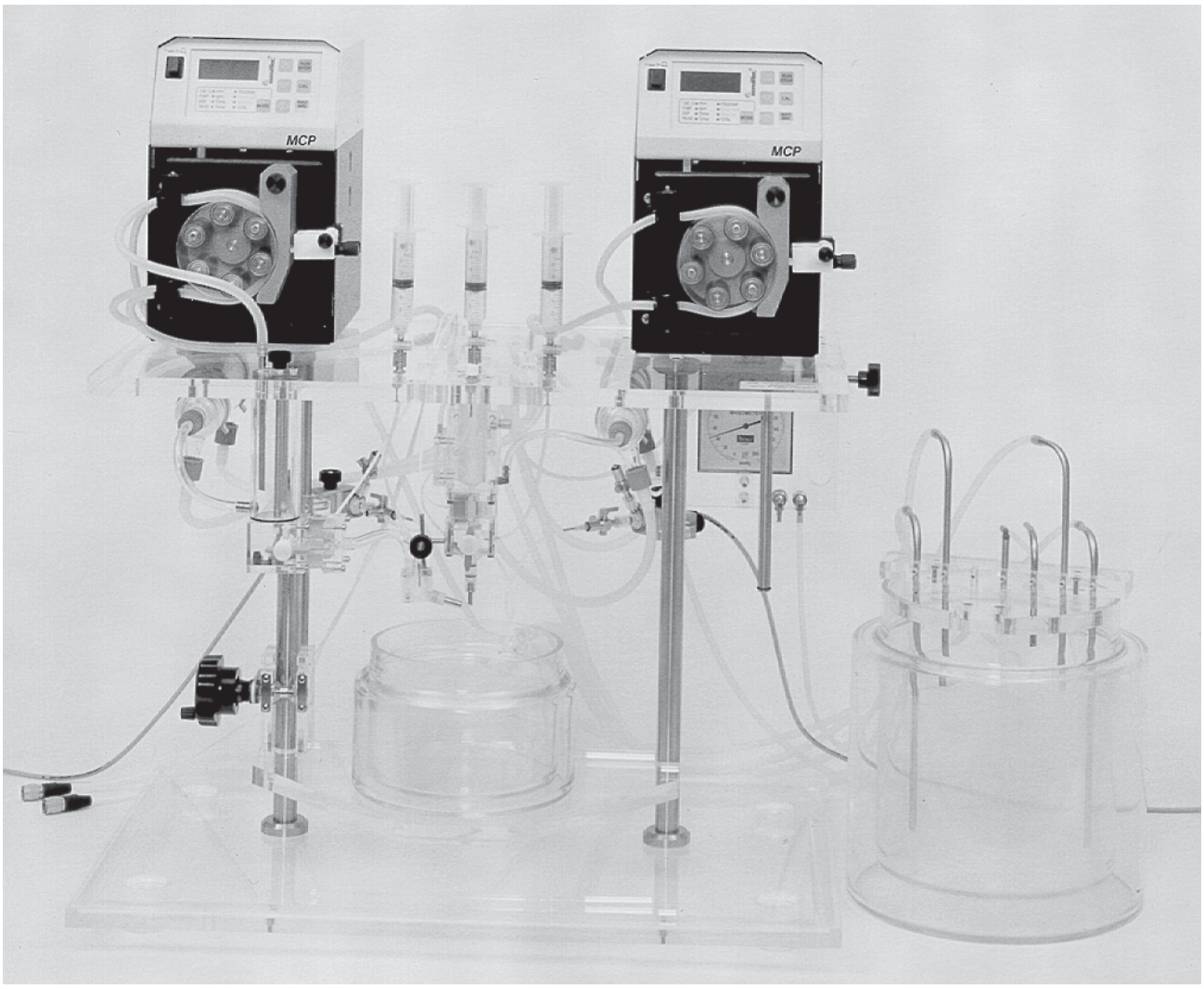
OPERATING MANUAL

Apparatus

ISOLATED HEART SIZE 5 Type 843

Operating modes: **WORKING HEART** and **LANGENDORFF HEART**



NOT FOR HUMAN USE

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Operating Manual

for the

Apparatus ISOLATED HEART SIZE 5, Type 843

for the heart of rabbits up to approx. 2.5 kg body weight

for the operating modes: **WORKING HEART** and **LANGENDORFF HEART**

Version 1.4 / printed : November 2012

Contents

1 Application of the Manual

This Manual applies to the Isolated Heart apparatus Size 5 (IH5) Type 843 for the heart of rabbits up to a body weight of approx. 2.5 kg. It can be operated both in the Langendorff mode (retrograde perfusion of the heart) and in the Working Heart mode. The size of heart which can be used is limited in the upward direction by the diameter of the tubes for inflow and outflow. This apparatus allows a maximum cardiac output of approx. 500 ml/min.

In the Working Heart mode the heart actually performs pressure-volume work; i.e. the left heart chamber umps liquid from the left atrium through the aorta cannula back into the apparatus. This is different from the usual Langendorff system where the heart is supplied with the perfusion solution through the coronaries; the heart does not perform any measurable volume work in the Langendorff mode.

2 Preliminary remarks

In this Manual a conscientious attempt has been made to explain all the items important for operating the apparatus and to describe and explain their use. The author has made every effort to tap the sources of information available to him and to incorporate the relevant details in this Manual. He is however fully aware that he has not dealt with all the points which are important when working with the apparatus. On the other hand, certain areas which are less important may have been overemphasised.

In order to improve both the apparatus itself and also this Manual we depend on appropriate information and suggestions from you. We therefore call for constructive comments from you the user of the apparatus.

- ❑ What is missing?
- ❑ What has been explained badly or incorrectly?
- ❑ What should be explained in greater detail?
- ❑ What illustrations or drawings should be included?

We are grateful for any suggestions and advice which you can offer.

3 Manufacturer and supplier of the apparatus

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4 Literature reference

Useful information on experimental setup, laboratory equipment, measuring methods, organ preparation and function testing of the isolated heart, as well as an extensive list of references can be found in the publication Biomechanics V: „The isolated perfused heart after Langendorff“ (ISBN 3-924638-04-7) by H.J.Döring and H.Dehnert, available from Biomesstechnik-Verlag March GmbH, 79232 March, Germany.

5 Safety, hazard notes and reservations

The text of this Manual refers to various hazards which may arise when using the product described. Special attention is drawn to the fact that this does not automatically exclude other dangers which are not mentioned here. It remains the responsibility of the user to assess his particular experimental setup, together with the auxiliary and test substances used, for any possible dangers. In case of doubt he is responsible for ensuring that advice is obtained from an appropriate expert.

☞ During setting up, installation and operation of the apparatus the appropriate safety regulations have to be observed. Below are listed a few notes which should be taken into account in order to ensure that hazards for the user do not arise or are averted

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

❑ Use only perfect, undamaged and dry mains power cables!

❑ 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!

❑ 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.

❑ When using toxic substances or gases, appropriate precautions must be taken in order to avoid any danger to the operator or contamination of the working area.

❑ Remember the fire hazard! Combustible substances in combination with Carbogen gas (= 95% oxygen) represent a fire hazard. Observe the appropriate guidelines and regulations!

I Operating Mode WORKING HEART

6 General description of the apparatus

The apparatus is designed mainly for use with hearts up to the size of rabbits with a weight of 2.5 kg. The following table summarises the limits of the apparatus:

Body weight:	up to 2.5 kg
Heart diameter:	30 - 40 mm
Heart length (apex-base):	40 - 50 mm
Aorta internal diameter:	5 mm
Pulmonary artery internal diameter:	5 mm
Aortic flow:	up to 500 ml/min

Description of operation:

Fig. 1 shows the principle of operation. Fig. 3 shows the same diagram but with the addition of item numbers to identify the various functional elements; these are described in the legend which follows.

With the exception of the solution reservoir and the thermostat, all parts illustrated are mounted or placed on a Plexiglass stand. Suitable stopcocks and movable connections are provided to permit and simplify the mounting of the organ, initially in the so-called Langendorff mode.

Perfusate is supplied to the apparatus in excess. Pumps provide the organ with more solution than it needs. The excess solution not required by the organ is pumped back into the reservoir. The perfusate is aerated by bubbling inside the main reservoir and inside the preload reservoir.

As shown in the schematic diagram, the perfusate ejected by the heart is returned to the large reservoir. By repositioning the return tubing, recirculation can also be arranged through the small preload reservoir or the apparatus can be operated without recirculation.

Adjustment of the perfusion pressure (in Langendorff mode, during the preparation phase) and of the aortic pressure (during the normal working phase) is provided by the artificial flow resistor operated by a rotary nob. The pressure gauge permits a rough indication of the set pressure.

The artificial flow resistor has a virtually linear characteristic and its resistance depends very little on the actual flow. The afterload pressure thus remains largely constant over a wide flow range.

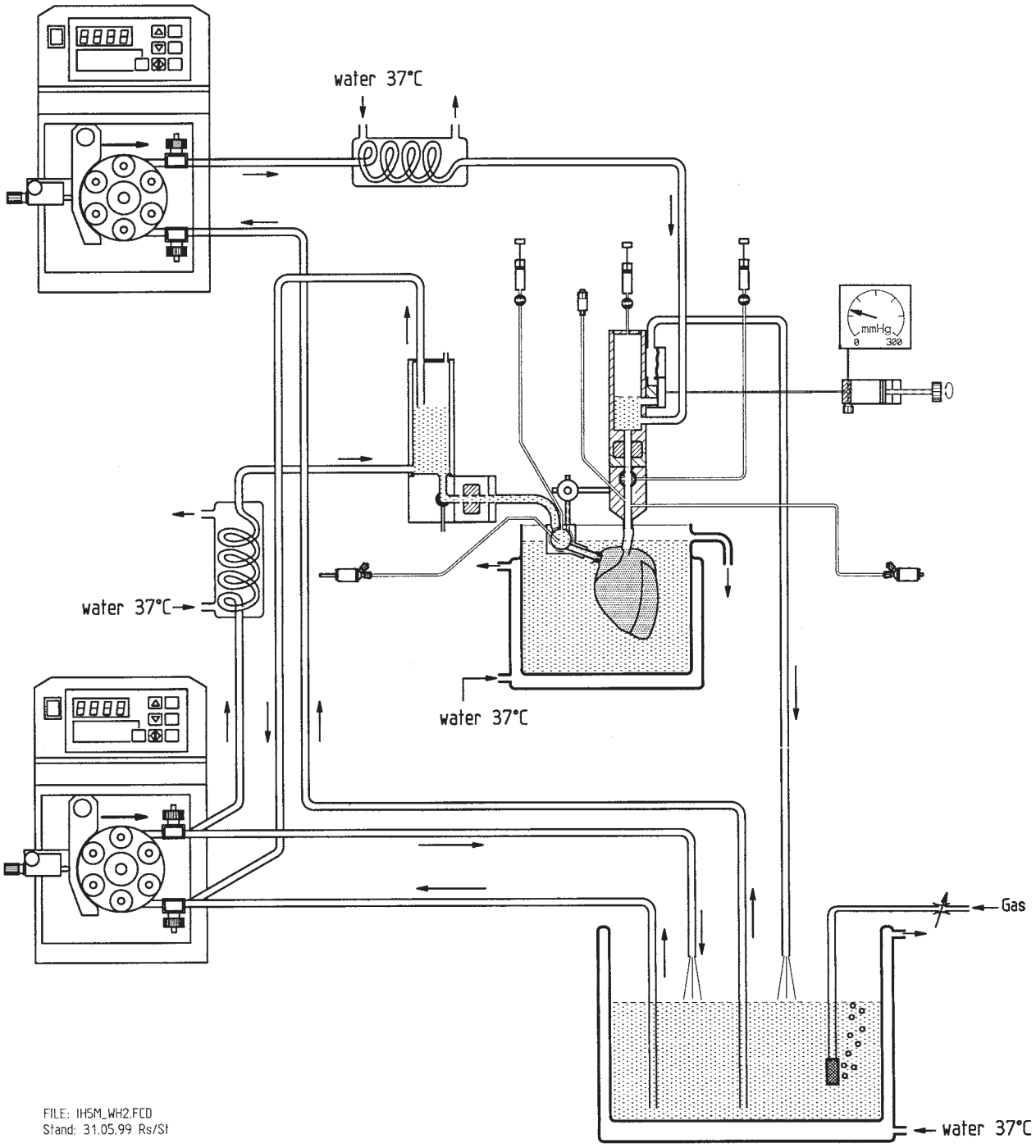

Fig. 1: Functional diagram of the apparatus Type 843 (Working Heart mode)

7 Starting up

The procedure suggested below has proved satisfactory in practical use. Depending on the type of experiment proposed it may be useful to proceed other than as described. The actual details of the experiment must therefore be left to the decision of the experimenter. 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 the possibility of working with a heart which right from the start is not fully functional. Carry out a check before the start of the experiment by using suitable functional tests (e.g. check Bayliss effect, note literature reference).

7.1 Preliminary notes

At the start of an experiment in the Working Heart mode (WH mode) the heart is initially being operated in the Langendorff mode and only switched over to the Working Heart mode when it has recovered from the stress of preparation. The flow through the coronaries is thus initially retrograde in the Langendorff mode and supplies the heart muscle without it having to perform any work. Pump (7) in conjunction with the afterload pressure regulator (2a) and (5) produces the necessary aortic pressure (e.g. 50 mm Hg).

Later, after the recovery phase and when the atrium cannula has been bound in correctly, the atrial flow is opened up at stopcock (13d) and the heart takes over the function of pump (7). The pump is then switched off. The afterload pressure (aortic pressure) can be adjusted at the pressure regulator (5) exactly as before. The pressure gauge (5a) indicates the pressure setting. The aortic flow is measured with the flow transducer (3a).

7.2 Preparation of the apparatus, provision of ancillary items

The following notes assume that the apparatus has been assembled complete with all necessary ancillary equipment and that all parts are connected together correctly as required.

☞ **NOTE:**
Notes with item numbers (NN) can be found in the legend to Fig. 3.

Preparation:

- ❑ When starting up for the first time and also after each experiment, all parts in contact with the perfusate have to be cleaned thoroughly. (Fill in hot cleaning solution and allow to stand e.g. overnight!). Observe the cleaning specifications!
- ❑ Remove the cleaning solution from the apparatus and thoroughly rinse with distilled water. Do not forget to rinse bubble trap and catheter tubing to remove all solution residues.
- ❑ Make up the perfusion solution, preferably fresh each day (filter it!). If possible warm it to the required temperature e.g. 37° C in a warming cabinet, and place it into the reservoir (8).
- ❑ Switch on the thermostat (37° C), allow the apparatus to warm up to the operating temperature.
- ❑ Switch on the aeration and adjust the bubbling through the perfusate reservoir at the appropriate needle valve (10).
- ❑ Switch on the measuring instruments and recorder and after a warm-up period (approx. 15 min) perform zero adjustment and calibration.
- ❑ Plug the preparation tubing with tubing adapter on to the connection for the aorta cannula (4d). Run the free end of the tubing back to the reservoir.

Filling the aorta block:

- ❑ Switch on pump (7) for Langendorff operation; ensure correct direction of rotation! Close the tubing pressure finger and adjust the correct pressure on the pump tubing. Note the information in the Operating Instructions of the pump. Set a low output, e.g. 10 ml/min.

- ❑ On the pressure regulator close the vent valve (5b); using the control of the pressure regulator (5) set the afterload pressure controller to about 50 mm Hg. The pressure is indicated on the pressure gauge (5a).

- ❑ If you are using a micro tip catheter pressure transducer (MTC) for later measurement of the LVP, release squeeze seal (4h) on the access port (4g) and insert the catheter. Advance it so far that it is located in the connection tube of the aorta cannula (4e) so that it is initially in a protected location. If you are not using a tip catheter, close the Luer taper (4g) with a Luer blind stopper.

- ❑ Using the syringe (2h) and its shut-off stopcock (2g), allow the air vessel (2e) to fill about half full with perfusate. Filling the air vessel can also be achieved by a different method:
  remove syringe (2h) and open the corresponding stopcock (2g). Then on pump (7) press the „MAX“ key and at the same time prevent flow through the preparation tubing by squeezing it shut. The liquid level in the air vessel now rises rapidly. When the required level is reached (half full), close the open stopcock (2f) again, release the squeeze on the preparation tubing and release the „MAX“ key. The previously set low flow (10 m/min, see above) now flows again through the preparation tubing back to the reservoir. The pressure in the air vessel is then approx. 0 mm Hg.

Filling the atrium supply:

- ❑ Close stopcock (13d)!

- ❑ Switch on pump (11) for the atrium supply; note direction of rotation! Close the tubing pressure finger and adjust the correct pressure on the pump tubing. Set the flow rate higher than the expected atrial flow, e.g. 20 l/min. The preload vessel (13) fills itself up to the level of the draw-off tube (13b). If the pump tubing diameters have been chosen correctly 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 (13d) and wash out all air bubbles. Then set the stopcock (13d) so that there is a small perfusate flow out of the atrium cannula and fill the bubble trap in the atrium head (15) using the syringe (15g) and the corresponding shut-off stopcock (15f). To fill the tubing to the pressure transducer (17) and the dome itself free from air bubbles you close off the atrium cannula with the finger and open the venting stopcock on the dome.

- ❑ Close the stopcock (13d) again!

- ❑ Adjust the aeration in the reservoir (8) with the corresponding needle valve (10).

7.3 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 (7). The test run in the Langendorff mode should be carried out first since this involves the use of pump (7) in the apparatus. If you then carry out the test run in the Working Heart mode you use pump (7) as heart replacement. The connection (2a) must of course be closed with a suitable piece of tubing so that a pressure can build up in the air vessel (2e) through the pumping action of the replacement heart.

7.3.1 Test run in the Langendorff 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 the coronary flow.
- ❑ Check that the return tubing (8c) is not kinked and that the return flow is not impeded.
- ❑ Switch on pump (7) and set a flow rate of approx. 50 ml/min.
- ❑ Set the pressure regulator (5) for the adjustable flow resistor (2b) to 50 mm Hg [vent valve (5b) must be closed; the pressure setting is read on the pressure gauge (5a)].
- ❑ The pressure in the air vessel (2e) should now rise to about 50 - 60 mm Hg. The rise in pressure is measured by the pressure transducer (6) and can be monitored on the pressure amplifier and the associated recorder.
- ❑ Check all connections for leakage. Rectify any leakage before continuing.
- ❑ Now change alternatively the pressure [by turning the pressure regulator (5)] and the output of pump (7) and note the corresponding changes in flow and pressure. When you increase the pump output you note an increase in pressure which you do not really expect. This pressure increase is caused by the flow resistance of the afterload pressure regulator (2b) and the return line (8c).
- ❑ Now check the effect of the air vessel (2e). Set the afterload pressure with the control of (5) to about 100 mm Hg, the pump (7) to a medium flow rate (e.g. 100 ml/min) and alter the filling level in the air vessel [use syringe (2h) and the corresponding stopcock (2g), remember the minimum filling level (=top edge of outlet bore!)]. On the recorder 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.

7.3.2 Test run in the working heart mode with 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. If you are using pump (7) for this test run you have to close the outlet (2a) with a short piece of tubing, for example.

- ❑ Connect the additional pump as heart replacement into the apparatus between atrium cannula (16) and aorta cannula (4e).
- ❑ Switch on pump (11) and set an output of approx. 200 ml/min.
- ❑ Open the stopcock (13d) and switch on the heart replacement pump. Initially set a pump output of about 100 ml/min. The pump output is measured with the flow transducer (14) or (3a) and can be read on the corresponding flowmeter.
- ❑ If you now set an afterload pressure of e.g. 50 mm Hg on the pressure regulator (5) [can be read on the pressure gauge (5a)], the aortic pressure should also increase due to the pump output.
- ❑ On the recorder you can see the various measurement signals:
  - Aortic pressure [pressure transducer (6)]. Because of the additional hydrostatic pressure of the liquid column (air vessel - pressure transducer) the recorded pressure is slightly higher (about 8 mm Hg) than the value read on the pressure gauge (5a). Pulsation is more or less pronounced depending on the air cushion set in air vessel (2e).

- Atrial pressure [pressure transducer (17)]. 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 transducer (3a)]. The associated flowmeter should indicate the flow set on the heart replacement pump (here: 100 ml/min). With pulsatile recording a strong pulsation can be seen, due to the action of the mechanical pump.

- Atrial flow [flow transducer in (14)]. The associated flowmeter should indicate the flow set on the heart replacement pump, as above (here: 100 ml/min). With pulsatile recording a strong pulsation can be seen, due to the action of the mechanical pump.

- Provided you have set the correct indication for zero flow and the calibration factors, the indications for atrial flow and aortic flow should differ very little (3 - 5%). 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.

❑ Now vary the output of the heart replacement pump and observe the behaviour of the different recorder traces. Aortic pressure increases slightly with an increase in flow, but not proportionately (see characteristic curves of the adjustable flow resistor [afterload] in Section 15).

During the variation check for the maximum possible atrial flow. If you exceed the flow rate set on pump (11) (in this example the setting was 200 ml/min!), the reservoir (13) will obviously run dry. If you later use a heart with a large ejection capacity you have to set the output of pump (11) 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.

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 in Section 7.2 (before the start of the test runs) before you proceed to the next stage.

7.4 Preparation and mounting of the organ

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 7.2. Summarising briefly, the apparatus should be in the following condition:

- the reservoir is filled with fresh perfusate and is at the correct temperature (pre-warm the perfusate)
- thermostat is switched on, all parts of the apparatus at the correct temperature
- pump (11) is switched on, the required output is set
- aeration of perfusate is adjusted
- atrium head and all tubing and transducer domes are free of air bubbles
- shut-off stopcock (13d) is closed
- pump (7) is switched on, the required initial output is set (10 ml/min)
- air vessel (2e) is half full of perfusate
- afterload pressure is set on (5) to 50 mm Hg as read on pressure gauge (5a)
- preparation tubing is placed on aorta connector (4d) and filled free from air bubbles; the small perfusate flow (10 ml/min) provided by pump (7) drips off the free end of the preparation tubing and can initially drip into a container at the operating table
- measuring instruments and recorder switched on, calibration and zero adjustment have been completed
- tubing to the two pressure transducers (6) and (17) and the two domes filled free from of air bubbles

Now start the organ preparation:

- ❑ 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 preparation tubing, clamp off the vena cava, open the pulmonary artery, bind the cannula into the aorta and immediately raise the output of pump (7) so that an aortic pressure of about 50 mm Hg is obtained. (Blood is washed out, heart muscle colour changes to light pink.)
  - ▸ 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 heart from the preparation tubing and mount it as rapidly as possible directly on the aorta connector (4d) in place of the preparation tubing. Ensure that no gas 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 minutes).
- ❑ The aortic flow sensed by the flow transducer (3a) corresponds to the coronary flow (in view of the flow direction the reading is negative!).
- ❑ Perform a test on the organ for perfect function (Bayliss effect, administer test substance, or similar).
- ❑ Place a suitable atrium cannula (16) on the atrium head (15) and fill it with fresh solution free from air bubbles [open stopcock (13d) at little so that perfusate drips off the cannula (16)].
- ❑ 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 chamber filling.
- ❑ Bind off open vessel stumps on the atrium. Now fully open stopcock (13d) and open up the atrial flow. Check the atrium for any open vessel stumps and bind these off.

The heart should now beat regularly and rhythmically and perform work. Perfusate is ejected at the aorta. The actual flow is measured by the flow transducer (3a) and can be read on the associated instrument. The polarity of the flow indication changes from negative to positive according to the flow direction in the aorta cannula. Now switch off pump (7) and monitor the aortic pressure. If the heart ejection is too small so that no positive aortic flow is produced, the pump (7) 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 with oxygen. Remedy: check aeration, perhaps you have forgotten to switch on the „bubbling“. Check gas composition! (has the correct cylinder been connected up?).

- 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!).

When the aortic flow is large enough (see above), you can switch off pump (7).
Note: do not release the tubing pressure finger on the pump; it must remain pressed down!

☞ Please note: the aortic pressure must not drop appreciably below 50 mm Hg to ensure the supply to the heart muscle.

❑ Adjust the control of the pressure regulator (5) 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 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 Micro Tip Catheter pressure transducer (MTC) for measuring the LVP you can now advance it into the left ventricle. The MTC is a sensitive and, as you must know, a very expensive item (price of SPR 407: DM 6000). Handle it with care and never use force when handling it. If it is damaged it is very rarely possible to have it repaired. You must then purchase a new one or abandon this excellent and precise method of measuring LVP (note Fig. 5 and the corresponding description).

  - To insert the MTC, release the pressure piece (4h2) of the squeeze seal (4h) 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.

  - Now advance it further until you can feel the movement of the aortic valve in your finger tips. Monitor the LVP trace on the appropriate recorder channel. Then quickly slide the catheter tip into the ventricle when the aortic valve is open during systole. The characteristic changes in the pressure curve show 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 preparation for the experiment are now completed. Adjust the filling level of the air vessel (2e) 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 in situ. The traces of the pressure and flow signals obtained on the apparatus can therefore never show an exactly „physiological“ form.

During the experiment you must monitor the bubble trap in the atrium head. Any gas bubbles washed in must be removed in good time before the trap is filled so far that gas bubbles can pass into the heart through the atrium cannula.

Now perform the experiment as planned: administer test substance as bolus or mixed into the perfusate, change perfusate, use a different aeration gas, alter temperature, or whatever.

7.5 Notes on measurement

7.5.1 Pressure measurement (see Fig. 2)

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

- 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 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!

- The pressure measuring system must be calibrated (see Operating Instructions for pressure transducer, electromanometer or bridge amplifier).

- 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.

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

- Perform zero adjustment correctly and in the correct order.

Performing zero adjustment:

- Allow instruments to warm up (at least 15 minutes).
- Move stopcock (6a) to position (6a2). IMPORTANT: stopcock (6a) must be mounted in such a position that the unused side connection is horizontal. The pressure in the dome is now atmospheric pressure.
- Now zero the pressure amplifier (bridge mplifier or electromanometer).
- For measurement, move stopcock (6a) back to position (6a1). Stopcock (6b) remains continuously closed during zeroing [position (6b1)]. It is opened only for filling the dome free from bubbles [position (6b2)]. Stopcock position (6a3) serves for filling the connecting tubing free from gas bubbles).

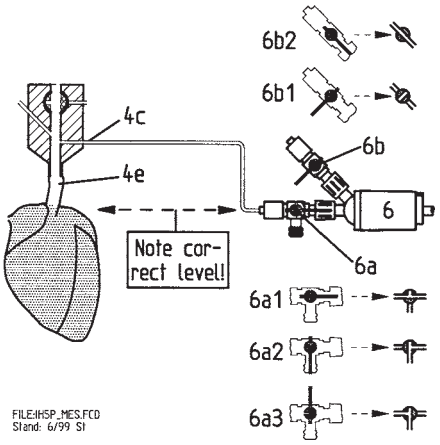

Fig. 2: Pressure measurement, stopcock positions

7.5.2 Flow measurement

Two different principles can be used for flow measurement: electromagnetic measurement and ultrasonics (Transit Time Flow Meter TTFM). In general, good results can be obtained only if certain points are observed carefully. Electromagnetic flow measurement is generally more troublesome than ultrasonic measurement. If there are any problems, the Operating Instructions for the flowmeter should be consulted first. The notes below provide some additional information concerning the use of any flowmeters built into this apparatus.

IMPORTANT: fill flow transducer free from gas bubbles! Irrespective of the principle of your measuring system it is important to ensure that the lumen of the flow transducer (measuring head) is filled free from gas bubbles. If the lumen is not filled properly, the flow indication is either completely absent or greatly disturbed. Bubbles can be removed by producing a sudden movement of the liquid inside the lumen, using a syringe (not too small!). With ultrasonic flowmeter heads it is particularly important to ensure freedom from bubbles as these interfere with the measurement. If there are any air bubbles in the ultrasonic flow probe the meter indicates „Ac.Er“ (acoustic error). These bubbles, too, can readily be removed by producing a sudden movement of the liquid using a syringe. Use the same syringe as is used for adjusting the air vessel. (cf. Fig. 14). Air bubbles should be removed immediately on filling the apparatus and before the start of the experiment.

IMPORTANT: check the calibration by volumetric measurement:

You require a calibrated measuring cylinder and a stopwatch

- Set the factory calibration factor manually, using the information supplied, as described in the Operating Instructions of the flowmeter.
- Check the zero (clamp off tubing or close stopcock!).
- Pass flow through the transducer at a rate which is of the same order as the factory calibration factor and should be as constant as possible.
- Collect the perfusate flowing through in the measuring cylinder for exactly one minute.
- Compare the volume obtained with the indicated measurement. If you find any deviation, correct the calibration on the instrument until the numerical indication agrees with the volume found.
  NOTE: in the case of the ultrasonic flowmeter (TTFM Type 700) the calibration of the measuring head is permanently programmed in the connector and can only be altered by the factory.
- Repeat the procedure, check the calibration found and note it in your records.

During the entire calibration procedure the set flow rate must of course remain unchanged and constant. The calibration factor found is valid only for the solution used for the calibration measurement. If you are using a different perfusate in your experiment you have to repeat the procedure described with this different perfusate.

7.5.3 Notes on electromagnetic flow measurement

The following information concerns measurement with the electromagnetic flowmeter EFM Type 693 and the appropriate flow transducers. The first source of information for working with this instrument should be the Operating Instructions for the EFM which are supplied with the instrument.

Basic details: do you remember?

Electromagnetic flow measurement is based on the measurement of minute electrical voltages (a few µV) which are induced through the movement of the liquid in the magnetic field of the transducer. The measurement voltage is picked off through very small-area electrodes in the lumen of the transducer. Flow measurement is in general a difficult procedure and requires detailed adherence to certain rules if good results are to be obtained.

For satisfactory flow measurement it is important that:

- ❑ electrical interference is kept away from the flow measuring head,
- ❑ surfaces of the pick-up electrodes inside the measuring head are properly maintained so that they are kept „clean“, i.e. that they have a low resistance.

The first is achieved by carefully earthing the liquid column before and after the measuring head by means of metal tubes. It is important that the cross-section of the connecting cables is sufficiently large (at least 1.5 mm2) so that the so-called stray currents are efficiently discharged and kept away from the flowmeter head. In addition the earth socket (blank socket for 4 mm banana plug on the front panel of the PLUGSYS housing) must be used as the earth point.

When servicing the pick-off electrodes inside the measuring head you should observe the following basic points:

- Allow the measuring head lumen to dry out only after it has been thoroughly rinsed with distilled water!
- When the liquid is stationary (flow = 0) switch off the excitation current of the measuring head at the flowmeter to avoid unnecessary heating of the measuring head which results in deposits on the electrode surfaces.

- 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.

- Clean dirty electrodes with a soap-free cleaning agent (a domestic detergent such as Ajax) using a suitable round brush or pipe cleaner. Take care that as little plastic as possible is „polished away“ during this operation. Then thoroughly rinse with distilled water.

☞ In principle: **if the zero is unstable or the signal is noisy, the cause is virtually always a dirty electrode surface.**

With trouble of this type the remedy is always the same: the measuring head must be cleaned, cleaned, and cleaned again ... (but do not polish away any plastic), or better still avoid any contamination in the first instance (see above)!

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

- Plugs of measuring head and magnet/signal 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.
- Measuring head and magnet/signal cables must not move! Moving cables produce electrical artifacts which are superimposed on the measurement signal and produce errors. Therefore ensure that the cables are not arranged together with the „pulsating“ pump tubing.
- Never arrange measuring head and/or magnet/signal cables close to a mains supply cable!
  The mains voltage and spikes superimposed on the mains voltage are some 100 000 times larger than the measuring voltage of the flowmeter head so that corresponding interference must be expected.

☞ **Calibration:**
In order to achieve correct flow measurement the calibration factor of the measuring head used has to be set on the flowmeter.

The calibration factor of each measuring head is normally evaluated by the manufacturer and marked on the packaging together with the serial number.

Example: K-5.0, Cal.fac.=275 ml/min, Serial No: 2834.

Serial number and lumen of the measuring head can be found on the connector (e.g. 2834 5.0) so that the transducer can always be clearly identified.

This value („0275 ml/min“ in this example) must be set on the associated flowmeter. The measured flow is then indicated directly in ml/min.

The calibration factor is measured by the manufacturer on the basis of a 0.9% saline solution. If you use a different solution or whole blood you have to check the calibration by the procedure described above and make an appropriate correction to the setting, since the sensitivity depends to a slight extent also on the liquid passing through.

7.5.4 Notes on ultrasonic flow measurement

The following information concerns measurement with the ultrasonic Transit Time Flow Meter TTFM Type 700 or the Transonic Flowmeters T106 and T206, together with appropriate flow transducers. The first source of information for working with this instrument should be the Operating Instructions for the TTFM which are supplied with the instrument.

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/sec or l/sec. Flow measurement by this principle is very simple and free from interference.

The only requirement for obtaining good results is to

❑ fill the flow probe free from bubbles.

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

In general, the following points have to be observed (as with electromagnetic flow probes):

- 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.

- 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 made in the introduction lead to the following additional requirements:

- Plugs of measuring head 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.

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

Unlike in electromagnetic flow measurement, connecting the flow probe to the flowmeter results in automatic identification of the transducer type and calibration values.

The serial number of the measuring head 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.

7.6 End of the experiment, cleaning the apparatus

After the experiment has been completed, drain any perfusate residue from the apparatus and rinse it thoroughly with distilled water. Use a short piece of tubing to link the atrium cannula (16) with the aorta cannula (4e), then fill the complete system with warm cleaning solution and allow the filled apparatus to stand overnight. Use only the permitted cleaning solutions!

☞ WARNING:
do not keep the apparatus filled with cleaning solution for more than 24 hours. If the solution is kept in the apparatus for longer periods there is a danger that cleaning solution diffuses into tubing and Plexiglass surfaces and can not be removed immediately on rinsing. The cleaning solution should be removed at the latest after 24 hours and the apparatus should then be rinsed thoroughly with distilled water.

7.7 Shutting down the apparatus

If you want to shut the apparatus down **for a few days** you should not allow it to dry out after cleaning but should let it stand filled it with distilled water. In order to avoid or at least reduce the growth of algae the apparatus should if possible not be placed in bright light and should be protected against the action of light (cover over with a dark cloth).

If you plan for a **longer shut-down period** all liquids should be removed from the apparatus after cleaning and rinsing, and the apparatus should be allowed to dry out. Also drain the thermostat circuit and rinse it with distilled water. Ensure that no liquid residue remains inside the apparatus, if necessary by tilting it. Liquid residues form a good substrate to algal growth. When starting up again you may then have a great deal of (unnecessary) trouble to remove the undesirable algae from the apparatus.

7.8 Re-starting

When the apparatus has been kept dry and is being placed back in use after a longer period, it is best to proceed as described in Section 7.2. As a first step carry out a thorough cleaning procedure. Check that all connections are tight. Replace any tubing which does not look good or is porous or brittle.

8 Measuring coronary flow and oxygen saturation

For accurate measurement of the coronary flow with an additional flowmeter it is necessary to cannulate the pulmonary artery. The perfusate outflow is measured with a flowmeter head. To measure also oxygen saturation, part of the coronary flow (approx. 1 ml/min) is pumped by a low-flow tubing pump in a bypass through a thermostated measuring chamber and evaluated there with an oxygen pO_2 electrode (ZABS No.1). Operation of the pO_2 electrode requires a suitable instrument, e.g. HSE PLUGSYS module OPPM Type 697.

When cannulating it is important that the pulmo-arterial outflow (= coronary flow) is not restricted (cannula lumen must be sufficiently large, avoid kinking the tubing) and that no suction is generated in the pulmonary artery through an outflow tubing hanging down low.

When measuring oxygen saturation it is also important to ensure that cannula and tubing are made from a material impervious to oxygen (stainless steel, thick-walled Tygon tubing) in order to prevent any oxygen exchange between the perfusate and the surrounding air. This would produce correspondingly large errors in oxygen measurement.

☞ **Please note**:
Silicone tubing is quite unsuitable because of its extremely high oxygen permeability! (See also Section 12, Tubing material).

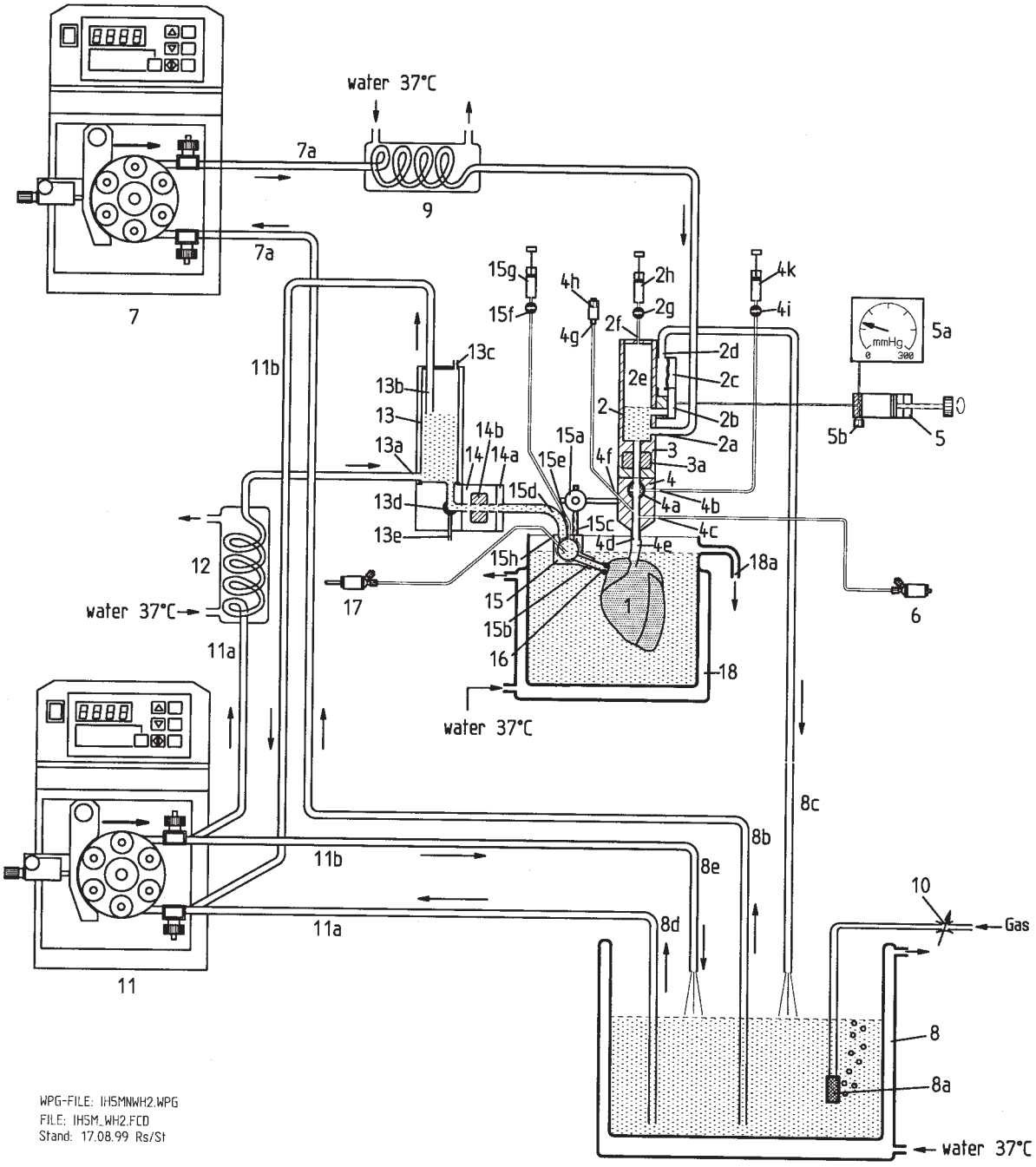

Fig. 3: Functional diagram of the apparatus with item numbers for the legends below

9 Technical description of the apparatus, legends for Fig. 3.

Item numbers (NN) in brackets refer to Fig. 4!

(1) **Mounted isolated heart**. Note that the heart is shown rotated out of the position in situ. The left atrium which normally points towards the right (viewed from the front) is on the left.

(2) **Aorta block**, central component, made from Plexiglass. It serves essentially as an artificial aorta. The block is supplemented by the flowmeter head adapter (3) and the stopcock block (4), with the necessary connections and devices, in order to meet the various requirements of the different possible experiments.

(2a) **Connection** for feeding the perfusate to the aorta block (in the Langendorff mode).

(2b) **Adjustable flow resistor**. In the WH mode is serves as an adjustable artificial circulation resistance. In the Langendorff Heart mode it is used to set the required perfusion pressure. It consists essentially of an elastic diaphragm with clamping cover. The pressure set on the pressure regulator (5) acts on the cover side of the diaphragm. If the pressure on the perfusate side of the diaphragm is larger than the set pressure, the diaphragm lifts and the perfusate can flow through underneath the diaphragm and passes via the oscillation damper (2c) and the tubing (2d) back to the reservoir.

Dismantling: The adjustable flow resistor is screwed to the left side of the aorta block (2). After releasing the four fixing screws it can be removed, for example for cleaning.
WARNING: do not damage the sealing edge in the cover! When re-assembling, ensure that the diaphragm is located accurately in the recess of the cover. Only tighten the fixing screws so far that the cover just rests on the aorta block without a gap; do not overtighten the screws!

☞ **Note**: the diaphragm thickness is essential for the correct functioning of the adjustable flow resistor. Cover and diaphragm are matched to each other. The correct diaphragm thickness in units of 0.01 millimetre is engraved on the cover (example: 50 = 0.5 mm). Fit only original diaphragms of the correct thickness!

(2c) **Oscillation damper**. The adjustable flow resistor (2b) is followed by an oscillation damper which serves to improve the dynamic characteristics of the system. The damper is mounted directly next to the flow resistor on the back of the aorta block (2) in order to achieve a favourable characteristic. The oscillation damper consists of an elastic diaphragm with clamping cover. With pulsing perfusate flow it reduces the resulting pulsation in the liquid system and dampens the reaction on the flow resistor (2b).

Operating note: Proper operation of the oscillation damper is only ensured if there is no appreciable positive or negative pressure on the diaphragm to prestress it. The tubing to the connection (2d) must therefore not be kinked and should terminate approximately at the liquid level inside the aorta block. The difference in height should not exceed 50 cm.

Dismantling: The oscillation damper is mounted on the back of the aorta block (2) directly next to the flow resistor. After releasing the four fixing screws it can be removed, for example for cleaning.
WARNING: do not damage the sealing edge in the cover! When re-assembling, ensure that the diaphragm is located accurately in the recess of the cover. Only tighten the fixing screws so far that the cover just rests on the aorta block without a gap; do not overtighten the screws!

☞ **Note**: cover and diaphragm are matched to each other. The correct diaphragm thickness in units of 0.01 millimetre is engraved on the cover (example: 50 = 0.5 mm). Fit only original diaphragms of the correct thickness!

(2d) Outlet connection. This tube connection (6 mm o.d.) forms the outflow of the oscillation damper (2c). In the Langendorff mode the excess volume supplied by pump (7) appears here. In the Working Heart mode the volume ejected by the heart is discharged from the aorta block through this connection and (during recirculating operation) back to the reservoir (8).

(2e) **Air cushion of the air vessel**. The upper volume of the aorta block (2) forms the air vessel. Its task is to reproduce as far as is possible the elastic properties of the aorta in situ. This desired replacement of the aorta can only be imperfect. For this reason the course of the aortic pressure trace measured on

the apparatus approximates to the curve measured in situ only if the air cushion has optimum adjustment, i.e. in accordance with the actual situation.

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 (2h). The air volume is limited by the position of the outlet bore to the adjustable flow resistor (2b). With maximum air volume (approx. 15 ml) the perfusate level in the aorta block is approximately 2.5 cm high.

(2f) **Air vessel connection**. A small tube with a female Luer taper is provided in the upper cover plate of the air vessel (2e). A syringe (2h) together with a shut-off stopcock (2g) is connected here. Syringe (2h) together with the shut-off stopcock serves to adjust the air cushion of the air vessel (2e).

(2g) **Shut-off stopcock**

(2h) **Syringe** e.g. 20 ml

(3) **Fitting adapter for flowmeter head**. A suitable flowmeter head (option) can be fitted into this adapter to measure the coronary flow (in the Langendorff mode) or the aortic flow (in the WH mode). If no flowmeter is ordered, a blank piece with a through bore is fitted at this point.

The adapter is mounted with 4 hexagon socket screws M3x12 each between aorta block (2) and stopcock block (4). After removing the 8 screws (use the screwdriver supplied!) the adapter can be dismantled. During re-assembly ensure that the O-ring seal (6x2 mm, Silicone) lies in the groove provided. This side must be towards the aorta block (2).

(3a) Flowmeter head (option), mounted permanently inside the fitting adapter (3).

(4) **Stopcock block with main stopcock** (4a). The main stopcock (4a) is used to close the outflow from the air vessel. In the Langendorff mode it can be used e.g. to perform a otal ischemia. Through the provision of a side access port (4b) the stopcock also permits feeding in a special solution, e.g. to perform cardioplegia experiments without mixing with the contents of the aorta block. The different stopcock plug positions and their relation to the plug handle are shown in Fig. 4.

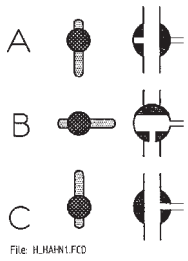

Fig. 4: Positions of the main stopcock

Dismantling: after releasing the 4 fixing screws the stopcock block can be removed from the intermediate adapter (3). During re-assembly it is important that the O-ring seal (6x2 mm, Silicone) is located in its groove. This side must be towards the adapter (3).

(4a) Main stopcock. For details of its function see under (4).

(4b) Side access port to main stopcock (4a).

(4c) Connection for measuring the aortic pressure by pressure transducer (6).

(4d) Connector for fitting the aorta cannula (4e). The connector carries 2 grooves to take two O-rings (7x1.2 m, Silicone). These O-rings perform two tasks; they provide a seal and they retain the aorta cannula with the heart attached to it.

(4e) Aorta cannula for binding into the aorta. Several cannulae of different dimensions are supplied in the accessories to suit the heart size.

(4f) Insertion port for a Micro Tip Catheter pressure transducer (MTC) size 2F to 3F. The small tube is extended by a length of thin guide tubing up to the apparatus top plate. Here there is a metal Luer connection (4g), female, to which the necessary insertion adapter (4h) is connected.

(4g) Connector to the insertion port (4f) (female Luer taper).

(4h) **Insertion adapter** (seal) **for micro tip catheter pressure transducer MTC**. This adapter is shown in Fig. 5. It serves to insert an MTC (size 3 French max.) from above into the aorta cannula and to seal it through a squeeze seal. The adapter is fixed with its male Luer taper to the connector (4g).

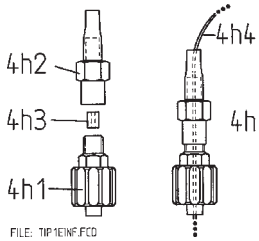

Fig. 5: Insertion adapter (4h) for MTC

The insertion adapter (4h) consists of three parts (see Fig. 5): the body (4h1) with Luer taper, the Silicone squeeze seal (4h3) (3 mm o.d., 1(1.6) mm i.d., 4 mm long), and the pressure piece (4h2) with ring nut and anti-kink extension. The Silicone seal is inserted into the body and compressed from above by the ring nut. This secures and seals the MTC passed through the adapter.

To introduce the MTC (4h4) remove the pressure piece (4h2) with ring nut, apply a little vaseline to the catheter tip and insert it a few centimetres (2-3 cm). Then carefully screw on the thread of the pressure piece and tighten it so far that the catheter is just secured in position. To move the catheter, release the pressure piece (about half turn anticlockwise) so far that the MTC can be moved easily.

☞ Warning: the micro tip catheter pressure transducers is a very expensive item which is extremely sensitive to excessive mechanical force; when damaged it usually can not be repaired.

Handle this delicate transducer with appropriate care:

- Observe the Operating Instructions!
- Never press or squeeze the transducer tip or allow it to impact a hard object (e.g. when pulling it out!)
- Do not kink the catheter or pull hard on it.
- If it is inserted in the apparatus but is not actually being used, it should be withdrawn into the guide tube (4f) in the stopcock block.
- During insertion and removal, release the pressure piece (4h2) sufficiently, move it gently, do not use force.
- When the tip catheter is not in use, e.g. when working only in the Langendorff mode, store it in its original package.

(4i) Shut-off stopcock.

(4k) Syringe, e.g. for bolus drug administration.

(5) **Pressure regulator** for generating the setting of the pressure in the adjustable flow resistor (2b). Rotation of the knob produces a corresponding movement of the plunger and the pressure is transmitted through the tubing connection to the diaphragm of the adjustable flow resistor. The pressure setting is indicated on the pressure gauge (5a). By opening the vent valve (5b) the pressure can be removed quickly and set to zero.

(5a) **Pressure gauge** to indicate the pressure set with the pressure regulator (5). Note that it indicates the pressure setting and not the actual aortic pressure. The aortic pressure is about 8 to 12 mm Hg higher, depending on the hydrostatic pressure.

(5b) **Vent valve** for rapidly removing the set pressure. To open it, turn it about half a turn anticlockwise. To close it, tighten it gently clockwise.

(6) **Pressure transducer for measuring aortic pressure**. The pressure transducer is mounted on the right side on the vertical column [below the pressure gauge (5a)] on a holder with height adjustment. The connecting tubing is connected to the tube (4c) below the main stopcock (4a).

☞ **Note**: for correct pressure measurement the tubing and the dome of the transducer must be filled free from bubbles and the transducer must be set at the level of the aorta.

(7) **Roller pump for Langendorff mode**. This pump is used to supply a retrograde flow to the heart in the Langendorff mode (during the preparation and recovery phases); the heart itself does not perform any pumping work.

Through the tubing (7a) the perfusate is drawn out of the reservoir at the suction tube (8b) and pumped through the heat exchanger (9) via the connection (2a) into the aorta block (2). The pump output must be set higher than the flow discharged through the coronaries of the heart. Excess pumped perfusate flows back through tubing (8c). The effective perfusion pressure is determined by the adjustable flow resistor (2b).

(8) **Perfusate reservoir**. The standard reservoir has a capacity of 6 litres and is a jacketted glass vessel. Thermostated water from the circulation thermostat required for the operation of the apparatus is passed through the reservoir jacket and warms the perfusate. The vessel cover lies loosely on the open top and ensures that the aeration gas introduced through the frit collects above the solution level and does not escape immediately to the surroundings.

☞ **Operating note**: do not fill the reservoir with cold (room-temperature) solution before the experiment! You will have to wait a long time before the solution has warmed to the temperature determined by the thermostat. 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.

The vessel capacity of 6 litres is not large enough to perform a (non-recirculating) experiment on a rabbit heart for appreciably longer than 30 minutes. It is therefore necessary to provide continuous replacement of the used perfusate, for example by the arrangement shown in Fig. 6. In this system, satisfactory and uniform thermostating and gas saturation is ensured at all times.

For obvious reasons it is at least 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 greater or lesser instabilities in the course of the experiment.

The cover of the reservoir carries the necessary tubing connections as adjustable tubes. Four connections are reserved for connecting the roller pumps (7) and (11), with the two longer tubes used as suction tubes. The two short tubes are used to connect up the return flow tubing.

Aeration is provided using the glass frit (8a). The glass tube of the frit is connected by a short piece of tubing to the shortest (straight) metal tube. Aeration operates through small Silicone tubing (2 mm i.d.) which is connected to one of the two needle valves (10). The intensity of aeration is adjusted on the needle valve.

Description of the re-fill system (Fig. 6):

The re-fill system consists of:

- level probe (PTC)
- power supply for submersible pump, and
- submersible pump placed at the bottom in the low-level container.

The complete equipment for providing the perfusate is conveniently mounted in a separate mobile rack with three shelves (option).

The immersion level probe is mounted in the cover of the reservoir so that its sensing tip is at the height of the desired perfusate level. The sensor cable is connected to the control input of the Power Supply (5-pin plug). At the Power Supply output „output to pump“ (12 V, 3 A) the submersible pump is plugged in through a 2-pole extension cable. The connecting tubing is connected to the shortest, bent tube on the reservoir.

☞ **Note**: it is essential to use this shortest **tube** which is **not immersed** in the perfusate during operation. If this precaution is disregarded, solution will be syphoned back from the reservoir into the low-level container when the pump is not working.

As the liquid level in the reservoir falls below the probe tip, the immersion pump is activated through the Power Supply. It then pumps solution from the low-level container to the higher-level reservoir until the liquid level has risen far enough for the level probe to respond and switch off the pump.
A switch on the Power Supply allows the immersion pump to be switched on manually at any time. The output of the immersion pump can be adjusted to suit requirements by altering the output voltage of the Power Supply. The voltage is adjustable over the range 0 to 12 Volt (normal setting 9 V approx.).

☞ **Operating note**: The level probe works with a PTC resistor which is heated by the sensor current (approx. 60° C) and utilises the heat dissipation to the surroundings for its measurement. The heat loss in air is appreciably less than in a liquid. The resulting different time constants for heating (slow) and cooling (rapid) lead to different times when the pump switches on and off. The pump switches off virtually immediately when the liquid level
reaches the probe tip. When the level falls below the probe tip it takes a few seconds (5 - 10 sec) until the pump is switched on. The switch-off sensitivity can be adjusted with the „SENS“ trimmer.

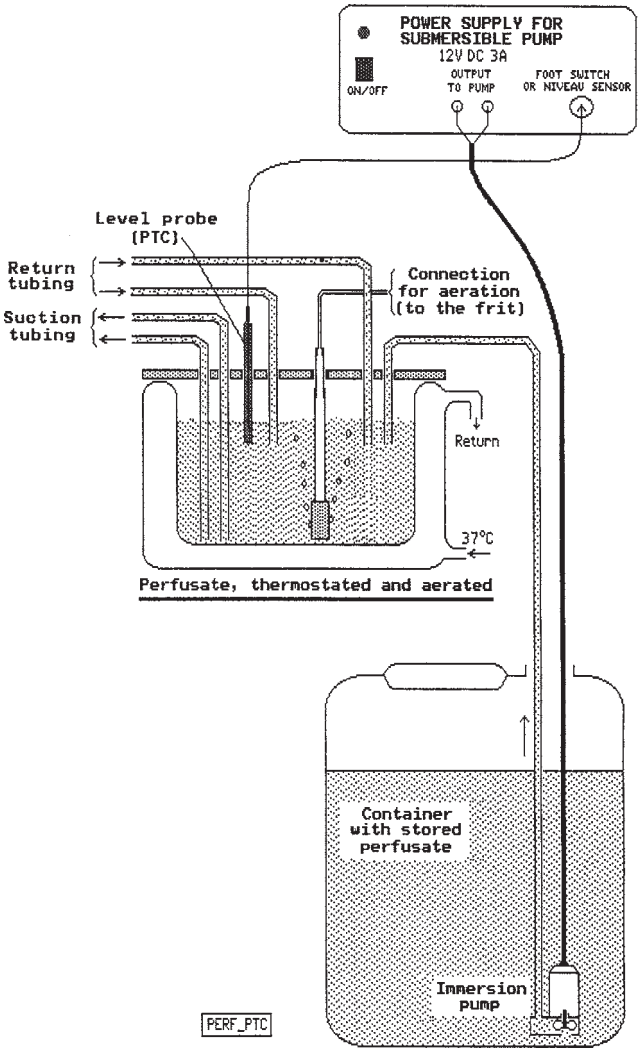

Fig 6: Provision of perfusate

(9) **Heat exchanger**. This heat exchanger is used to compensate the heat lost as the perfusate flows through the pump tubing (7a).

(10) **Needle valve**. The needle valve is used for adjusting the aeration rate in the perfusate reservoir (8). It is mounted on the short vertical Plexiglass panel below the pressure gauge (5a). The tubing to the frit is connected to the small side tube on the valve body. The connection for the gas supply (Carbogen) is accessible from back. The supply pressure should be limited to about 1 bar for safety reasons. The aeration rate is adjusted by turning the knurled knob on the needle valve; clockwise rotation reduces the aeration rate. The valve can be locked against unintentional movement by means of the small knurled nut (spindle lock).

☞ **Note: keep the needle valve dry!** Do not allow any perfusate to pass into it! Danger of oxidation!

(11) **2-channel roller pump for atrium supply**. This pump serves to provide perfusate in the preload vessel (13) at a constant level. Excess perfusate is supplied so that more perfusate is pumped in to the preload vessel than is required by the organ. The excess pumped volume is drawn off by a second pump channel [tubing (11b)] and pumped back.

In order that overflowing of the preload vessel is prevented under all operating conditions the two tubes in the two pump channels are of different size: the supply tubing (in 11a) should be slightly smaller than the suction return tubing (11b). When working with a rabbit heart, Silicone tubing of 4 mm i.d. and 5 mm i.d. can be used for example

☞ **Note on adjusting the pump output**: as indicated above, the outflow of pump (11) must always be larger than the atrial flow from the preload vessel. The pump should however not be run at maximum speed to reduce wear on the pump tubes. It is better to adjust the pump so that some perfusate is always pumped back in the return tubing (11b).

(12) **Heat exchanger**. This heat exchanger compensates for the heat loss as the perfusate flows through the pump tubing (11a).

(13) **Atrium reservoir (preload vessel)**. This vessel provides the perfusate flowing into the atrium. The height difference between the liquid levels in the vessel and in the atrium (hydrostatic pressure) corresponds to the filling pressure of the atrium (= preload pressure). Excess perfusate solution is provided in the preload vessel. Pump (11) provides more solution than flows into the atrium of the attached heart. The excess perfusate pumped in at the inflow connection (13a) is drawn off by the second channel of the same pump through the suction tube (13b). [see also under (11)]. The filling level in the preload vessel can be adjusted by moving the suction tube (13b). Atrial pressures in the range of about 5 to 10 mm Hg are obtained depending on the adjustment. The short tube (13c) acts as a vent.
The preload vessel is mounted on the upper horizontal Plexiglass plate to the left of the aorta block. After releasing the knurled screw and the two hexagon socket screws (M4x16) as well as the clamping mount (15a) for the atrium head (15) the complete atrium supply system with the preload vessel can be removed from the apparatus.

(13a) Inflow connection for the preload vessel. It projects at the bottom edge on the side of the vessel (Plexiglass tube).

(13b) Suction tube, sliding, for adjusting the liquid level and maintaining it constant.

(13c) Vent tube

(13d) Control stopcock for the atrium supply. The positions of the stopcock plug and the corresponding handle positions are shown in Fig. 7. In addition to shutting off the atrium inflow (handle position A, upwards) and opening it up (handle position B, to the right) there is a further setting (handle position C, downwards) in order to relieve the load on the atrium by means of the tube (13e) when the atrium is cannulated and the heart is operated in the Langendorff mode. Slight return flow from the aorta to the left atrium may lead to an undesirable pressure increase inside the atrium if this relief position is not selected.

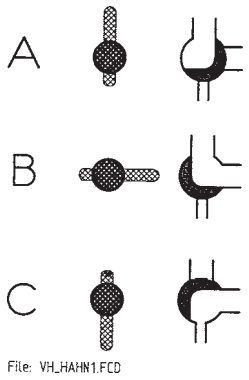

Fig. 7: Control stopcock for the atrium supply

(14) **Fitting adapter for flowmeter head**. For measuring the atrial flow a suitable flowmeter head can be fitted into this adapter (option). If no flowmeter has been ordered, a blank piece with through bore is fitted in this position.

The adapter is secured with 4 hexagon socket screws M3x12 to the underside of the preload vessel (13) after the stopcock (13d). After releasing the screws (use the screwdriver supplied) the adapter can be dismantled together with the connection plate (14a) with tubing connection. On re-assembly, please ensure that the O-ring seal (6x2 mm, Silicone) is located in its groove. This side must be towards the stopcock (13d).

(14a) Flowmeter head (option), mounted permanently in the adapter (14).

(15) **Atrium head**. The atrium head contains all the components required for the functionally correct connection of the left atrium close to the atrium cannula (16). Connection (15d) (tube projecting inwards) is provided for atrial pressure measurement by the pressure transducer (17). Syringe (15g) is connected through the shut-off stopcock (15f) to the short tube which terminates at the inner top edge of the atrium head inner space. This syringe is used for removing any air/gas bubbles which may have been swept into the atrium head.

The perfusate passes through a short piece of tubing (Tygon tubing 6.4 mm i.d.) from the tubing connection of the connection plate (14a) to the inlet connection (15d) of the atrium head. From there the solution passes through the connection (15b) to the atrium cannula (16). To improve filling of the atrium, the back side wall of the atrium head carries an elastic diaphragm whose outer surface is connected to atmospheric pressure [via support tube (15c)].

☞ **Operating note:** the free end of the support tube (15c) must not be closed and no liquid must enter it! The atrium head is mounted with a clamping fitting (15a) by the support tube (15c) on a rod (4 mm dia., 40 mm long) which is located at the side on the aorta block (2). After releasing the clamping fitting 15a) the atrium head can be adjusted as required.

(15a) Clamping fitting for supporting and adjusting the atrium head.

(15b) Connection for fitting the atrium cannula (16). 2 grooves to take two O-rings (7x1.2 mm, Silicone) are provided for sealing.

(15c) Support tube for securing the atrium head. Operating note: the free end of the support tube (15c) must not be closed and no liquid must enter it!

(15d) Connection for feeding in the perfusate, suitable for Tygon tubing 6.4 mm i.d.

(15e) Metal tube (1.3 mm o.d., short tube!) for connecting the syringe (15g) via the stopcock (15f). Syringe (15g) serves to remove any bubbles which have been swept into the atrium head.

(15f) Shut-off stopcock

(15g) Syringe, e.g. 10 ml, for removing gas/air bubbles from the atrium head (15).

(15h) Metal tube (1.3 mm o.d., longer tube projecting inwards) for connecting the pressure transducer (17) for measuring atrial pressure.

(16) Cannula for binding into the left atrium. Note: atrium and aorta cannulae are interchangeable, they fit both here and on the connection (4) of the aorta block! Always use the largest possible cannula in order to achieve optimum atrium filling.

(17) **Pressure transducer for measuring atrial pressure**. The pressure transducer is mounted on a holder with vertical adjustment. The connection tubing is connected to the longer, inwards projecting tube (15d) of the atrium head (15).

☞ **Note:** for correct pressure measurement both the tubing and the transducer dome must be filled free from bubbles and the transducer must be adjusted to the level of the atrium.

(18) **Thermostated heart chamber, movable**. The jacketted heart chamber serves to provide a moist and warm environment for the organ.

There are two sizes of heart chamber:

A): The larger chamber (capacity approx. 1.3 l) is required when MAP signals have to be picked off from the heart surface with a circular electrode arrangement, or when multi-electrode ECG recordings after Einthoven and Wilson are made using an „electrode basket“.

B): The smaller chamber (capacity approx, 0.6 l) is recommended when neither of the above recordings are made.

Both chambers are provided with an overflow tube. The smaller chamber (B) also has a drain connection so that the effluate dripping off can be discharged from the chamber without having to fill the chamber volume.

The heart chamber is mounted on a platform whose height can be adjusted with a rotary control. The jacket of the vessel is included in the thermostating circuit of the apparatus so that thermostated water flows through it.

(18a) Overflow tube. The perfusate overflowing here (coronary effluate) is usually discarded and not returned to the reservoir (8).

(18b) Discharge connection for emptying the chamber (18). This connection is provided only on the small heart chamber (chamber B, see above).

10 Tubing and connections on the apparatus, dimensions

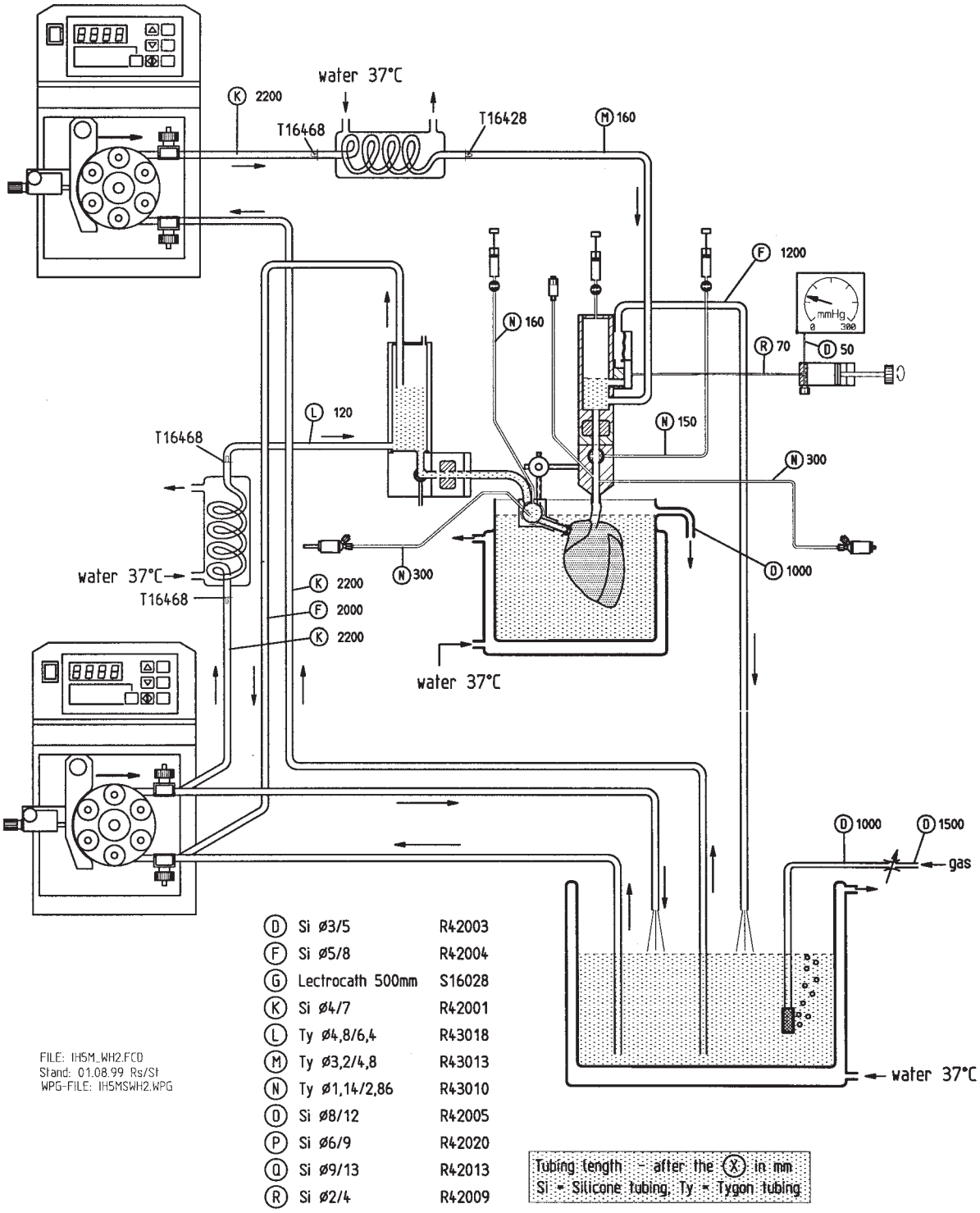

Fig. 8: Dimensions of the tubing connections (without thermostating)

The tubing diameters for perfusate transport as indicated in Fig. 8 apply for medium flow rates up to about 300 ml/min aortic flow. Under these conditions the dwell time in the tubing is short so that losses of heat and oxygen remain small. With low perfusate flows it may be useful to use smaller diameter tubing than those suggested here in order to reduce the dwell time of the perfusate in the supply tubing. Additionally it is then better to use Tygon tubing instead of Silicone. Apart from reduced heat losses on the way from the reservoir to the organ there is then also less reduction in oxygen saturation.

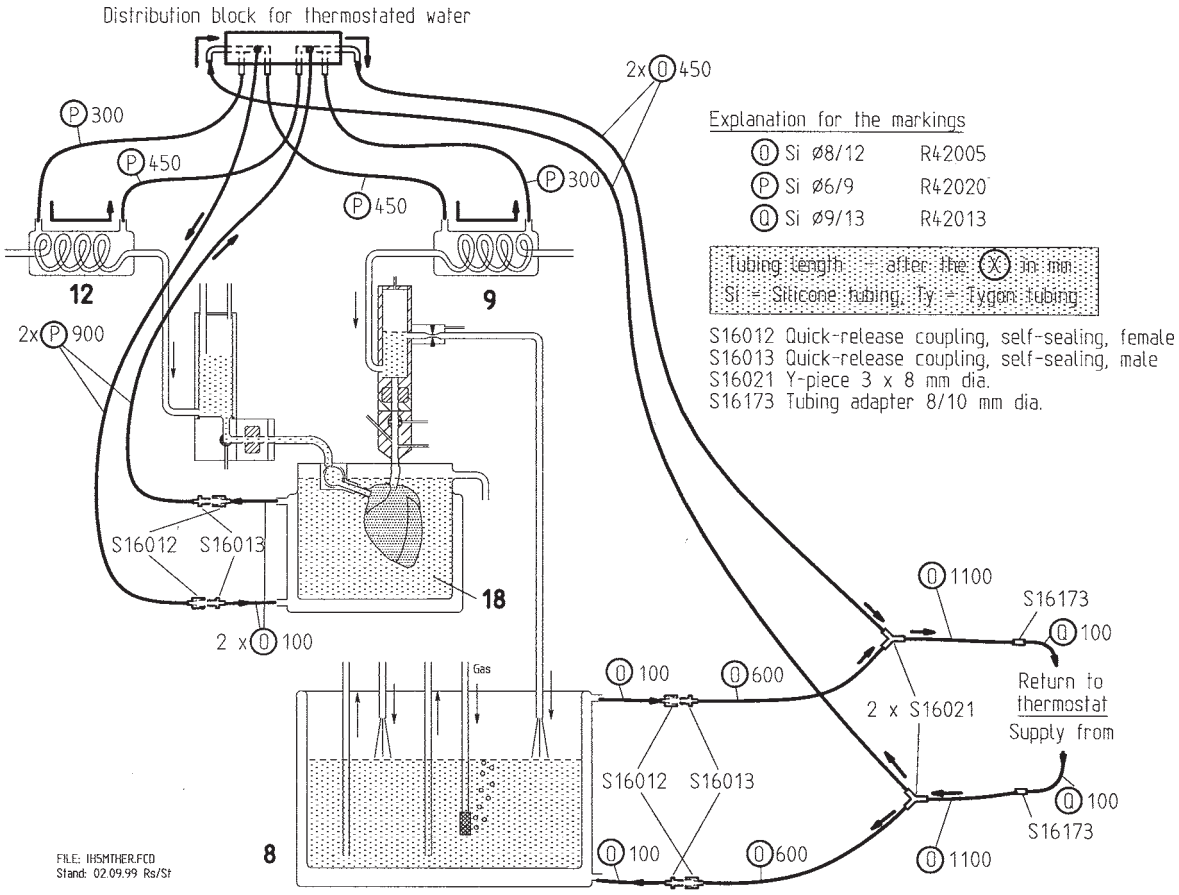

Fig. 9: Tubing connections of the thermostating circuit.

Fig. 9 shows the tubing sections of the thermostating circuit. Silicone tubing only is suggested. The distribution block for the thermostated water is located on the back of the top Plexiglass plate. When connecting the individual jacketted vessels please ensure that the flow is from the bottom upwards where possible. The larger vessels (8) and (18) are connected through self-sealing quick-release couplings. They can readily be disconnected for cleaning without first having to drain their water jacket.

☞ **NOTE**: the reference numbers starting with R or S (e.g. R42005) are HSE part numbers.
If you require any spares please quote where possible the part number indicated.

11 Servicing and maintenance

11.1 Perfusate circuit

Before the first start-up and daily after the experiment has been completed, the apparatus has to be cleaned. It is particularly important that the perfusion circuit and all parts in contact with the perfusate are kept clean.

☞ **Warning:** In order to avoid damage to the apparatus only the cleaning agents mentioned below may be used without special testing.

Daily cleaning is important in particular because of bacteria. Apart from other effects, bacteria in the apparatus lead to early oedema in the organ! Bacteria also grow overnight! Therefore leave the cleaning agent inside the apparatus overnight.

For cleaning you first rinse the perfusate circuit thoroughly with distilled water. Use the pumps for the purpose. First connect the atrium cannula (16) to the aorta cannula (4e) with a short piece of tubing.

Then fill the various vessels [preload vessel (13) and aorta block (2)] with hot cleaning solution.

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

If the apparatus is not in use over the weekend it should be filled with distilled water after cleaning and be kept in the dark (or covered over against excessively bright light).

With longer breaks in the experimental work it is better to keep the apparatus dry after cleaning; before the next experiment it has to be filled with cleaning solution which is allowed to act overnight.

In the morning, switch on the thermostat in order to warm up the cleaning solution. Then remove the cleaning solution from the apparatus and rinse thoroughly with distilled water. Do not forget the side branches [tubing to the pressure transducers (6) and (17) and to the syringes (4k) and (15g)].

Alternatively to the above suggestion:

- ❑ rinse with 0.1N HCl (hydrochloric acid 0.1 normal), then
- ❑ rinse with distilled water, then
- ❑ fill with 0.1 N H_2O_2 (3% hydrogen peroxide)
- ❑ leave overnight.

Perfusate system servicing, summary:

- Drain and remove perfusate!
- Rinse with distilled water!
- Fill vessels with hot cleaning solution!
- Allow to stand for a few hours or overnight (24 hours max.)!
- In the morning heat up apparatus to 37° C with the thermostat!
- Then remove cleaning solution!
- Rinse with distilled water, do not forget side branches!

11.2 Thermostatic circuit

The thermostatic circuit must also be serviced regularly. If you are filling the thermostat with pure distilled water without any additive you have to replace the water every week in order to avoid algal growth inside the system. When adding Thermoklar or sodium azide (danger, toxic!) you should replace the contents at least every month.

Thermostatic circuit servicing, summary:

- Thermostatic circuit without additive, replace water weekly!
- With additive, replace at least every month!
- Fill only with distilled water (with or without additive)!
- Before filling, rinse with cleaning solution and clear water!

11.3 Recommended cleaning agents

Use only recommended cleaning agents.

In order to avoid damage to the apparatus, only the cleaning agents listed below can be used without special testing. If you require a different cleaning agent for any particular reason you must first test it for compatibility before using it. In case of doubt contact the manufacturer of the apparatus.

RBS 50 or RBS 35

Manufacturer and supplier: Carl Roth GmbH & Co KG, Chemische Fabrik,
Schoemperlenstr. 1-5, D-76185 Karlsruhe 21, Germany
Phone: (+49) (0)721 / 56 06-0, **Fax**: (+49) (0)721 / 56 06-49,
E-Mail: Carl@t-online.de, **Internet**: http://www.Carl-Roth.de

MUCASOL

Manufacturer: Merz + Co GmbH & Co., Bereich Dr. Kramer,
Eckenheimer Landstrasse 100-104, D-60318 Frankfurt/Main 1, Germany
Phone: (+49) (0)69 / 1 50 31, **Fax**: (+49) (0)69 / 5 96 21 50, **Telex**: 414 031.

Supplier: Rudolf Brand GmbH & Co.,
P.O. Box 11 55, D-97861 Wertheim, Germany
Phone: (+49) (0)9342 / 8 08-0. **Fax**: (+49) (0)9342 / 8 08-236.

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

Alternative rinsing agent: 0.1N HCl (hydrochloric acid 0.1 normal)

Overnight in the apparatus: 0.1N H_2O_2 (hydrogen peroxide 0.1N, 3%).

Cleaning agent for Plexiglass surfaces:

☞ **WARNING**: not all the cleaning agents commonly used in the laboratory are suitable for cleaning the apparatus. For example, Mucocit F supplied by Merz, Frankfurt, attacks Plexiglass.

12 Tubing material

The tubing used in the apparatus has to meet various requirements. It has to be selected so that its material properties best meet the requirements. In addition to chemical, mechanical and optical properties it is also necessary to take account of the gas permeability of the various materials. The selection aid for certain materials as listed below is really intended to do no more than provide some assistance. The user must decide for himself which type of tubing is most suitable for his particular application. In case of doubt it is necessary to carry out tests and make appropriate measurements.

When considering permeability to gas, the time the liquid remains in the tubing is of course not unimportant. Longer periods result in more gas exchange with the surrounding air than shorter periods. In case of doubt it is necessary to carry out appropriate measurements.

Silicone tubing:

Silicone is the tubing material most widely used in the laboratory. It is largely inert and resistant to most chemicals used in the laboratory. Natural Silicone is not glass clear but is sufficiently translucent so that the lumen can be examined visually. A further advantage is its favourable mechanical properties. When used in roller pumps there is relatively little wear.

Silicone exhibits a high permeability to oxygen. Whenever oxygen interchange between atmosphere and lumen has to be avoided you must not use Silicone tubing.

Tygon® tubing (R-3603)

Tygon® is also frequently used in the laboratory. It is manufactured under strictly controlled conditions. It is largely inert and resistant to most chemicals used in the laboratory. Tygon is glass clear and permits optimum visual checking of the lumen. Tygon tubing is flexible and the remaining mechanical properties are also favourable. When used in roller pumps it suffers somewhat more wear than Silicone.

Tygon has a low permeability to oxygen. It is therefore a better tubing material than Silicone when exchange of oxygen between the surroundings and the lumen has to be avoided. Thick-walled tubing is preferable to thin-walled tubing in this case.

PharMed™ tubing (65)

PharMed™ tubing has been specially developed for medical applications and has only recently become available. Unfortunately only little information is available on this material. Chemical and mechanical properties are said to be excellent. Its permeability to oxygen is said to be practically zero. The disadvantage is that the material is not transparent. Visual examination of the lumen is not possible.

13 Wetted materials

Glass: reservoir, heat exchanger, heart chamber, aeration frits.

Plexiglass (PMMA): aorta block assembly, atrium connection system, stopcock blocks, adapter for flowmeter heads.

Stainless steel (V2A, V4A): aorta and atrium cannulae, various connection tubes, supply and discharge lines of reservoir and preload vessel.

Tygon: connecting tubing from preload vessel to atrium head (possibly also all pump tubing, depending on the user).

Silicone: diaphragms in adjustable flow resistor, oscillation damper and atrium head, possibly also pump tubing depending on the user.

14 Technical data

Dimensions:	baseplate 60 cm x 40 cm height 65 cm
Lumen of perfusate lines:	4.8 to 5 mm
Perfusate flow, max.:	500 ml/min
Aortic pressure:	up to 300 mm Hg
Internal size of large heart chamber:	depth 10 cm, diameter 145 cm
Internal size of small heart chamber	depth 10 cm, diameter 10 cm
Ambient operating temperature:	20 - 30° C
Storage temperature:	10 - 40° C
Humidity:	20 - 80% rel. humidity
Weight (empty):	approx. 14 kg

Trademarks:

Teflon is a registered trade mark of du Pont de Namours & Co.
Tygon and PharMed are registered trademarks of Norton Co.

15 Characteristic curves of the adjustable flow resistor (afterload)

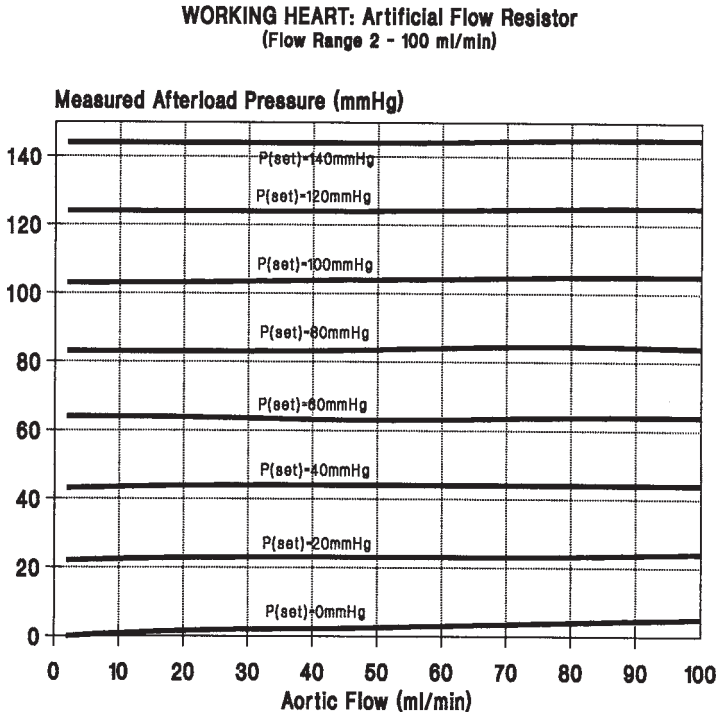

Fig. 10: Characteristic curves of the adjustable flow resistor over a flow range of 2 - 100 ml/min

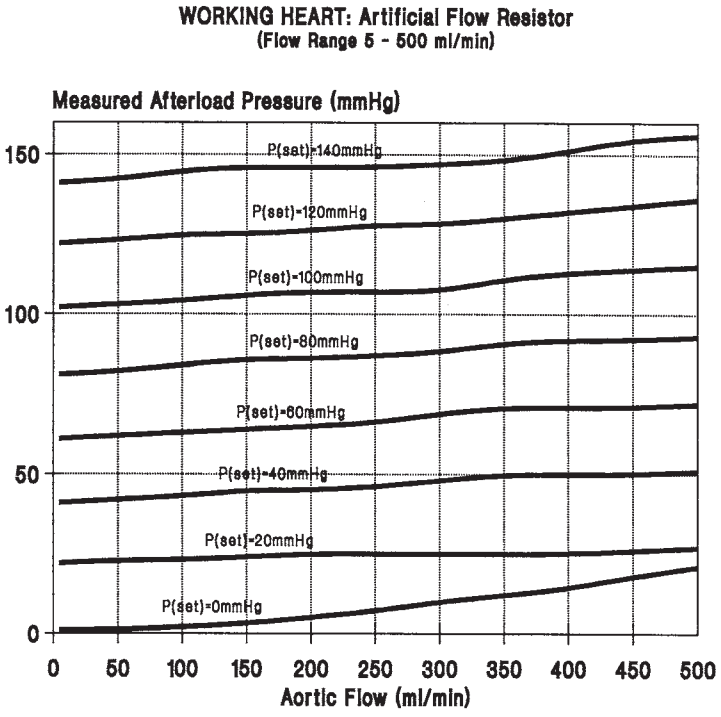

Fig. 11: Characteristic curves of the adjustable flow resistor over a flow range of 5 - 500 ml/min

16 Appendix 1: various illustrations of the apparatus

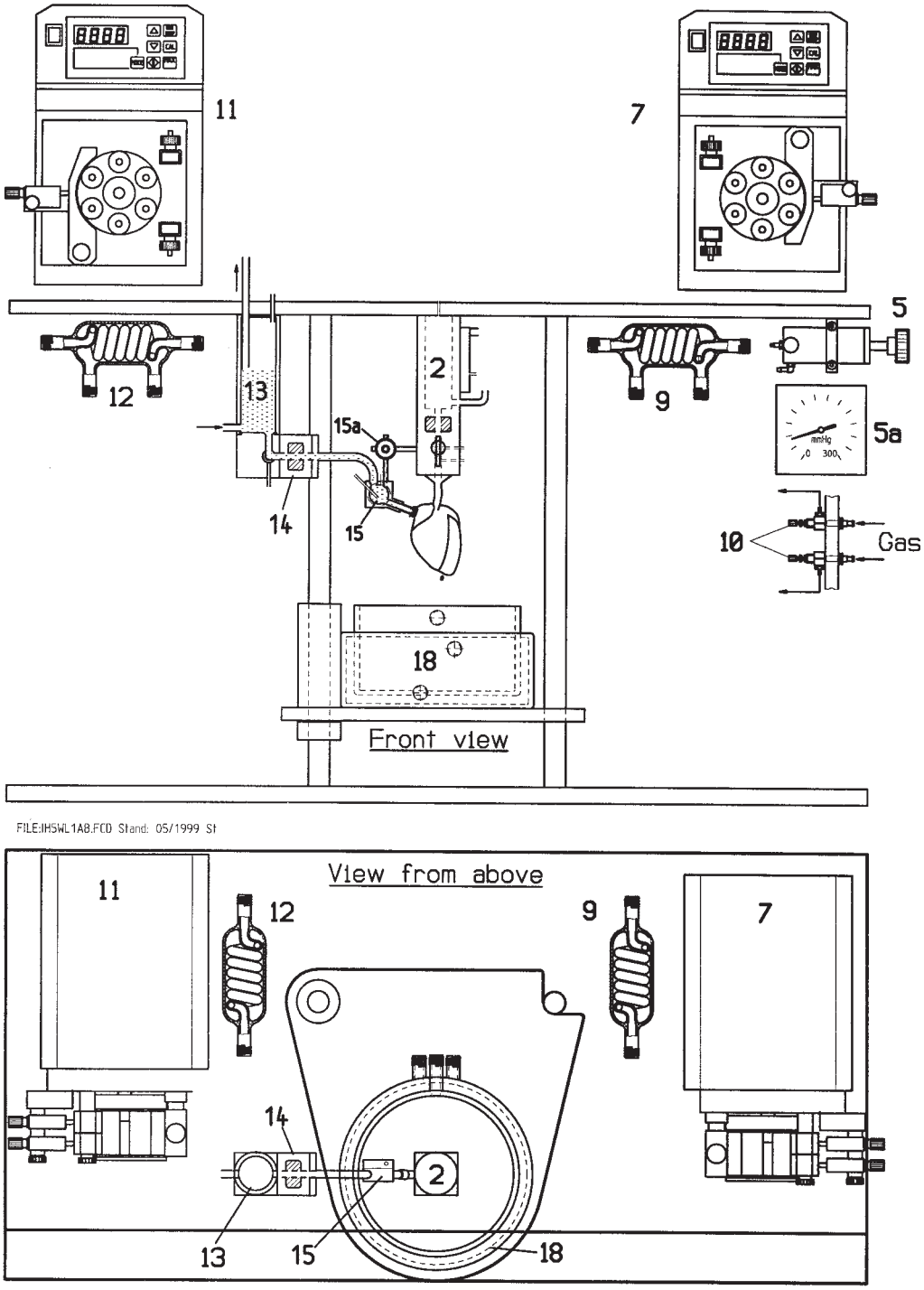

Fig. 12: Mechanical arrangement of the WH apparatus

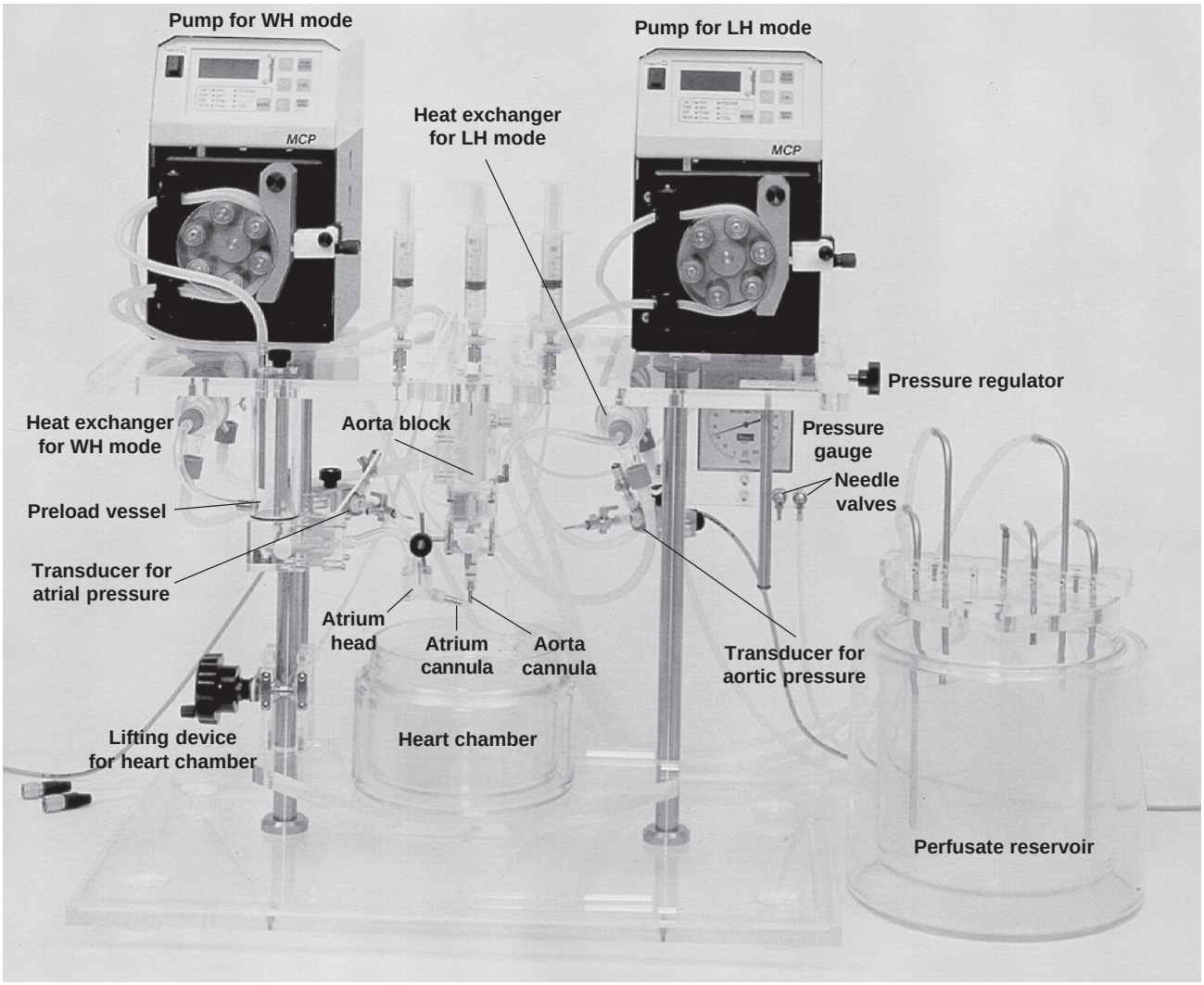

Fig. 13: General view of the apparatus in the Working Heart (WH) mode

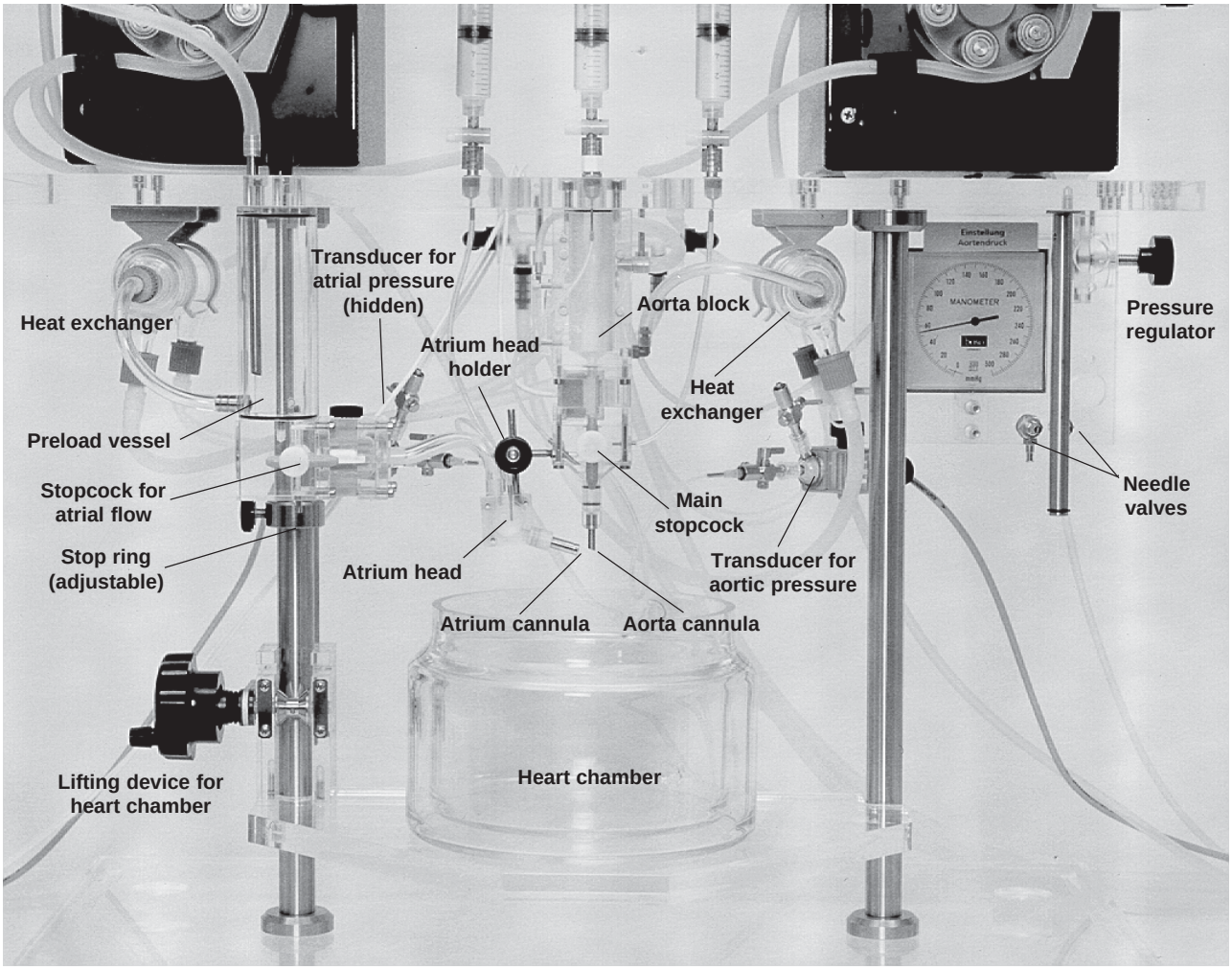

Fig. 14: Detail view in Working Heart (WH) mode

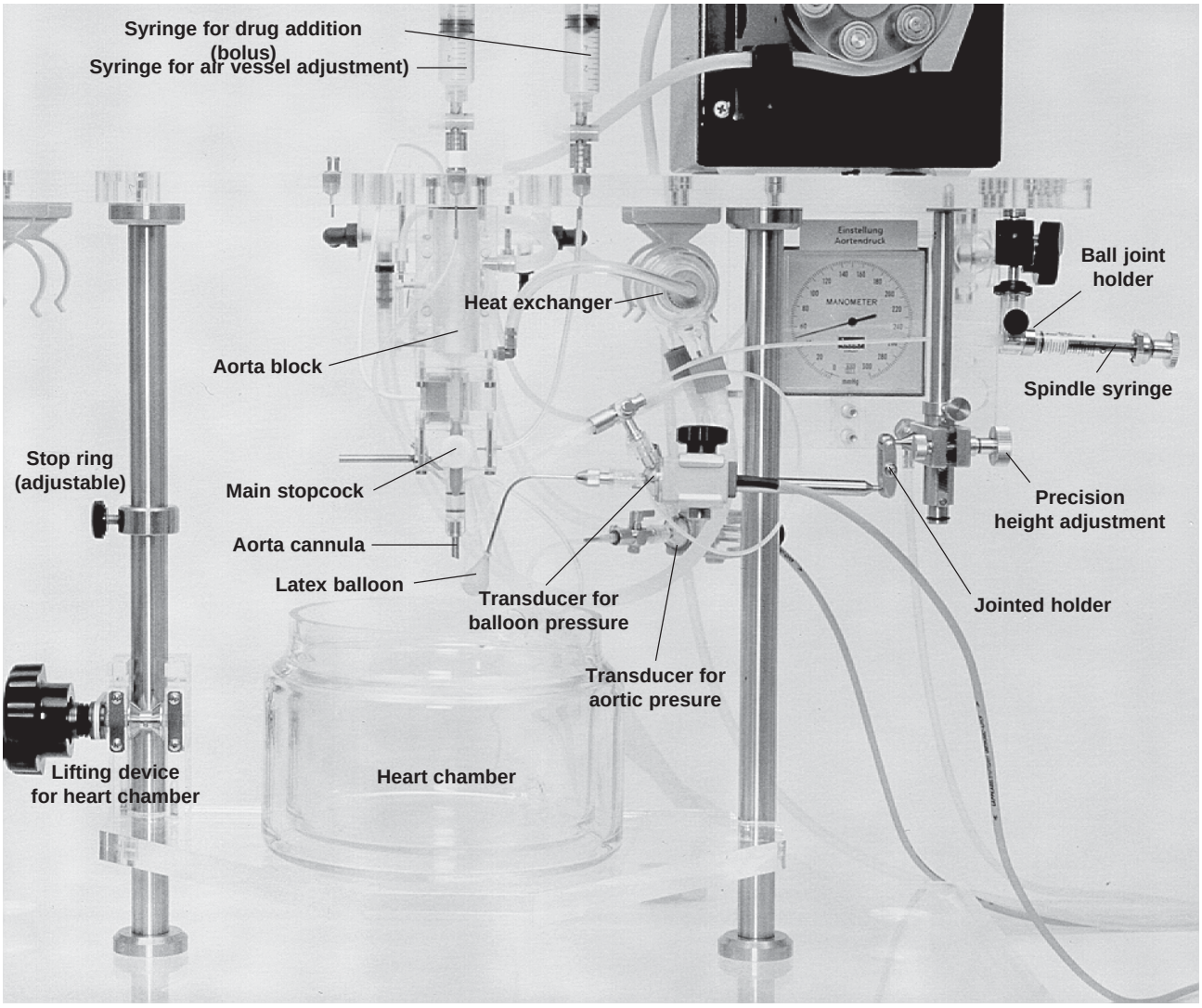

Fig. 15: Detail view in Langendorff Heart (LH) mode

II Operating Mode LANGENDORFF HEART

17 Description of function

In the Langendorff Heart operating mode the heart beats „empty“; it does not perform any pressure-volume work and is supplied by retrograde perfusion.

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

The complete system consists of the basic apparatus as described in Part I, but reduced essentially by the elements required for the atrium supply. In addition there is usually a device for measuring the LVP by the so-called balloon method.

The 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 of the pressure regulator and indicated on the pressure gauge (set value). The roller pump (7) pumps the perfusate into the aorta block and generates the necessary pressure. When the pressure in the aorta block reaches the set value, the flow resistor opens up the return line to the reservoir. By suitable adjustment of the pump output and the flow resistor the heart can be perfused either under constant-pressure or under constant-flow conditions.

17.1 Perfusion at constant pressure

The output of the roller pump is 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. 50 mm Hg, is set on the pressure regulator. 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, or only a very small one in the case of high return flow rates. In the case of vascular dilation the reverse action takes place. A reduction in pressure is only observed if the pump output has been set too low, so that all the pumped perfusate flows through the organ and there is no return flow.

17.2 Perfusion at constant flow

Under constant-flow conditions the entire perfusate pumped by the roller pump flows through the organ. The setting of the adjustable resistor has to be selected higher than the highest expected or permitted perfusion pressure (e.g. 100 mm Hg). The adjustable 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 (here 100 mm Hg).

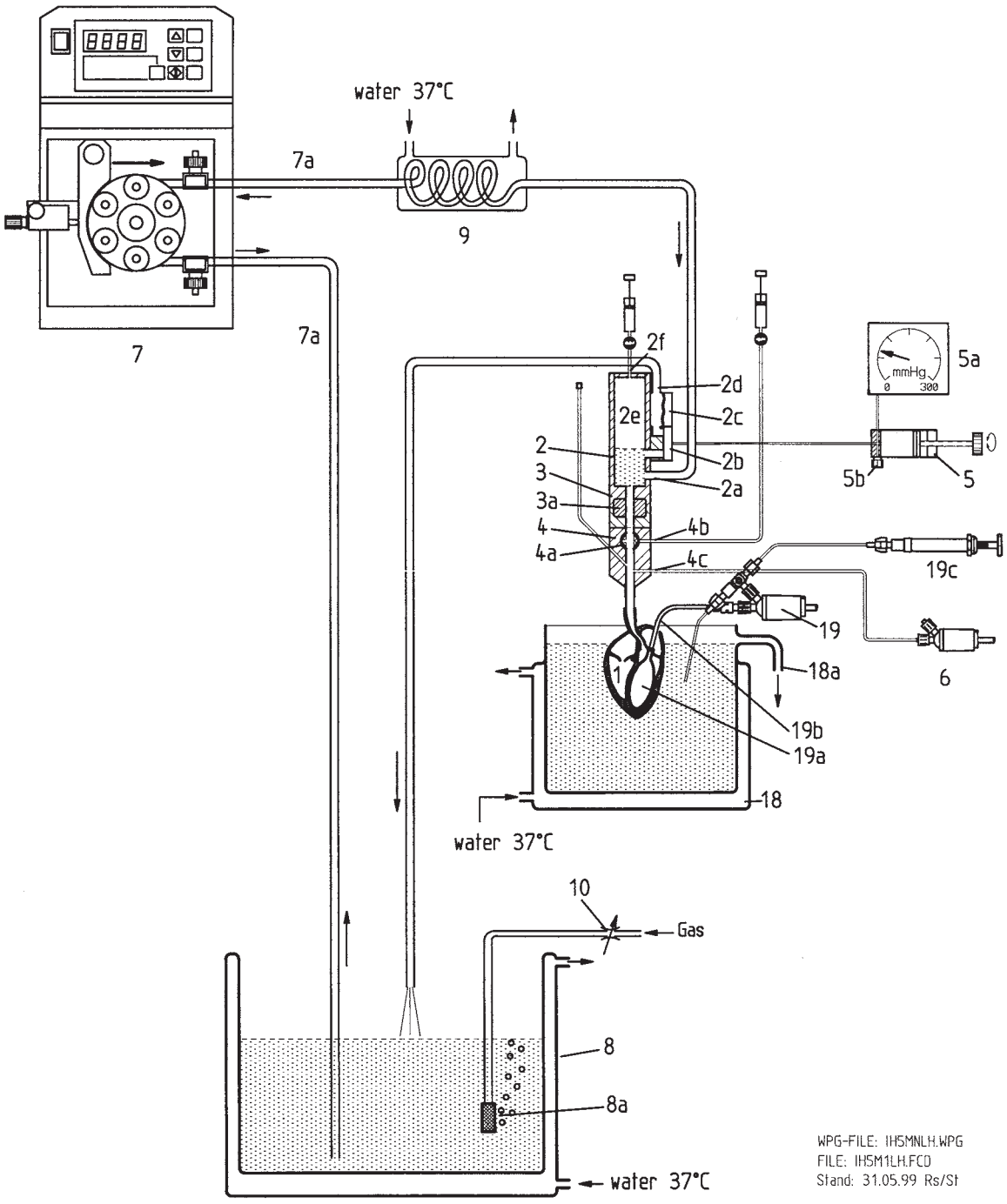

Fig. 16: Schematic arrangement for experiments „Isolated perfused heart after Langendorff“. Except for Item 19, all the descriptions can be found in Section 9.

18 Isovolumetric measurement of the internal cardiac pressure

The measuring system is shown in the diagram of Fig. 16. It consists of the following elements: pressure transducer (19), e.g. Isotec, with latex balloon (19a), catheter (19b) and 3-way stopcock, as well as the 2 ml spindle syringe (19c). Details of this measuring system are contained in Fig. 17, Fig. 18 and the corresponding legends.

The latex balloon is positioned in the left ventricle of the heart by means of the steel catheter. The pressure transducer is mounted by a ball joint holder on the precision height adjustment which itself is mounted on the vertical column to the right of the heart. This allows the transducer system (pressure transducer with catheter and balloon) to be moved accurately to the required position by the precision height adjustment; in this way the vertical position of the balloon in the ventricle can be accurately adjusted (for list of available balloon sizes see Appendix.)

The spindle syringe is mounted with a ball joint holder on the top horizontal Plexiglass plate and is connected by catheter tubing and the 3-way stopcock to the pressure transducer. The syringe has a threaded spindle. By rotating the spindle the balloon can be filled sensitively to the required diastolic pressure, e.g. 10 mm Hg. The 3-way stopcock is used for filling balloon, catheter and dome free from bubbles, and then to provide a pressure-tight seal between the filled measuring system and the spindle syringe.

The measuring system is best filled with a mixture of alcohol and distilled water (mixing ratio approx. 1:1). This mixture has good wetting properties so that filling free from bubbles is readily possible. In addition this mixture produces only few gas bubbles through „outgasing“ during measurement, a problem found with other filling media.

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

Legend for Fig. 17:

(1) Latex balloon, size depending on organ size

(2) Balloon catheter (with thread on Luer taper, metal or plastics)

(3) Pressure transducer (Isotec or P23XL or similar)

(4) Metal 3-way stopcock with Luer connections

(5) Pressure equilibration tube, required for exact zeroing of pressure transducer

(6) Connecting tubing for setting the filling pressure with the spindle syringe

(7) Spindle syringe

(8) Connecting cable to electromanometer or bridge amplifier

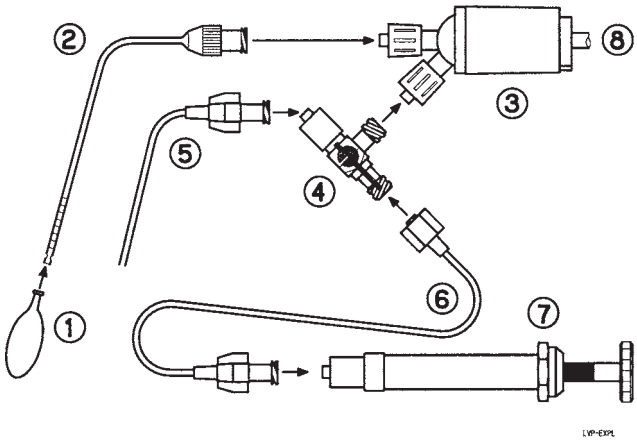

Fig. 17: Components for measuring the isovolumetric LVP. Note the legend!

Legend for Fig. 18:

This illustration shows schematically the arrangement for measuring the isovolumetric internal heart pressure using the so-called balloon method. Note in particular the positions of the 3-way stopcock as shown in the diagrams A - C.

☞ **Please note**:
always ensure first that the measuring system is filled free from bubbles (Section 19)!

(A) Perform zero adjustment of the pressure transducer!

Zero adjustment is performed before inserting the balloon into the left ventricle. Move the 3-way stopcock into the position shown. To compensate the hydrostatic pressure (the liquid column between balloon and transducer 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 full of liquid!

(B) Set the balloon pre-load (diastolic pressure!

After the balloon has been inserted into the ventricle the required 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 „fast" recorder or an oscilloscope connected to the pressure amplifier so that the dynamic balloon (LVP) signal can be monitored.

(C) Normal operation with measurement of isovolumetric LVP

After the pre-load has been set (B) the dome is closed with the 3-way stopcock. 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. Note the information in Section 20.

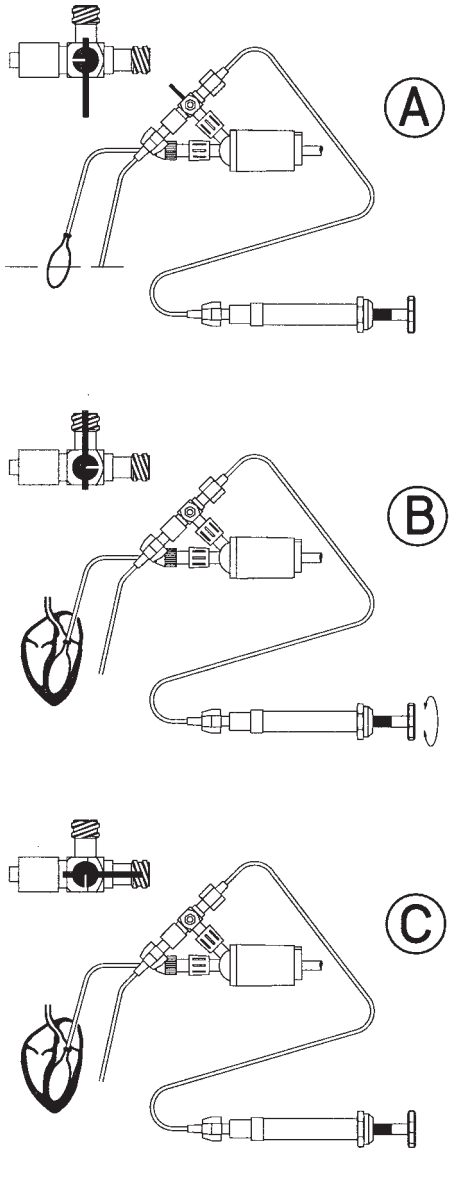

Fig. 18: Isovolumetric LVP measurement. Note the legends (A) - (C)!

19 Filling the balloon measuring system free from bubbles

When measuring the isovolumetric internal heart pressure by the balloon method it is necessary to have the complete measuring system consisting of latex balloon, steel cannula and pressure transducer completely filled and free from air bubbles. A possible procedure is to initially accept air bubbles during assembly and then to remove them as described below.

In order to remove air bubbles from the measuring system a 3-way stopcock must be inserted between spindle syringe and transducer dome (side connection of the pressure transducer). The steel catheter with the balloon mounted on it is filled first; using a syringe applied directly to the catheter cone, the balloon is alternatively filled and emptied, with catheter and syringe held so that the balloon hangs downwards and some of the air rises into the syringe each time the balloon is emptied. With small balloon sizes it may be necessary not only to fill the balloon but also to extend it slightly so that sufficient liquid reaches the balloon and air bubbles are transported sufficiently far upwards inside the catheter during emptying. The rising of the bubbles can be assisted by simultaneously tapping against the catheter.

As shown in Fig. 17, the spindle syringe is connected through catheter tubing and a 3-way stopcock to the side connection of the transducer dome.

After the balloon with catheter has been filled free from bubbles as described above, it is connected to the central connection of the transducer dome (the dome has already been filled free from bubbles). Care must be taken to ensure that no air bubbles are introduced at the connection point. Remaining bubbles can be removed by filling the balloon by means of the spindle syringe (if necessary extending the balloon slightly!) and then releasing the pressure by operating the 3-way stopcock so that any air bubbles are washed out. Washing out can be assisted by carefully squeezing the balloon manually. Care must be taken that no fresh air bubbles are introduced into the measuring system. Do not forget to refill the spindle syringe in good time by drawing in filling solution through the equilibration tube (Part 5 in Fig. 17), again without air bubbles. For this operation the 3-way stopcock must be moved to position
(C) (Fig. 18).

20 Notes on the stability of the pre-load inside the balloon

In order to obtain defined measuring conditions it is essential that the balloon inserted into the ventricle is filled at a pressure of e.g. 10 mm Hg (= pre-load) which is maintained as constant as possible. When the ventricle is operating normally the pressure falls to this value during each diastole and can be monitored on the recorder. Any decrease of the pre-load below the initially set value may have various causes:

Rapidly decreasing pre-load (within a few minutes) suggests a leak in the measuring system. In this case check in particular the 3-way stopcock and its Luer connection, also the metal catheter. The metal catheter must have a leak-tight solder joint to the Luer connector.
IMPORTANT: be sure that the spindle syringe is separated from the measuring system [stopcock position, Fig. 18 (C] as it can never be completely leak-tight!

A slowly decreasing pre-load (e.g. over an hour) usually has a different cause. The pressure decrease is not due to any leakage in the balloon but arises because rubber, like smooth muscle, is not only elastic but in fact has appreciable plastic components so that it flows under pressure. Under the pressure the balloon extends its volume, causing a loss of pressure in the measuring system. This effect is particularly noticeable when the balloon has been chosen too small in relation to the ventricle size.

Balloons made up from **condom rubber or thin plastic foil** are somewhat better. Due to the folded surface structure they suffer relatively little extension or none at all. The pre-load simply unfolds the balloon and there is very little force or none at all on the rubber or plastic. As a result there is no flow and the pre-load does not alter. However, these home-produced balloons often suffer from the disadvantage that due to their irregular shape they take up an undefined position inside the ventricle which may change during each extrasystole. This results in sudden jumps in the diastolic pressure.

21 Table of micro balloons available

Species	Body weight	Latex balloon		
	(standard value) (kg)	Size No.	Volume *) (ml)	Dimensions d x l (mm)
mouse, small rat	0,02 - 0,1	2	0,01	2 x 5
rat, guinea-pig	0,1 -0,2	3	0,03	3 x 7
rat, guinea-pig	0,3 - 0,4	4	0,06	4 x 8
rat, guinea-pig	0,5	5	0,1	5 x 9
guinea-pig, small rabbit	0,7	6	0,2	6 x 10
rabbit	1	7	0,27	7 x 11
rabbit	1,2	8	0,35	8 x 12
rabbit	1,5	9	0,5	9 x 13
rabbit, cat	1,7	10	0,7	10 x 14
rabbit, cat	2,2	12	1,3	12 x 17
rabbit, cat	2,7	13	1,6	13 x 18
cat	3	14	1,9	14 x 19
cat	3,5	15	2,4	15 x 20
cat, small dog		16	3,0	16 x 22
cat, small dog		17	3,5	17 x 23
cat, dog		18	4	18 x 24
dog	4	19	5	19 x 25
dog	up to 20	24	10	24 x 32
dog	up to 40	30	20	30 x 40

*) volume without pressure

the shape of the balloon is not spherical but is adapted to the cone-shaped interior of the ventricle by gibing it a long, oval shape.

22 ECG (EG) recording with miniature suction electrodes

The ECG signal can be recorded from the ventricle in different ways. Miniature suction electrodes supplied by HSE represent one of the recording methods. Two suction electrodes are mounted by suction at the required points on the ventricle and connected to the amplifier inputs A and B. An electrical connection is made to the perfusate column in the aorta cannula from the amplifier null to provide the reference potential for the amplifier. This can be done, for example, through the lower insertion tube (4f) for the MTC.

☞ **WARNING**: handle the suction electrodes with care! Do not pull at the thin suction tubing! The suction tubing contains thin flexible wires which may break under stronger tension. This renders the electrode **unusable**.

A vacuum is required to operate the suction electrodes. A suitable source of vacuum is a water jet pump. If this pump is operated directly from the water supply the vacuum produced is so strong that the ventricular tissue underneath the suction electrode is damaged. Instead of the required ECG signal you then record injury potentials which are superimposed on the ECG signal and produce erroneous results. The vacuum must therefore be limited.

The combination of a water jet pump with a water flow regulator (2 l/min) as offered by HSE produces a vacuum of about 200 mm Hg. With this vacuum the electrodes adhere firmly and tissue damage is avoided.

23 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 the 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

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