably before the weekend,
- after each experiment if substances are used which strongly adhere to the wall and cannot be flushed out with water. In extreme cases the apparatus has to be dismantled for cleaning. Tubing conducting the perfusate may have to be replaced.
- Before shutting down the apparatus for more than a few days. In that case the apparatus should be allowed to stand completely dry.
- After a very careful flushing with distilled water and leaving the apparatus filled at least one night.

Important:
If any protein has passed into the apparatus (recirculation!) do not use hot cleaning solution, **never warmer than 40°C.**

Recommended daily simple cleaning
Dispose first the heart and remove the remaining perfusate from the apparatus and the reservoir. Then the entire perfusate system is flushed through with distilled water:
- Use roller pump(s). In the WH mode link first the aorta cannula (2) to the atrium cannula (29) using a short piece of tubing.
- Ensure that all hollow spaces are completely filled and flushed through [air vessel (8) in the aorta block (1) and preload reservoir (26)]
- Do not forget any side branches and flush them through also [tubing connections to the syringes (9) and (17) as well as to the pressure transducers (18) and (30)].
- After flushing, fill the apparatus with distilled water and allow to stand overnight.

8.1.2 Thorough cleaning with cleaning solution

First the perfusate circuit is flushed through with distilled water as described above. Then fill the perfusate system with warm cleaning solution using the roller pump. Ensure that all hollow spaces [aorta block (2) and preload vessel (26)] are filled completely. IMPORTANT: use only the cleaning agents recommended (see below).

The apparatus filled with cleaning solution is allowed to stand for a few hours or better overnight. Remember however: the longer the solution remains in the apparatus the longer it takes until all solution residues have been flushed out again. For this reason the solution should not be kept in the apparatus for more than 24 hours.

If the apparatus is out of use over the weekend, it should after cleaning be filled with distilled water and kept in the dark, or at least covered over to protect it from bright light.

For longer intervals between experiments (longer than a few days) it is better to allow the apparatus to stand dry after cleaning; before the next experiment the cleaning solution should be allowed to act overnight.

Before the next experiment
In the morning, switch on the thermostat in order to warm up the distilled water or the cleaning solution. Then remove the distilled water or cleaning solution kept inside the apparatus and flush through thoroughly with fresh distilled water. Do not forget the side branches (tubing connections to the pressure transducers and to the syringes).

Then remove all the distilled water and immediately fill the apparatus with perfusate.

Perfusion system maintenance, summary
Daily
- Remove perfusate from the apparatus!
- Flush with distilled water!
  When used every day, leave distilled water in the apparatus overnight.

Weekly and before longer intervals between experiments
- Remove perfusate from the apparatus!
- Flush with distilled water!
- Fill the vessels with warm cleaning solution not warmer that 45°C.
- Allow to stand for a few hours or overnight (24 hours max.).
- In the morning warm up the apparatus to 37°C with the water circulation thermostat!
- Then remove the cleaning solution!
- Flush with distilled water, do not forget side branches!

8.2 Thermostatic circuit

The thermostatic circuit also requires regular maintenance. If you fill the thermostat with distilled water without any additives you should replace the water every week in order to prevent algal growth inside the system. With the addition of Thermoklar or Aquaclean (for suppliers see below) it is advisable to replace the contents at least every month.

Please Note:
Don´t use anti-algae solutions which contain alcohol.

Thermostat circuit maintenance, summary:

- Thermostat circuit without additive, replace water weekly!
- With additive, replace at least monthly!
- Use only distilled water (with or without additive)!
- Before filling, flush with cleaning solution and fresh water!

8.3 Water additives for the thermostatic circuit

In order to suppress algal growth inside the thermostatic circuit we recommend adding the following products to the distilled water:

Thermoklar #103108

Manufacturer: Biomed Labordiagnostic GmbH
Bruckmannring 32
D-85764 Oberschleissheim, Germany
Phone: (+49) (0)89/3151618
Fax: (+49) (0)89/3153242
Internet: http:\\www.biomed.de

Aquaclean No. 3170/1

Manufacturer: Glaswarenfabrik
Karl Hecht “Assistent”
Stettener Str. 22-24
D-97647 Sondheim/Rhoen 1, Germany
Phone: (+49) (0)9779/8080
Fax: (+49) (0)9779/80888

AQUA RESIST Art. No. 462-7000

Supplier: VWR International

Waroklar

Supplier:

Warning:

Please don´t use the Anti-Algae solution from SIGMA, this contains alcohol and damages the Plexiglass.

8.4 Recommended cleaning agents

IMPORTANT: use only the cleaning agents recommended!

RBS 50 or RBS 35

Manufacturer: Carl Roth GmbH & Co KG
Chemische Fabrik
Schoemperlenstr. 1-5
D-76185 Karlsruhe 21, Germany
Phone: (+49) (0)721/5606-0
Fax: (+49) (0)721/5606-49
E-mail: Carl@t-online.de
Internet: http://www.carl-roth.de

Supplier: see above (Carl Roth GmbH & Co KG)

MUCASOL

Manufacturer: Merz + Co GmbH & Co
Bereich Dr. Kramer
Eckenheimer Landstr. 100-104
D-60318 Frankfurt/Main 1, Germany
Phone: (+49) (0)69/15031.
Fax: (+49) (0)69/5962150.

Supplier: Rudolf Brand GmbH & Co
P.O.Box 1155
D-97861 Wertheim, Germany
Phone: (+49) (0)9342/808-0.
Fax: (+49) (0)9342/808-236

UK, IRL, Supplier: Evelyn Fitzgerald
2 Mount Cottages
The Mount
Barley Hertfordshire SG8 8JH
Phone: +44 1763 849517
Fax: +44 1763 849723
E-mail: fitzgerald@brand.de

USA, Supplier: Brand Tech. Scientific
25 Middlesex Turnpike
Essex, CT 06426-1479
Phone: 860-767 2562

In order to avoid damage to the apparatus you must therefore use only the cleaning agents recommended by the manufacturer.

If for certain reasons you require some other cleaning product you have to test it first to check that it is compatible. In case of doubt contact the manufacturer of the apparatus.

9. Mechanical details

9.1 Aorta block

Figure 25 shows the aorta block as an exploded view, with flow probe holder (C) and flowprobe for ultrasonic flow measurement. The screws and seals (O-rings) required are also indicated.
If you have to dismantle the aorta block, for example for cleaning, make sure that you use all seals and screws as indicated for reassembling.

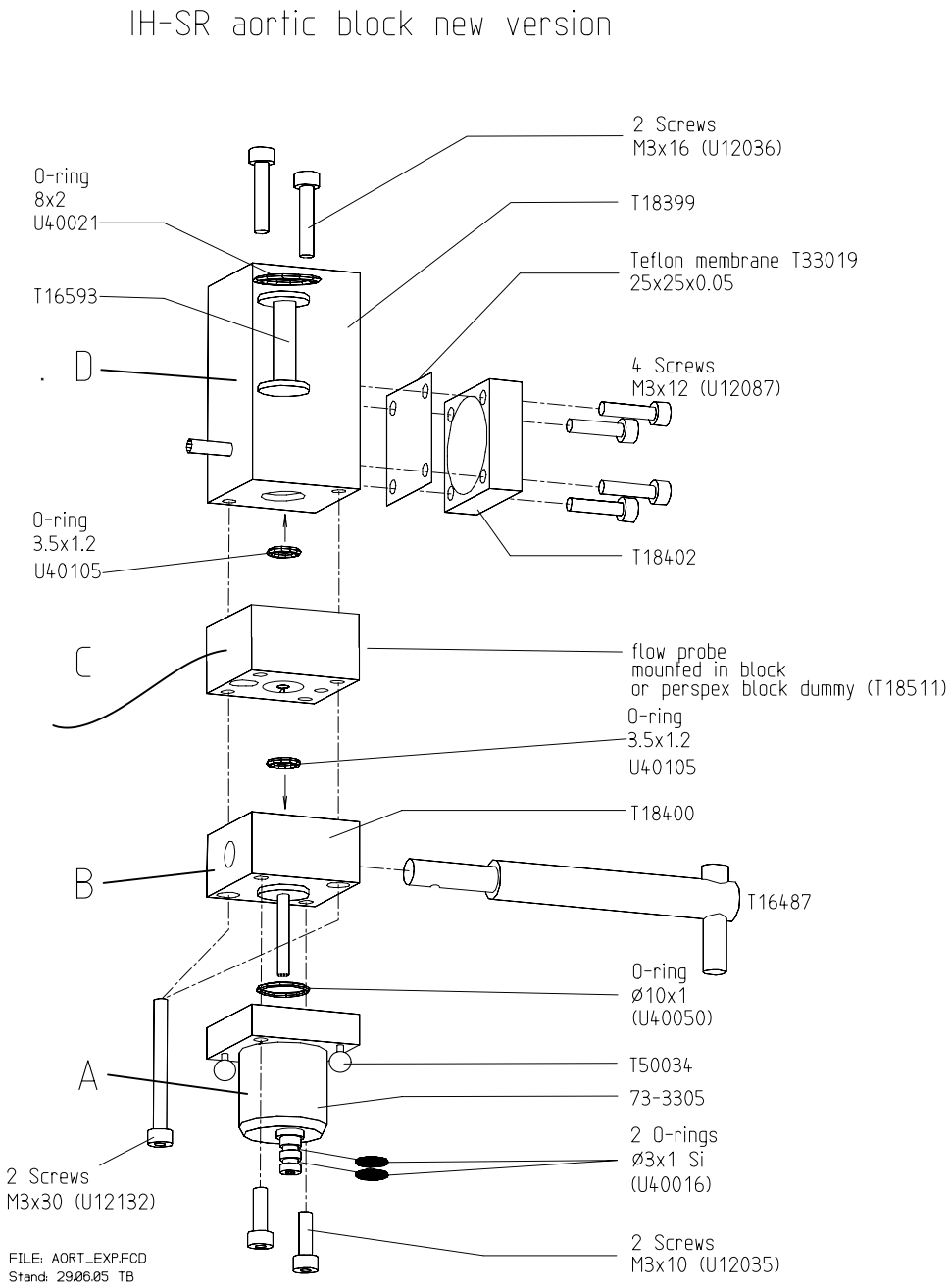


Fig. 25: Aorta block

A Air vessel.
B Intermediate piece with shut-off stopcock.
C Holder for ultrasonic flow probe.
D Upper part with flange-mounted adjustable flow resistance.

For further notes on parts A to D see page 58.

Notes on parts A to D (Figure 25):

A Air vessel
downwards: connection for the aorta cannula with grooves for two O-rings (3 dia. x 1 Si).
top, centrally (not visible): recess for O-ring (10 dia.x 1)
towards the back (not visible):
bottom: connection tube for pressure measurement und drug addition (bolus)
top: connection tube for adjusting the air vessel air volume.
The two screws (M3x30) serve for mounting the parts A, B and C on the top part D (see dotted lines).
3 X 5 mm balls are mounted on the air vessel.

B Intermediate piece with shut-off stopcock
top: central recess (not visible) for O-ring (2.5 dia. x 12).

C Holder for ultrasonic flow probe
The flow probe is mounted in a perspex block. If the direction indicated on the flowmeter is not correct, you can turn round the holder together with the transducer or use the switch “POLARITY“ on the flowmeter. The flowprobe should never by removed; correct assembly is impossible without special tools.

D Upper part with flange-mounted adjustable flow resistance.
The completely assembled aorta block is secured to the top Plexiglass plate of the apparatus using the screws (M3x16) shown at the top.
bottom: there is a central recess suitable for an O-ring (2.5 dia. x 1.2)
The adjustable flow resistance is fitted on the right side face. The Plexiglass flange is secured with 4 screws (M3x12) with the PTFE membrane (25 x 25 x 0.25mm) underneath the flange.
If you have to dismantle the flow resistance, e.g. for cleaning, you should be sure that the sealing edges on the flange are not damaged.
A tube nipple is visible on the left side; it is used to pump in perfusate in the LH mode (see 1 and 2). At the back at an angle (not visible) is the discharge tube of the adjustable flow resistance.

9.2 Atrium block

Figure 26 shows an exploded view of the atrium block with flow probe holder (B) and a flowprobe for ultrasound measurement. The necessary screws and seals (O-rings) are also indicated.

If you have to dismantle the atrium block, e.g. for cleaning, you should be sure that all seals and screws as indicated are used during re-assembly.

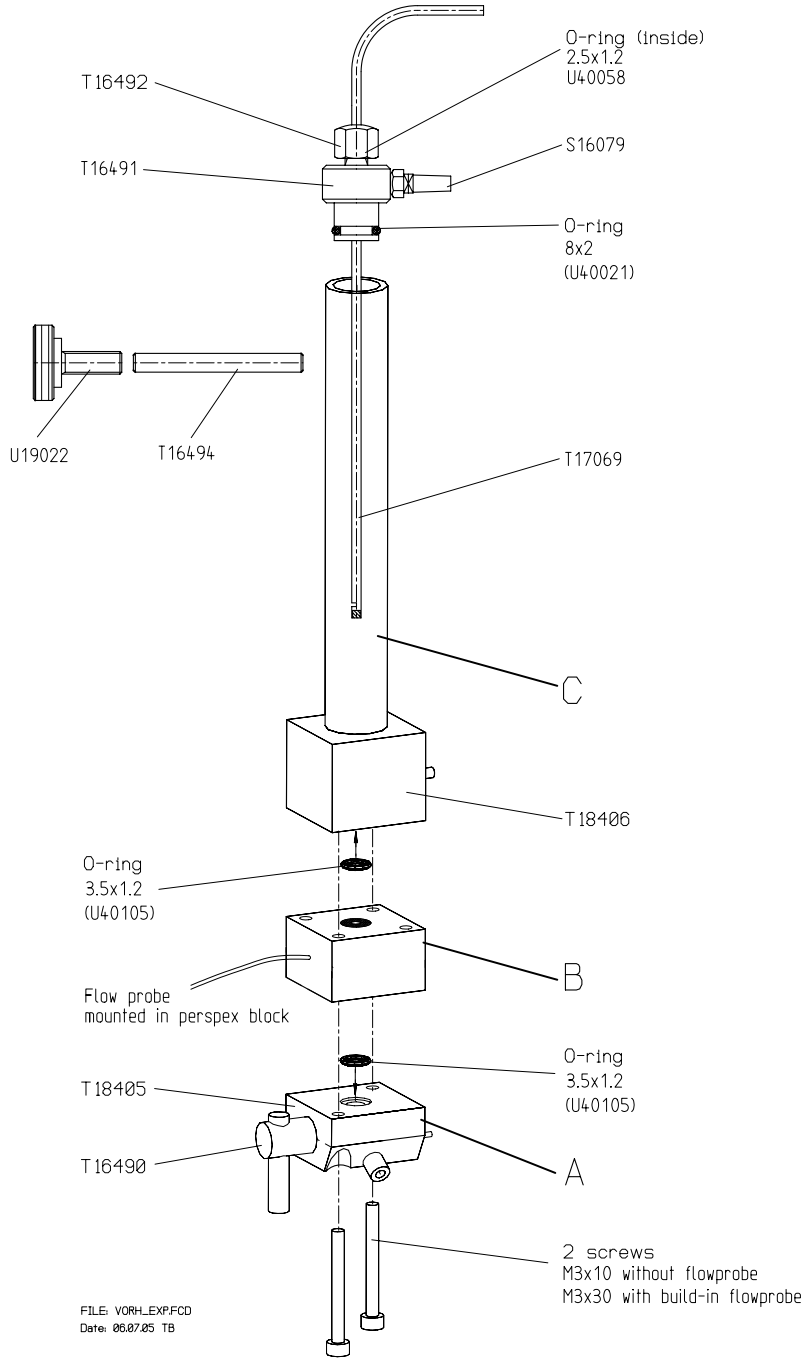


Fig. 26: Atrium block.

A Atrium connection part with changeover stopcock.
B Holder for ultrasonic flow probe.
C Preload vessel.

For futher information on parts A to C see page 59.

Notes on parts A - C (Figure 26):

A Atrium connection part with changeover stopcock.
Note: lever positions and function of the changeover stopcock are described in Section XX.
to the right front: nipple suitable for adapter piece for atrium cannula.
downwards: atrium relief bore (for explanation see Section XX).
top centre: recess for O-ring (2.5 dia. x 1.2).
to the right (at the back): connection tube for pressure transducer to measure the atrial pressure.
The two screws drawn at the bottom (M3x10) are used for securing parts A and B on the preload vessel C (see dotted lines).

B Holder for flow probe
for explanation see previous Section under C.

C Preload vessel
bottom centre (not visible): recess for O-ring (2.5 dia. x 1.2).
right at the back: connection tube for pumping in the perfusate (see 2).

10. Photographic illustrations

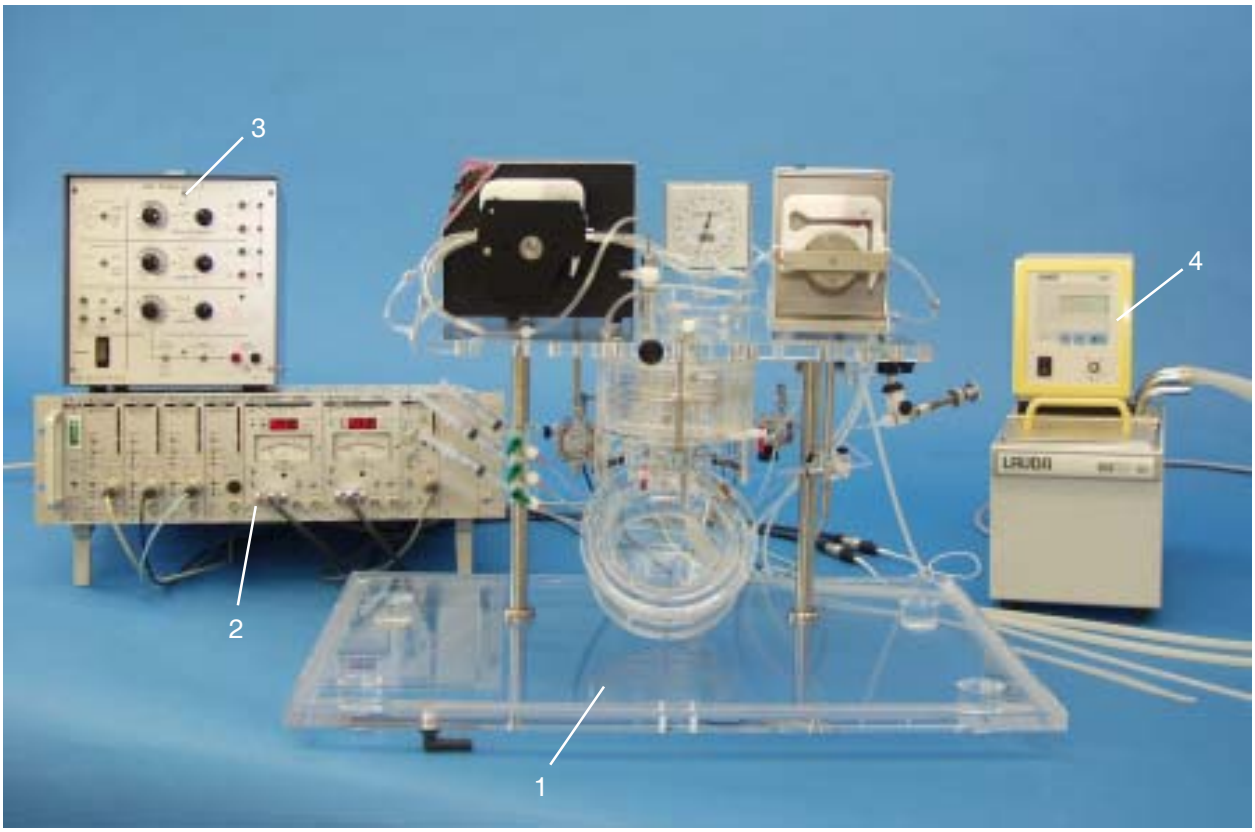


Fig. 27: IH-SR, Working Heart version, example of use.

1 IH-SR. The two roller pumps are positioned on the upper Plexiglass plate. Only one pump is required in the LH mode. The pressure gauge to indicate the set afterload pressure (in the WH mode) is mounted between the pumps. In the LH mode this pressure gauge shows the set perfusion pressure. The left vertical metal column carries two holders to take the syringe for adjusting the air vessel volume in the aorta block and for bolus addition of a test substance. The heart chamber is opened.

2 PLUGSYS Housing. ThePLUGSYS Housing is equipped with different amplifier modules according to the experiments to be performed.

3 Stimulator for stimulating the isolated heart.

4 The Thermocirculator pumps water through the thermostating circuit.

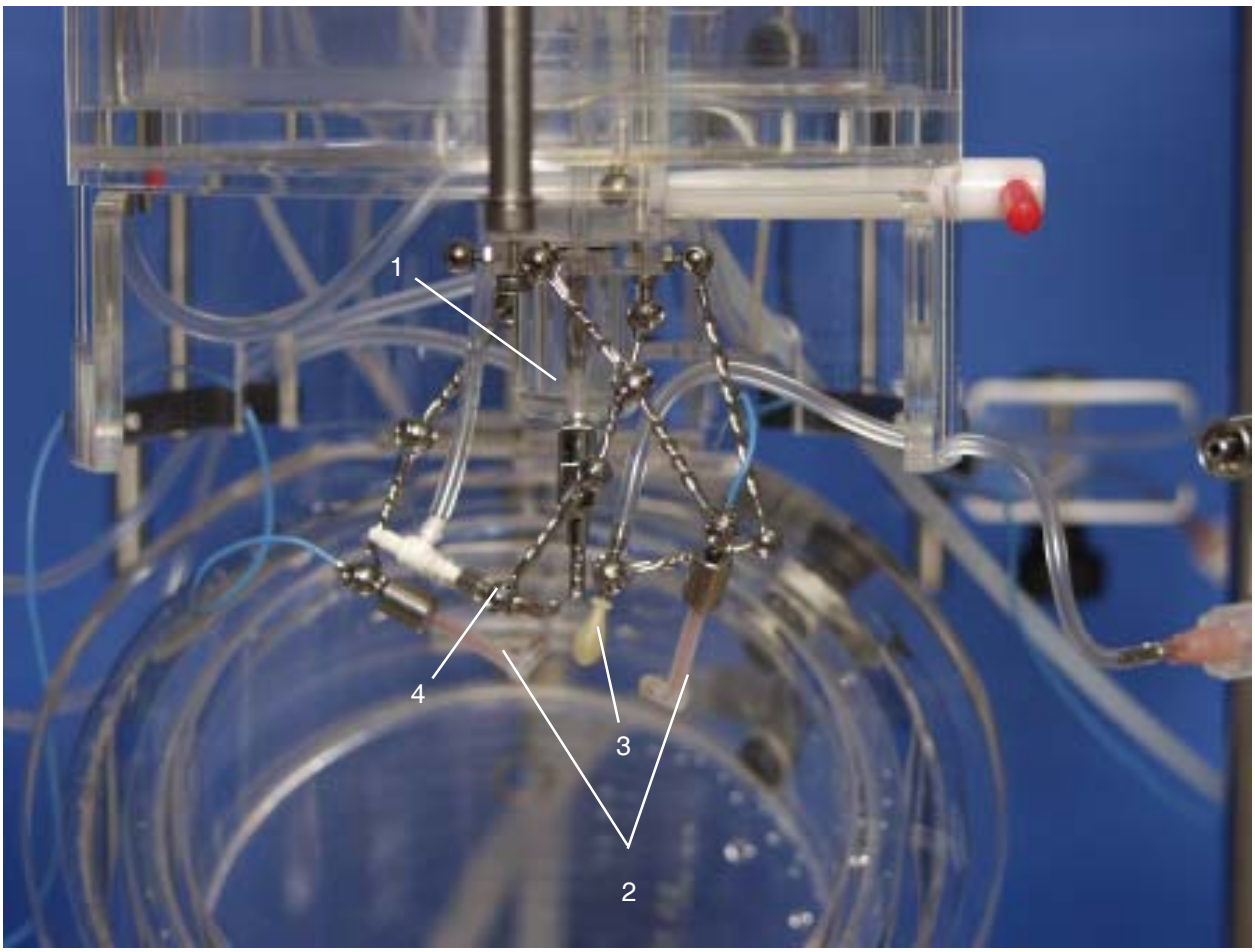


Fig. 28: IH-SR. LH mode for rat heart. The system is equipped with a mounted cannula system for pulmonary artery cannulation and a rat balloon kit for LVP measurement. Heart chamber opened.

1 Aorta block with aortic cannula.
2 ECG electrodes. The ECG electrodes are secured in the required position on the myocard using miniball joint holders. Due to the flexible end these electrodes follow the beating heart. One electrode is positioned at the apex and the second near the right atrium.
3 Balloon for rat hearts (LVP measurement).
4 Cannulating system for pulmonary artery. Using a mini ball joint holder a precise fixation without stress load from the cannula on the heart and vessel is possible.It is used for collecting the effluate of the heart for measuring gas concentrations or metabolsm analysis. It also allows to measure coronary flow on a working heart by connecting an appropriate flow sensor. The system can be used for rats and guinea pigs but not for mice.

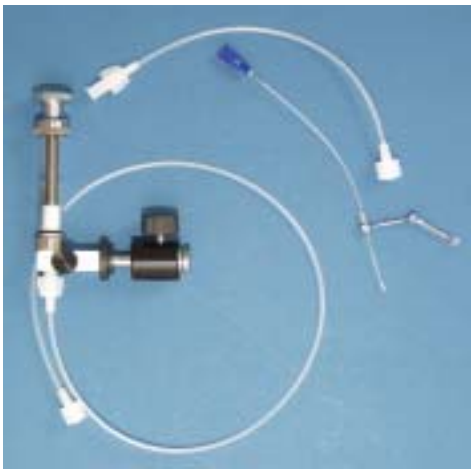
These measurements can also be performed on mice hearts exept PA cannulation (4).

Fig. 29: IH-SR. WH mode (mouse), detail view. Heart chamber opened.

1 Atrium cannula with atrium connector for mice.
2 Atrium block with preload vessel.
3 Aorta block.
4 Aortic cannula.
5 J-holder for LVP measurent. The J-holder is designed to maintain a small PE catheter forced through the left ventricle apex for LVP measurement in the isolated working heart. In combination with the mini ball joint holders a precise fixation without stress load from the PE catheter on the heart is possible.

The experiments can also be performed on rat or guinea pig hearts.

11. Acessory kits



73-0143 **IH-SR Mini Balloon Kit for Mice Hearts**
includes 10 PE catheter, for connecting the balloon to the pressure transducer, Luer stub cannula, holder with ball joints for fixation of the balloon in the left ventricle, spindle syringe for sensitive filling of the balloon.

Without picture

73-2787 Mouse Ventricular Balloon Assembly Kit
Balloons must be handmade from ceran wrap or cling film

73-2813 **IH-SR Balloon Kit for Rat / Guinea Pig Hearts**
includes ball joint holder, steel catheter, 1 balloons No. 5 (0.1ml), spindle syringe for sensitive filling of the balloons to adjust preload (balloon pressure), blunt Luer cannula, holder with ball joints for spindle syrindge

12. Reply form

Please take a few minutes of your time in order to write to us on any difficulties in understanding the Manual or in the use of the apparatus. With your feedback you will help to improve our products and the system documentation and make them more user-friendly.

Please tell us

- where you have found mistakes,
- where the arrangement was not clear and what you did not understand,
- and where you would like to see improvements.

Your critical notes will always be welcome.

Many thanks for your kind assistance.
Yours HUGO SACHS ELEKTRONIK-
HARVARD APPARATUS GmbH.

Your name _____

Organisation _____

Street _____

Town _____

Phone/Fax _____

_____

_____

_____

_____

_____

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_____

Please send this sheet or a copy to:
HUGO SACHS ELEKTRONIK - HARVARD APPARATUS GmbH,
Gruenstr. 1, D-79232 March-Hugstetten, Germany
Fax. (int. + 49) (0) 7665-9200-90

13. Literature references

Useful information on experimental setup, laboratory equipment, measurement methods, organ preparation and functional tests on the isolated heart, together with extensive literature references, can be found in the following booklets which have been published by Biomesstechnik-Verlag March GmbH, D-79232 March, Germany:

DOERING, H.J., DEHNERT, H.:
Das isolierte perfundierte Herz nach LANGENDORFF (The isolated perfused heart after Langendorff) 1985 (ISBN 3-924638-04-7), Biomesstechnik Series No. 5, published 1985 by Biomesstechnik-Verlag March, D-79232 March, Germany.
DOERING, H.J.:
The isolated perfused heart after Langendorff, updated shortened version (in German and English) 1990, by the same publisher (ISBN 3-924638-11-X)

Literature reference, experiments on the murine heart:

CRAIG J. HARTLEY, LLOYD H. MICHAEL AND MARK L. ENTMAN:
Noninvasive measurement of ascending aortic blood velocity in mice. American Physiological Society (1995) H499-H505

INGRID L. GRUPP, ARUN SUBRAMANIAM, THIMOTHY E. HEWETT, JEFFREY ROBBINS, GUNTER GRUPP:
Comparison of normal, hypodynamic and hyperdynamic mouse hearts using isolated work-performing heart preparations. American Physiological Society (1993) H1401-H1410

Willie A. Ng., Ingrid L. Grupp, Arun Subramaniam, Jeffrey Robbins:
Cardiac Myosin Heavy Chain mRNA Expression and Myocardial Function in the Mouse Heart. Circulation Research (1991) 68:1742-1750

Further literature references:

BAYLISS, W.M.:
On the local reaction of the arterial wall to changes of internal pressure. J.Physiol. 28, 220 (1902)

GREGG, D.E.:
Effects of coronary perfusion pressure or coronary flow on oxygen usage of myocardium. Circulation Res. 13, 497 (1963)

GREGG, D.E., E.M.KHOURI and C.R. RAYFORD:
Systemic and coronary energetics in the resting unanaesthesized dog. Circulation Res. 16, 102 (1965)

NEELY, J.R. and H.E. MORGAN:
Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Ann.Rev.Physiol. 36, 413 (1974)

NEELY, J.R., H.LIEBERMEISTER, E.J. BATTERSBY and H.E. MORGAN:
Effect of pressure development on oxygen consumption by isolated rat heart. Am.J.Physiol. 212, 804 (1967)

14. Index

15. Chemical Behavior of PLEXIGLAS®

The data given refer to a test temperature of 23° C and presuppose stressfree installation. The behavior of the material in practice depends largely on the temperature in use. In case of doubt, we advise you to consult us as to the chemical resistance for particular applications.
The results obtained for all products, especially the branded ones, refer to production batch tested in each case.

The symbols signity:

+ resistant
- not resistant
o limited resistance

Antistatics

+	HB 155
+	Antistatic fluid and cleaning agent

Technical baths

+	Electroplating baths
+	Photochemical baths

Chemicals, solvents, etc.

a) General

-	Acetic acid, concentrated
o	Acetic acid, up to 25 %
-	Acetone
+	Alum
+	Aluminium chloride
+	Aluminium oxalate
+	Aluminium sulphate
-	Ammonia water
+	Ammonium sulphate
-	Amyl acetate
-	Aniline
+	Arsenic
+	Arsenic acid
+	Battery acid
-	Benzaldehyde
+	Benzine, pure
-	Bromine
-	1-Butanol
-	Butyl lactate
+	Butyric acid, up to 5 %
+	Calcium chloride
+	Calcium hypochlorite
-	Carbon disulfide
-	Carbon tetrachloride
-	Chlorinated hydrocarbons
-	Chlorine, liquid
o	Chlorine water
-	Chloroethyl ether
-	Chlorophenol
o	Chromic acid
+	Citric acid, up to 20 %
+	Copper sulphate
-	Cresol
+	Cyclohexane
-	Diacetone alcohol
o	Diamyl phthalate
-	Dibutyl phthalate
+	Diethylene glycol
-	Dioxane
-	Ether
-	Ethyl acetate
-	Ethanol, concentrated
o	Ethanol, up to 30 %
-	Ethyl bromide
-	Ethyl butyrate
-	Ethylene bromide
+	Ferric chloride
+	Ferrous chloride
+	Ferrous sulphate
+	Formic acid, up to 2 %
o	Formic acid, up to 40 %
+	Glycerol
+	Glycol
+	Heptane
+	Hexane
+	Hydrochloric acid
+	Hydrofluoric acid, up to 20 %
+	Hydrogen peroxide, up to 30 %
+	Iodine, metallic
+	Lactic acid, up to 20 %
+	Magnesium chloride
+	Magnesium sulphate
+	Manganese sulphate
+	Mercury
-	Methanol, concentrated
o	Methanol, up to 30 %
-	Methyl ethyl ketone
-	Methylated spirits
+	Milk of lime
+	Monobromonaphthalene
+	Nickel sulphate
+	Nitric acid, up to 40 %
+	Nitric acid, over 40 %
+	Oxalic acid
-	Perchloroethylene
+	Petroleum
+	Petroleum ether
-	Phenols
+	Phosphoric acid, up to 50 %
-	Phosphorus trichloride
-	Phosphorus, white
+	Picric acid, 1 % in water
+	Potassium bichromate
+	Potassium carbonate
+	Potassium chloride
+	Potassium cyanide
+	Potassium hydroxide solution
+	Potassium nitrate
+	Potassium permanganate
o	2-Propanol
+	Propylene
-	Pyridine
-	Silicon tetrachloride
+	Silver nitrate
+	Sodium bisulfite
+	Sodium carbonate
+	Sodium chlorate
+	Sodium chloride
+	Sodium hydroxide solution, 30 %
+	Sodium hypochlorite
+	Sodium sulphate
+	Sodium sulphide
+	Stannous chloride
+	Stearic acid
+	Sulphur
-	Sulphur dioxide, liquid
+	Sulfuric acid, up to 30 %
o	Sulphurous acid, conc.
+	Sulphurous acid, up to 5 %
+	Sulfuryl chloride
+	Tartaric acid, up to 50 %
-	Thionyl chloride
-	Toluene
+	Triethylamine
-	Trichloroacetic acid
+	Turpentine
+	Turpentine substitute
+	Urea, up to 20 %
-	Xylene
+	Zinc sulphate, aqueous
+	Zinc sulphate, solid

b) Branded products:

+	® CLOPHEN T 55,A60
o	® DEKALIN
o	® FRIGEN A 12(CF_2 CL_2)
-	® GLYBAL A
+	® PALATINOL K
o	® PALATINOL O, BB new
+	® SANGAJOL
+	® TERAPIN
-	® TETRALIN

Disinfectants

a) General

- Carbolic acid
+ Chlor. lime paste
- Hydrogen peroxide, up to 40 %
o Hydrogen peroxide, over 40 %
- Iodine tincture, 5 %
+ Lugol solution
- Methylated spirits
+ Sublimate

b) Branded products

o ® ÄTHROL, up to 5 %
+ ® BAKTOLAN, up to 5 %
- ® BAKTOLAN, conc.
+ ® CHINOSOL, up to 1 %
- ® CHLORAMIN, suspension
+ ® CHLORAMIN; solution
+ ® ELMOCID GAMMA, up to 2 %
- ® LYSOFORM
+ ® MEFAROL, up to 1 %
+ ® MERCKOJOD, up to 1 %
+ ® MERFEN
+ ® PERHYDROL
+ ® PERODIN
+ ® SAGROTAN, up to 2 %
o ® SAGROTAN, up to 5 %
o ® VALVANOL, up to 2 %
+ ® ZEPHIROL; up to 5 %

Fats, oils, waxes

+ Animal
+ Mineral
o Silicone oil
+ Vegetable

Gases and vapours

+ Ammonia
o Bromine vapours, dry
+ Carbon dioxide
+ Carbon monoxide
+ City gas
o Chlorine vapours, dry
+ Exhaust gases containing HCl
+ Exhaust gases containing HF
+ Exhaust gases containing H_2SO_4
+ Hydrogen sulphide
+ Methane
+ Nitrogen dioxide
+ Nitrogen monoxide
+ Oxygen
+ Ozone
+ Sulphur dioxide, dry

Beverages, etc.

+ Beer, Wine
+ Camomile extract
+ Chocolate
+ Fruit juice, milk, coffee
o Spirits, up to 30 %
+ Vinegar
+ Water, mineral water

Cosmetics, etc.

- Camphor
+ ® DIPLONA -hair oil
+ Face tonic
+ Glycerine
+ Hair setting lotion (PRIMAWELL)
- Nail varnishes
- Nail varnish removers
+ Ointments
+ Peat water
+ ® POLYCOLOR
+ Seawater
+ Soaps
o Sprays

Plastics

+ Foam plastics
- Foam plastics, plasticised
+ Polyamide
+ Polyethylene
+ PVC
- PVC, plasticised
+ Rubber
- Rubber, plasticised

Foods and spices

+ Aniseed, bay leaf, nutmeg
- Cloves
+ Common salt
+ Honey, pure
+ Ice cream
+ Meat, fish
+ Pepper, cinnamon, onions
+ Pickles

Cleaning agent

a) General

Acids, see under chemicals
- Alcohol, concentrated
o Alcohol, up to 30 %
Alkalis, see under chemicals
+ Ammonia solution
- Benzine, mixture, containing aromatics
+ Benzine, non-aromatic
+ Bleach
- Carbon tetrachloride
- Methylated spirits
- Perchloroethylene
+ Petroleum
+ Petroleum ether
+ Soap solution
+ Soda water
- Stain remover
- Trichloroethylene
+ Turpentine
+ Turpentine substitute

b) Branded products

+ ® AJAX
+ ®Antistastischer KUNSTSTOFF-REINIGER und Pfleger
+ ® BFK cleanser
+ ® BOLIMENT
+ ® BÖTTCHERIN
+ ® BURMAT
+ ® BURNUS
+ ® CILLIT-GRÜN
+ ® DOR
+ ® DOSYL
+ ® DOSYLAN
+ ® FAKO-Polish
+ ® FAKO-Polishing paste
+ ® FEWA
+ ® FRAPPIN
+ ® FÜLLBOX
+ ® LAWAPLEX
+ ® NULL-NULL
+ ® PERSIL
+ ® PLEXIKLAR
+ ® PRIL
+ ® REI
+ ® SEIFIX
- ® SIDOLIN
- ® SPECTROL
+ ® SPÜLI
+ ® WC-00

c) Cleaning agents for pipes and tanks

+ ® CALGONIT D, DA, S
+ ® NEOMOSCAN M, M powder
+ ® NIROKLAR GR liquid
+ ® NIROKLAR GR powder
+ ® P 3
o ® P 3 basic cleaner
+ ® P 3- dix

Pesticides

- Sprays (applied directly)
o Sprays (applied in the air)
o Pesticides in aqueous solutions
+ ® NEXION stable spray
+ ® RABOND stable spray

Protective coatings (strippable)

+ ® DIEGEL liquid film 23922
+ ® KOPPERSCHMIDT covering paste
o ® SPRAYLAT

Other substances

+ Urine
- Fuel for petrol engines
o Fuel for diesel engines