AN APPARATUS FOR THE CULTURE OF WHOLE ORGANS

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PLATE 15

(Received for publication, June 12, 1935)

The apparatus described in this paper was designed to maintain a sterile, pulsating circulation of fluid through living organs. More than twenty-six experiments, with various organs, have been made up to the time of writing. Some of the results have already been reported in brief.[1]

The apparatus may be considered to consist of two portions. One, the perfusion pump, contains the organ and perfusion fluid, and must be handled with aseptic technique. The other is for the purpose of creating and transmitting a pulsating gas pressure to the perfusion fluid contained in the first portion. It is unnecessary to keep the second portion sterile. The perfusion pump is made of pyrex glass,[2] and has only three openings which communicate with the exterior. These openings are protected against infection by filter bulbs containing non-absorbent cotton. Neither the organ nor the perfusion fluid comes in contact with any corks or joints which communicate with the exterior. The perfusion pump involves the use of three glass chambers, one above another. The organ lies on the slanting glass floor of the highest chamber. Fluid from the lowest, or reservoir, chamber is driven through the nutrient artery by pulsatile gas pressure. After passing through the organ, the fluid returns through the central chamber, back to the reservoir. The central chamber exists for pressure equalization.

The apparatus is actuated by compressed air controlled by a rotating valve. The pulsations created are transmitted through an oil

[1] Carrel, A., and Lindbergh, C. A., *Science*, 1935, **81**, 621.

[2] All glass contained in the apparatus was blown by O. Hopf of The Rockefeller Institute.

column to controlled gas which passes back and forth through the cotton filter bulbs and causes the perfusion fluid to circulate.

The composition of all gas in contact with the organ and the perfusion fluid is controlled. Filming and evaporation of the fluid are prevented. The maximum and minimum pulsation pressures and the pulsation rate are adjustable. The pressure at various points in the pulse cycle can be controlled. The temperature of operation is adjustable. The rate of flow of perfusion fluid can be measured. Changes in rate of flow through the organ are adjusted for automatically with a minimum effect on pulsation pressures. The perfusion fluid is filtered during its circulation and before it enters the organ. Organs can be removed from one apparatus and installed in another aseptically. The perfusion fluid can be removed and replaced aseptically. The organ and the perfusion fluid can be observed at all times.

Principle of Circulation and Pulsation

The basic principle of the pulsating circulation is built upon the use of three chambers placed in vertical line. The lower chamber (*R*) is the fluid reservoir. The upper chamber (*O*) is the organ chamber, and the center chamber (*E*) is the pressure equalizing chamber.

The pulsation and circulation in the apparatus is actuated by a pulsating gas pressure in the gas line (*G*). For the purpose of this explanation the pressure in the gas line (*G*) is set to vary from approximately 0 mm. Hg to 120 mm.

The gas line (*G*) branches just before entering the reservoir and equalization chambers (*R* and *E*). One branch leads directly to the equalization chamber (*E*) and transmits its pressure (approximately 0 mm. to 120 mm.) to the chamber (*E*). The other branch divides again and carries the pulsating gas through a one-way valve (*V*) in one line and a constricted opening (*C*) in the other. Then the two divisions come together again into a single line which enters the reservoir chamber (*R*). The one-way valve (*V*) offers no resistance to gas entering the reservoir chamber (*R*) and consequently, the maximum pressure of the gas line (120 mm.) is exerted directly on the reservoir chamber (*R*). The constricted opening (*C*) bypassing the one-way valve (*V*) is, for this explanation, so adjusted that only enough gas can leak back to reduce the pressure in the reservoir chamber (*R*) to 60 mm. Hg before the next pulsation brings it again to 120 mm.

By varying the maximum pressure in the gas tube line (*G*), and the size of the constricted opening (*C*), bypassing the one-way valve (*V*), the maximum and minimum pressures exerted on the organ can be set at any amount desired.

For the purpose of explanation of the operating principle, the organ chamber (*O*) may be considered as always under a pressure of approximately 0 mm. Hg. The reservoir chamber (*R*) has been adjusted to a pulsating pressure varying from 60 mm. minimum to 120 mm. maximum. Consequently, the equalization chamber (*E*) is under 120 mm. pressure when the reservoir chamber (*R*) is under 120 mm.; and is under approximately 0 mm. when the reservoir chamber (*R*) is

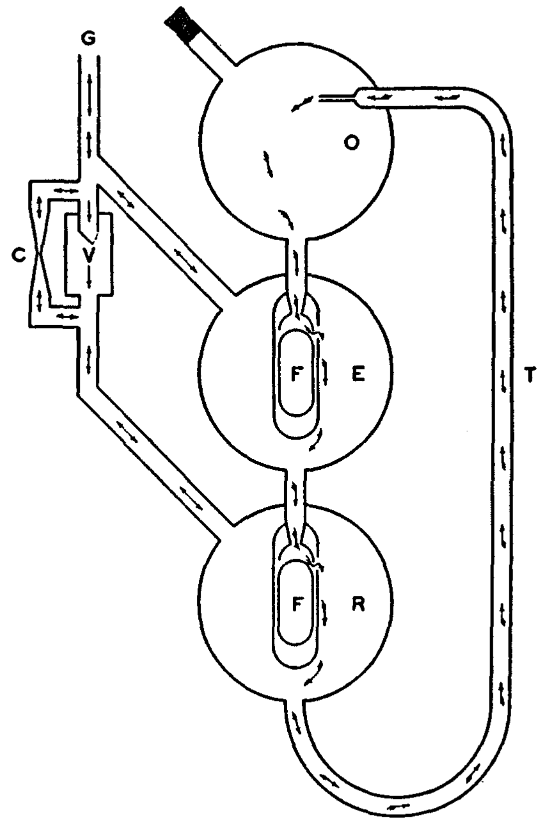


CHART 1. Working diagram of pulsating perfusion pump.

under 60 mm. In other words, during one portion of each pulsation the pressure in the equalization chamber (*E*) equals the pressure in the reservoir chamber (*R*). During another portion of the pulsation the pressure in the equalization chamber (*E*) equals the pressure in the organ chamber (*O*). The artery of the perfused organ is attached to a cannula leading from the reservoir chamber (*R*), and consequently is always approximately under the pressure of the reservoir chamber (60 mm. to 120 mm. Hg).

After the perfusion fluid passes through the organ it flows out into the organ chamber (*O*) at approximately 0 mm. pressure. When the pressure of the equalization chamber (*E*) is approximately 0 mm., the fluid in the organ chamber (*O*) flows past a one-way floating valve (*F*) and into the equalization chamber (*E*). When the pressure in the equalization chamber (*E*) is 120 mm., the fluid flows past a second one-way floating valve (*F*) and into the reservoir chamber (*R*), thereby completing the cycle.

In actual operation, there is a back pressure in the organ chamber (*O*). Also, the lowest pressure in the gas line is always above 0 mm. This is due to the rapidity of pulsation, pressure of fluid columns, floating valves, etc.; and must be taken into consideration in adjusting the relative internal and external pressures on the organ.

Detailed Principles of Operation

Pulsation Rate.—The pulsation rate is controlled by the speed of rotation of the pulsation valve (35). This valve is operated by an electric motor (34) which is geared down, and the speed of which can be controlled by a rheostat (33). There are two complete pulsation cycles per revolution of the valve.

Pressure during Pulsation Cycle.—The rate at which maximum pressure is reached and released, is controlled by the relative size of the slots in the pulsation valve rotor (62). Decreasing the length of the pressure slots (64) causes the maximum pressure to be reached more quickly. Control of pressure during the entire pulse cycle may be obtained by varying the length and width of the pressure and release slots in the rotor. Adjustments of this kind can be made only by stopping the apparatus and changing rotors. A relatively long release slot reduces the back pressure in the organ chamber, and a short release slot increases it.

Sterility.—Sterility in operation is maintained by passing the pulsating gas, which enters the perfusion pump, through two glass bulbs filled with non-absorbent cotton (12, 22). Two more glass bulbs filled with non-absorbent cotton (1, 2) protect the gas inlet and outlet to the organ chamber. No part of the apparatus beyond these filter bulbs is kept sterile. The filter bulbs and all parts of the apparatus on the organ side of them, are sterilized with heat.

After the perfusion fluid and the organ to be perfused have been aseptically inserted in the apparatus, all openings are closed with rubber stoppers, through which have been inserted glass tubes leading to the cotton filter bulbs. All joints between rubber and glass on the sterile side of the filter bulbs are sealed with waterproof cement. This leaves the only communication to the exterior protected by four cotton filter bulbs. All filter bulbs are placed in an approximately vertical position with the sterile opening high, to prevent the possibility of condensation water carrying infection through the filter.

Temperature.—A constant and controllable temperature is maintained by keeping the major portion of the apparatus in an incubator. The pulsating air enters the incubator immediately after leaving the pulsation valve (35) and passes through a coil of copper tubing (38) to an oil flask (40). The copper coil (38) has a capacity greater than the volume of air moving back and forth through it during pulsation. Consequently, its narrow diameter prevents the rapid mixing of cool air from the pulsation valve (35) with the warm air from the oil flask (40). The control gas is brought up to incubator temperature as it passes through the tubing leading to the oil flask (40).

Control of Gas in Contact with Perfusion Fluid.—An oil flask (40) is used to separate the pulsating air, which actuates the apparatus, from the controlled gas which is in contact with the perfusion fluid and the organ. This flask (40) is made of one piece of pyrex glass with three openings to the outside (23, 24, 26). The pulsating air from the pulsation valve (35) passes through the lower opening (26) to the outer chamber (27). The pulsating control gas to the perfusion pump (49) passes through the middle opening (24) from the inner chamber (25). The fresh control gas from the steel gas cylinder (58) passes through the upper opening (23) to the inner chamber (25).

In operation, the pulsating pressure of the air from the pulsation valve (35) is transmitted through the oil to the control gas. Air is always in contact with the oil surface in the outer chamber (27), and control gas always in contact with the oil surface in the inner chamber (25). Fresh control gas passes through the opening (23) on top of the inner oil chamber (25). Its rate of flow is regulated by a Hoffman clamp (39) on the rubber gas line leading from the gas cylinder (58). The replaced gas bubbles out into the outer chamber (27) during the pressure release portion of the pulsation cycle and is carried away with the released air. In this way, the composition of the gas in contact with the perfusion fluid is kept constant. All surfaces of the fluid are in contact with controlled gas.

Prevention of Evaporation.—Evaporation of the perfusion fluid is prevented by maintaining the temperature of the oil flask (40), and the oil check valve and one-way valve assembly (41, 42, 43, 44, 45, 46), slightly higher than that of the perfusion pumps (49). In consequence, there is no movement of moisture away from the pumps, and whatever condensation there is takes place within the perfusion pumps. If this becomes appreciable, it runs down the walls and back into the perfusion fluid.

Filming.—Filming of the perfusion fluid is prevented by avoiding any bubbling of gas through the fluid, any spraying of the fluid, or any dropping of the fluid during its circulation. The perfusion pump is so constructed that the fluid always follows and adheres to a glass surface.

Measurement of Rate of Flow.—Rate of flow is measured by stopping the return circulation from the organ chamber (4), and opening the organ chamber to atmospheric pressure. Either the rate of rise of fluid in the organ chamber (4), or the rate of fall in the reservoir chamber (18) can be timed.

Automatic Adjustment for Changes in Rate of Flow.—The perfusion fluid in the cannula (3) leading to the organ is under a constant pulsating pressure due to the compression and release of the control gas. The pressure exerted by this control gas is practically unaffected by the rate of flow through the organ. Consequently, as long as the capacity of the cannula, which is inserted in the artery of the organ, is materially greater than the rate of flow through the organ, the pressure exerted by the perfusion fluid is practically constant, regardless of changes in rate of flow through the organ.

Filtration of the Perfusion Fluid.—Two 150 mesh platinum screens (21) are located in the glass feed tube (20) which carries the perfusion fluid from the reservoir chamber to the organ. These screens are located at the top of the tube, and filter the fluid shortly before it enters the organ. After passing through the organ, the perfusion fluid is again filtered in passing through silica sand (6), held between two 52 mesh platinum screens (5).

Separation of Organ and Perfusion Fluid from Non-Sterile Substance by One Piece of Glass and Sterile Control Gas.—The perfusion pump is made of a single piece of glass except for the filters and floating glass valves (5, 6, 21, 9, 15). There are three openings to the outside. One of these is the mouth of the organ chamber (4); one is the neck (11) of the equalizing chamber (13); the third is the neck of the reservoir chamber (18). During operation the perfusion fluid does not come in contact with any of these openings. Consequently, the organ and perfusion fluid are separated by solid glass walls and sterile gas from all infected surfaces. Contact between the fluid and the outside is made only through the pulsating sterile control gas.

Simplicity in Sterilization.—The organ, equalization, and reservoir chambers (4, 13, 18) are separated from other parts of the apparatus by cotton filter bulbs (1, 2, 12, 22). No part of the apparatus beyond these filter bulbs is kept sterile.

Aseptic Installation and Removal of Organs.—The organ chamber (4) is placed at an angle approaching horizontal to reduce the mouth opening exposed to the air. The mouth is long enough to be flamed without injury to the organ and is sealed by a rubber stopper.

Aseptic Installation and Removal of Perfusion Fluid.—The necks to the reservoir and equalization chambers (18, 13) are of small diameter and can be flamed. A pipette can be inserted, through the neck of the reservoir chamber (18) to the bottom of the chamber, for removing the perfusion fluid.

Description of Construction and Operation

Perfusion Pump.—The reservoir chamber (18) is under a pulsating pressure due to the compression and release of the control gas passing through the cotton filter bulb (22). Consequently, the perfusion fluid from the reservoir chamber (18) passes into the mouth of the feed tube (19), and through the feed tube (20). While passing through the feed tube (20) the perfusion fluid is filtered by two 150 mesh 0.0016 wire platinum screens (21). The fluid then passes through the cannula (3) to the organ. It exerts a pressure on the organ which is approximately equal to the pulsating pressure in the reservoir chamber (18), less the back pressure of the column in the feed tube (20) and the back pressure in the organ chamber (4). After passing through the organ, the fluid arrives in the organ chamber (4), which is under a pressure of approximately 20 mm. Hg (depending on pulsation rate,

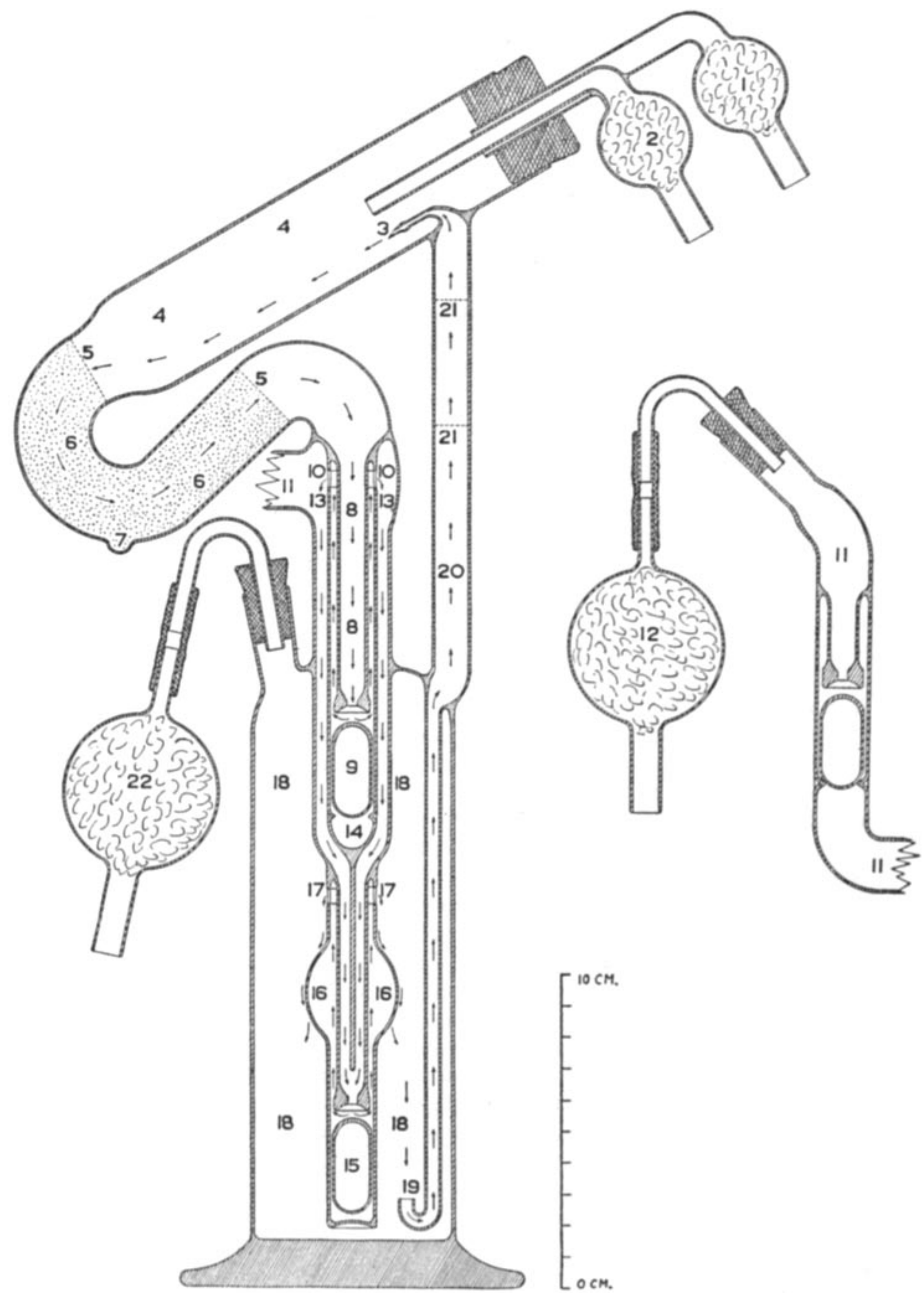


CHART 2. Cross-section of pulsating perfusion pump, (1–22).

pulsation pressures, diameter and lengths of tubing, depth of oil flask outer reservoir, volume of pulsating gas, etc.). When the pump is in operation, the organ chamber filter bulbs (1, 2) are closed. Consequently, the organ chamber is sealed gas tight.

During the portion of each pulsation in which the upper floating valve (9) is closed, the perfusion fluid collects in the organ chamber after passing through the organ. During the portion of each pulsation in which the upper floating valve (9) is open, the fluid from the organ chamber passes (partly due to gravity and partly to the added pressure its volume has created in the organ chamber) through the 52 mesh 0.004 wire platinum screen (5), through the silica sand filter (6), through the second platinum 52 mesh screen (5), down through the inner tube (8), past the upper floating valve (9) seat, up the floating valve reservoir (14), through the holes (10) at the top of the floating valve reservoir (14), and into the equalization chamber (13).

The perfusion fluid remains in the equalization chamber (13) during the portion of each pulsation cycle in which the lower floating valve (15) is closed. During the portion of each pulsation cycle in which the lower floating valve (15) is open, the fluid in the equalization chamber flows (partly due to gravity and partly to the relative rate of pressure equalization in the reservoir and equalization chambers) down past the lower floating valve (15) seat, up through the floating valve reservoir (16), through the holes (17) at the top of the floating valve reservoir (16), and into the main reservoir chamber (18), thereby completing the cycle of circulation.

The opening and closing of the floating valves (9, 15) is regulated by the pressure in the equalization chamber (13), relative to the pressure in the reservoir chamber (18) and the organ chamber (4). When the pressure in the equalization chamber is minimum, and approximately equal to the pressure in the organ chamber, the upper floating valve (9) is open and the lower floating valve (15) is closed. When the pressure in the equalization chamber is maximum, and approximately equal to the pressure in the reservoir chamber, the lower floating valve (15) is open and the upper floating valve (9) is closed.

The pulsating gas passes back and forth through the filter bulbs (22, 12) leading to the reservoir and equalization chambers (18, 13). It exerts its maximum pressure on both chambers (18, 13) almost equally. Its minimum pressure is exerted only on the equalization chamber (13) because the outflow from the reservoir chamber (18) is restricted by a one-way gas valve (44) and adjustable constricted bypass (45). Consequently, under normal operation, the pressure in the reservoir chamber (18) never drops as low as the pressure in the equalization chamber (13).

The rate of flow of pulsating gas, in and out of the equalization chamber (13), is adjusted by means of a Hoffman clamp (48) placed on the rubber tube gas line leading to the equalization chamber filter bulb (12). This adjustment is necessary because of the small gas capacity of the equalization chamber (13) in relation to the gas capacity of the reservoir chamber (18). Without adjustment, the pulsating gas fills the equalization chamber more quickly, and raises its pressure enough above that in the reservoir chamber to push gas past the floating valve (15), separating the two chambers (13, 18), and cause bubbling and filming in the reservoir chamber (18).

The floating valve in the equalization chamber neck (11) is for the purpose of stopping an overflow of the perfusion fluid if the lower floating valve (15), separating the equalization (13) and reservoir (18) chambers, becomes jammed due to the consistency of the perfusion fluid, or to infection, or to a fragment of degenerated tissue. When the fluid level in the equalization chamber (13) reaches and closes the floating valve in the equalization chamber neck (11), the entire pressure difference between the minimum pressure in the reservoir chamber (18) and the maximum pressure in the equalization chamber (13), plus the weight of the fluid column in the equalization chamber and neck (13, 11), is exerted on the jammed lower valve (15).

The filter bulbs (1, 2), leading to the organ chamber, are to permit the sterile gassing of the chamber with control gas. The organ chamber is of tubular construction for simplicity, and to permit sealing the mouth with a rubber stopper. It is set at an angle of about 30 degrees with the horizontal to maintain a slight tension on the artery leading to the organ, to prevent the perfusion fluid from touching the rubber stopper, and to simplify flaming and the aseptic installation of the organ. The large size of all external glass tubing is for strength of construction.

The open end of the equalization chamber neck (11) is set at an angle which permits the introduction of perfusion fluid and still minimizes the possibility of infection. The neck of the reservoir chamber (18) is set at an angle which permits the insertion of a pipette to the bottom of the chamber for the purpose of withdrawing the perfusion fluid. The filter bulbs leading to the equalization and reservoir chambers are flexibly connected by rubber tubing to lessen the possibility of breakage in handling. The floating valves are ground with a round, rather than a flat, seat. The guide tubes, in which they operate, and which form a reservoir for the perfusion fluid in which they float, have an inside diameter of 1 mm. to 1.2 mm. greater than the outside diameter of the floating valve. Too little clearance imprisons gas below the valve and makes the action sluggish. Too great clearance or a flat seat prevents proper closing.

The aperture in the valve seat is about 3 mm. The ground portion of the seat is of sufficient area to prevent leakage. The holes at the top of the floating valve reservoir (10, 17) are large (about 5 mm. diameter) to prevent spurting of the perfusion fluid. There are four holes at the top of each reservoir.

The clearance, between the outside of the valve reservoir tube (14) and the inside of the equalization chamber (13), is sufficient to prevent the fluid, which passes through the holes (10) at the top of the valve reservoir (14), from touching the inner wall of the equalization chamber (13). The perfusion fluid should run down the outer surface of the valve reservoir tube (14). If it touches the inner surface of the equalization chamber (13), filming may occur. A solid glass rod at the bottom of the floating valve reservoir (14) guides the fluid to the lower

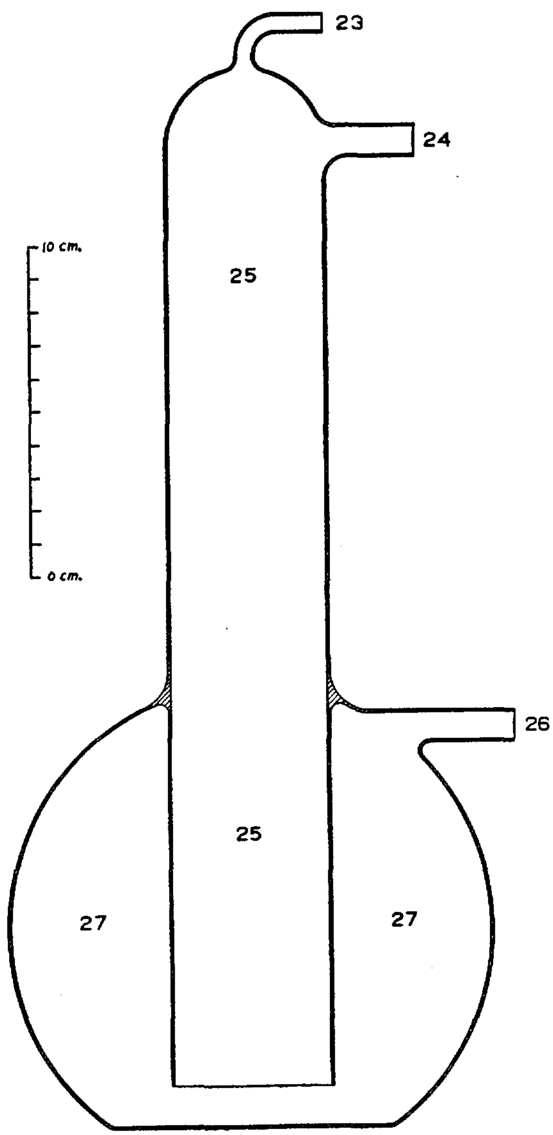


CHART 3. Cross-section of oil flask, (23–27).

portion of the equalization chamber, and prevents filming if the fluid level in the equalization chamber (13) is too low due to improper adjustment.

The lower floating valve reservoir tube (16) is blown out to a wider diameter near the middle. This is to prevent the rapid emptying of the tube if the valve does not always seat perfectly, and to keep all the fluid from being blown out of the floating valve reservoir while the rate of flow of pulsating gas to the equalization chamber is being adjusted. The lower position of the lower floating valve (15) is governed by making the end of the valve reservoir tube (16) concave. The lower position of the other two floating valves is governed by three indentations made around the glass guide tubing. From 3 to 4 mm. is sufficient range of valve travel. The inlet (19) of the feed tube (20) is bent upward, to prevent the loss of perfusion fluid from the feed tube (20) while the fluid in the reservoir chamber is being changed.

Crushed quartz sand is used in the filter (6). The sand must be sufficiently coarse to prevent its passing through the 52 mesh platinum screens (5). All dust and small particles of sand are thoroughly washed out before it is placed in the pump. Then the glass tube (7), through which the sand is inserted, is sealed.

Oil Flask.—The oil flask (40) is made of pyrex glass and contains two chambers (25, 27). The outer chamber (27) is partially filled with oil (liquid petrolatum U.S.P.). Air passing the pulsation valve (35) enters the outer chamber (27), and creates a pressure in the outer chamber which forces the oil into the inner chamber (25), thereby compressing the control gas in the inner chamber and transmitting pressure from the air pressure line to the perfusion pumps (49). When the pulsation valve (35) is in the release position, the compressed control gas in the inner chamber (25) forces the oil back into the outer chamber (27), thereby completing the pulsation cycle. Fresh control gas enters the top opening of the inner chamber (23). The replaced control gas bubbles from the inner chamber into the outer chamber during the release portion of the pulsation cycle, and is carried away through the release outlet (65) of the pulsation valve (35). The oil flask should be filled to a level which will not cause overflow from the outer chamber (27) when gas is bubbling from the inner chamber (25) into the outer chamber.

Pulsation Valve.—The pulsation valve (35) consists of a steel rotor (62) turning inside of a stationary bronze cylinder (60). During one portion of rotation the valve permits air from the air pressure line to pass into the oil flask outer chamber (27). During another portion of rotation, it releases the air from the outer chamber (27) of the oil flask. The valve is so constructed that each rotation causes two complete pulsation cycles. The pressure during the pulsation cycle may be governed by changing the lengths and widths of the slots (63, 64), milled in the steel rotor (62). For example, shortening the pressure slots (64) causes the maximum pressure to be reached more quickly and reduces the average pressure of the cycle.

Oil Check Valve.—The oil check valve (41) contains a sealed glass cylinder (59) with a slender taper at one end. This cylinder (59) is held in a second glass cylinder (41), of a diameter sufficient to permit the free flow of pulsating gas

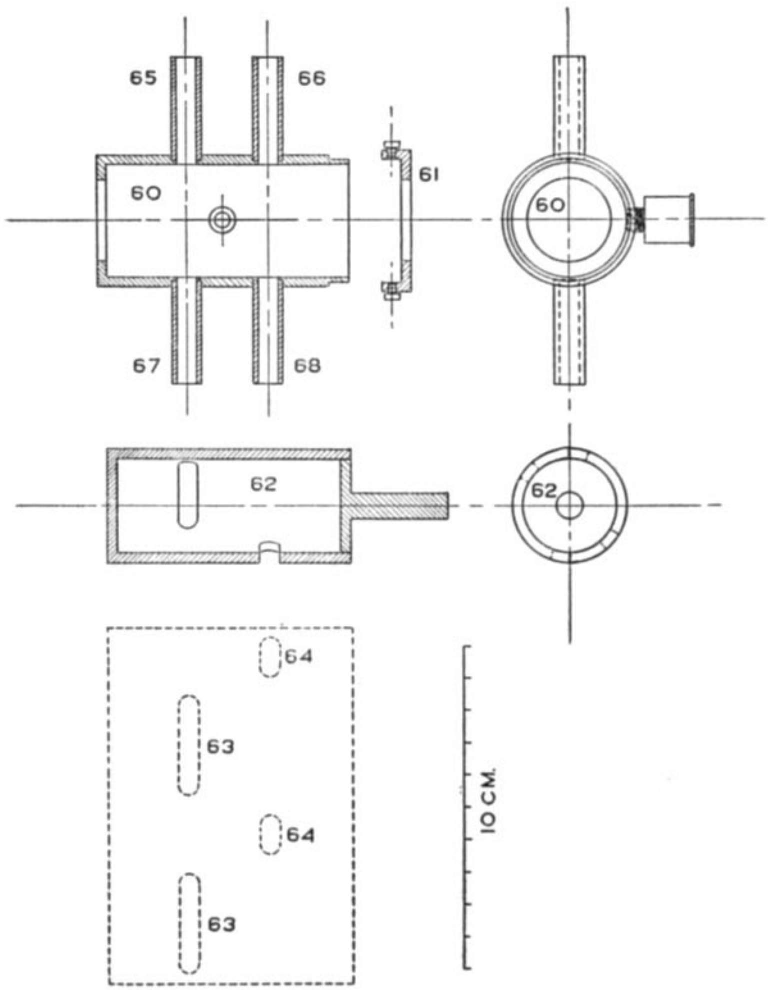


CHART 4. Pulsation valve, (60–68).

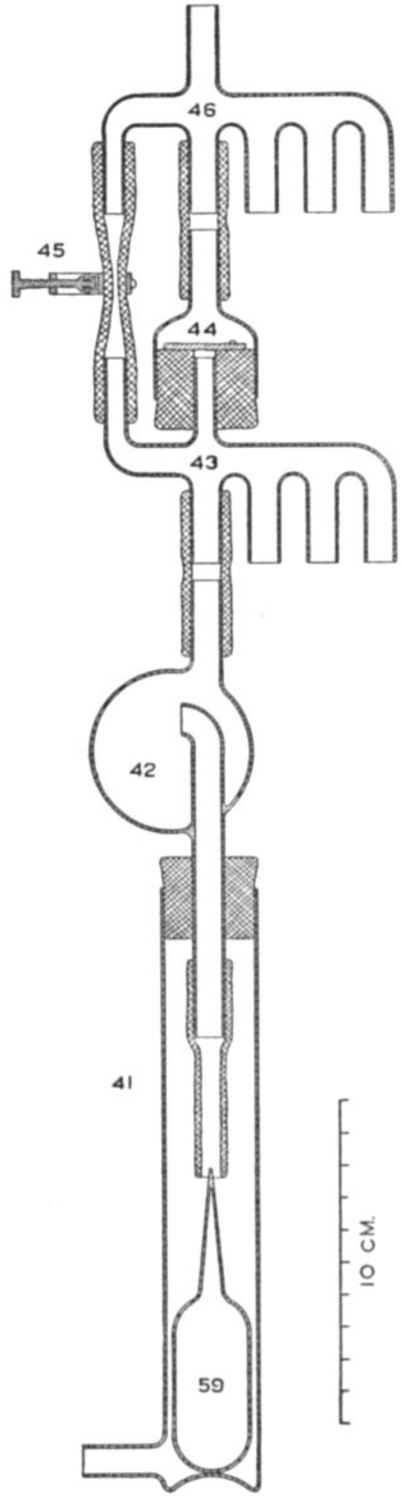


CHART 5. Cross-section of oil check valve, oil trap, and one-way gas valve assembly, (41–46, 59).

between the walls of the two cylinders (41, 59). The top of the outer cylinder (41) is closed with a rubber stopper, through the center of which runs a glass tube. A short length of rubber tubing is connected to the inside end of this glass tube. It is so adjusted that, when the rubber stopper is in place, the tapered end of the sealed inner cylinder (59) enters the end of the rubber tubing about 5 mm. The tapered end of the sealed inner cylinder (59) is covered with vaseline before assembling. The lower end of the outer cylinder (41) is connected by rubber tubing to the middle opening of the oil flask (24). The upper end of the outer cylinder (41) is connected directly to the oil trap (42).

The pulsating gas passes freely back and forth through the oil check valve (41); but if oil overflows the oil flask inner chamber (25), it flows into the bottom opening of the oil check valve (41), and floats the inner cylinder (59) until the tapered end closes the rubber tubing in which it is inserted, thereby preventing the oil from passing farther into the system.

Oil Trap.—The oil trap (42) is made of glass and is directly above the oil check valve (41). It is for the purpose of preventing any leakage of oil, past the oil check valve (41), from passing farther into the system.

One-Way Gas Valve.—The one-way gas valve (44) is made of glass, rubber, and metal pins. A hole (about 6 mm. diameter) is drilled through the center of a rubber stopper. A rubber flap is placed over this hole on the inside of the stopper and pinned at one edge. The rubber stopper is then inserted in the large end of the glass tube (44). This forms a one-way valve which permits the pulsating gas to pass in one direction only.

Assembly of Apparatus

The incubator used should have the heating element under the floor. It should have an inner glass door to permit observation of the interior without cooling the apparatus.

The perfusion pumps (49) should be placed in the front part of the incubator with the organ chamber mouths pointing toward the rear. They should be placed on a platform which thoroughly insulates them from the hot floor of the incubator. A sheet of asbestos on legs, which raise it about 3 cm. above the incubator floor, is satisfactory. This stand should be narrow enough so that the oil flask (40) can be placed directly on the hot floor of the incubator near the rear. The oil check valve and one-way valve assembly (41, 42, 43, 44, 45, 46) should be hung in a vertical position on the side wall of the incubator and near the rear. It should be placed to minimize the length of rubber tubing required to connect the oil check valve (41) to the oil flask (40). It is desirable to minimize the length of tubing used wherever possible throughout the apparatus.

The coil of copper tubing (38) should be placed directly on the hot floor of the incubator, next to the side or rear wall, and immediately adjoining the oil flask (40). This coil (38) should have a minimum inside diameter of 10 cm. The tubing should have a minimum inside diameter of 8 mm. The capacity of the coil must be greater than the volume of gas which pulsates back and forth through

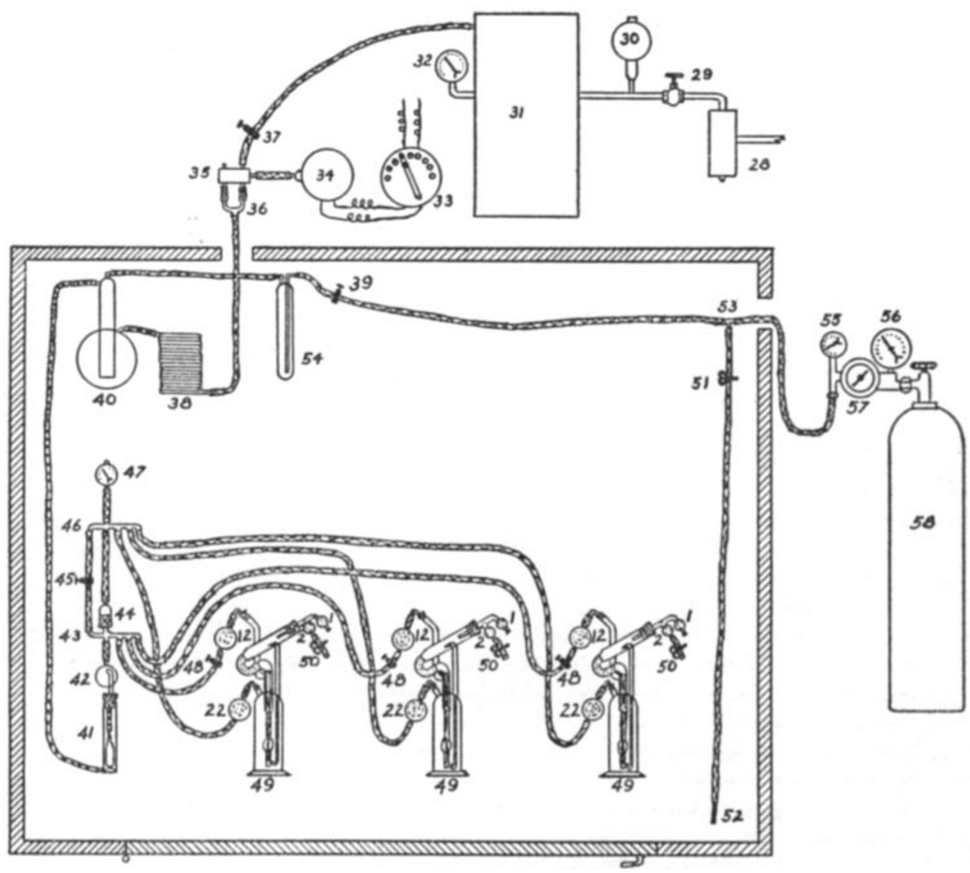


CHART 6. Assembly sketch, (1, 2, 12, 22, 28–58).

it. It is essential that the perfusion pumps (49) be placed in the coolest part of the incubator, so that the temperature of the oil flask (40) and the oil check valve and one-way valve assembly (41, 42, 43, 44, 45, 46) be higher than that of the pumps (49).

The pressure gauge (47) may be placed near the front of the incubator and connected with a longer tube, if desired. The bottom of the copper coil (38) is connected by rubber tubing to a glass Y (36), which is connected by two rubber tubes to the pressure outlet (68) and relief inlet (67) of the pulsation valve (35). The pulsation valve (35) should be placed close to the outside wall of the incubator, opposite the copper coil (38). A hole is drilled through the wall for the connecting tubing.

The pulsation valve (35) is connected by a flexible shaft to a 1/24 h.p. electric motor (34), which is geared down. The motor (34) is controlled by a rheostat (33) and so geared that the desired range of pulsation rate can be obtained. There are two complete pulsation cycles with each revolution of the pulsation valve (35).

The relief outlet (65) of the pulsation valve (35) can be left open or carried into a muffler, if desired. A satisfactory muffler can be made by coating the inside of a glass jar with the same cement used for sealing the corks on the perfusion pumps, and then lining the jar with non-absorbent cotton. If a muffler is used, care must be taken not to create excessive back pressure on the relief outlet (65) of the pulsation valve (35).

The pressure inlet (66) of the pulsation valve (35) is connected to an air reservoir tank (31). This tank should have sufficient capacity to avoid more than 1 cm. Hg change in pressure during each pulsation. A pressure gauge (32) is connected to the air reservoir tank (31). A pressure relief valve (30) is connected to the air line leading to the air reservoir tank (31). This relief valve should have sufficient capacity to minimize any changes of pressure in the air pressure line or changes of flow through the pulsation valve. A needle valve (29) is located on the air pressure line beyond this relief valve (30). Beyond the needle valve (29) is located a dirt and water trap (28) which contains a copper screen in the top, and a drain plug in the bottom. This trap is connected to the outside air pressure line.

The oil check valve and one-way valve assembly (41, 42, 43, 44, 45, 46) must be placed in a vertical position. When three perfusion pumps (49) are run on one system, a six-way glass fitting (43) is placed above the oil trap (42). One tube connects this fitting (43) to the oil trap (42); a second tube connects it to the one-way valve (44); a third, to the constricted opening (45) which bypasses the one-way valve (44). The remaining three tubes lead to the equalization chamber filter bulbs (12) of the three perfusion pumps (49).

A second six-way glass fitting (46) is placed above the one-way valve (44). One tube connects this fitting (46) to the one-way valve (44); a second tube connects it to the constricted opening (45); a third, to the pressure gauge (47). The remaining three tubes lead to the reservoir chamber filter bulbs (22) of the three perfusion pumps (49).

The one-way gas valve (44) is placed between the two six-way glass fittings (43, 46), and must be in a vertical position. The direction of flow is upward. The constricted opening (45) is formed and adjusted by a Hoffman clamp placed on a rubber tube which bypasses the one-way valve (44). Hoffman clamps (48) are placed on each of the rubber tubes leading from the lower six-way fitting (43) to the equalization chamber filter bulbs (12) of the perfusion pumps (49). These clamps (48) should be placed at a high point in the line, either near the filter bulb (12) or near the six-way fitting (43). This is to avoid possible interference by condensation water.

Controlled gas is led into the incubator from a steel gas cylinder (58). A gas pressure regulator valve (57) is used to reduce the pressure at the cylinder outlet. The two gauges (55, 56) attached to this gas regulator, indicate the pressure in the cylinder and the pressure at the gas outlet. A rubber tube leads from the gas outlet into the incubator to a glass T (53). From this T (53) one tube leads to a saturation flask (54) where the gas is bubbled through water. From the saturation flask (54) a rubber tube leads to the upper opening of the oil flask inner chamber (23). A Hoffman clamp (39) is placed next to the saturation flask (54) on the gas inlet rubber tube. A second rubber tube from the glass T (53) leads to a capillary glass tube (52) (about ½ mm. opening and 10 cm. long). This rubber tube is long enough to permit the capillary glass tube (52) to be inserted in the short rubber tube which is connected to the inner filter bulb (2) of the organ chamber (4) of the most distant perfusion pump (49). When not in use, it is closed by a Day pinch-cock (51).

The air reservoir tank (31) is connected to the outside air pressure line by ½ inch metal pipes and fittings. The rubber tubing used between the air reservoir tank (31) and the oil check valve (41) has a minimum inside diameter of 7 mm. and an outside diameter of 14 mm. The rubber tube inside the oil check valve (41) has an inside diameter of 6 mm. and an outside diameter of 10 mm. The rubber tubes, connecting the oil trap (42) to the lower six-way glass fitting (43), and connecting the one-way gas valve (44) to the upper six-way glass fitting (46), have an inside diameter of 7 mm. and an outside diameter of 14 mm. The rubber tube which bypasses the one-way gas valve (44), and which is used to form the constricted opening (45), has an inside diameter of 4 mm. and an outside diameter of 13 mm. All rubber tubes leading from the two six-way glass fittings (43, 46) to the perfusion pumps (49) have an inside diameter of 6 mm. and an outside diameter of 10 mm. Similar tubing is used on the inner filter bulbs (2) of the perfusion pumps (49), and to connect the upper six-way fitting (46) to the pressure gauge (47). The rubber tubing, between the control gas cylinder (58) and the oil flask (40) and capillary glass tube (52), has an inside diameter of 3 mm. and an outside diameter of 7 mm.

It is desirable to have all rubber tubing elastic enough to simplify making the connections between rubber and glass. However, it should not be so elastic that its diameter changes appreciably with the pulsations. Too small a diameter of the tubing, carrying the pulsating gas, will cause high back pressure and inefficient operation of the apparatus. Wherever practicable the glass tubing used in the pulsation line has an inside diameter of at least 8 mm.

Operating Directions[3]

Washing Perfusion Pump.—Rinse the pump with water. Place a rubber stopper, with a glass tube through its center, in the mouth of the organ chamber. Attach a rubber hose to the glass tube, and force water, under low pressure, through the sand filter (6) and cannula (3). Pour the excess water out of the pump and fill with chromic and sulfuric acid. The reservoir chamber neck must be closed with a rubber stopper when it is full, and then the upper portions of the pump filled with acid. The acid can be distributed through the sand filter more quickly by filling the organ chamber and closing it with a cork, which has a glass tube through the center. A light air pressure on this glass tube will force the acid quickly through the sand. After the acid has been in the pump for about 2 hours, pour it out and rinse the pump with water. All washing water, run through the pump after the acid has been removed, must be thoroughly filtered. After rinsing, place the rubber stopper, containing a glass tube through its center, in the mouth of the organ chamber; and pass water under low pressure through the pump for about 1 hour. It is essential that sufficient time be given for all organic material to be removed by the acid, and for all acid to be removed by the washing water. Pour out the water and dry the pump by vacuum. The equalization chamber neck should be partially closed by a cork with a small hole through its center. The vacuum hose is connected to the neck of the reservoir chamber. Drying should continue until the moisture is removed from all parts, including the sand filter. Care must be taken that the vacuum used is not strong enough to cause the floating valves to chatter. They chatter more easily when dry than when wet. Consequently, a negative pressure which does not cause chatter at first, may cause destructive chatter as the valves become dry. After the pump is dry, it is sterilized in dry heat. The glass filter bulbs, which are lightly packed with non-absorbent cotton, and which are attached to rubber, are sterilized in steam heat.

Preparing for Organ Installation and Sealing Pump after Installation.—After installation of the perfusion fluid, and prior to installation of the organ, a rubber tube is attached to the reservoir chamber filter bulb (22); and enough air pressure placed in the reservoir chamber (18) to force perfusion fluid through the cannula (3). Sufficient fluid is blown into the organ chamber (4) to wet the sand filter (6) and fill the upper floating valve reservoir (14). Then while perfusion fluid is still passing through the cannula (3), a pinch-cock is placed on the rubber tube connected to the reservoir chamber filter bulb (22). This holds the perfusion fluid level even with the mouth of the cannula (3), while the organ is being in-

[3] Great assistance has been received from J. Zwick in developing the technique connected with the operation of this apparatus.

stalled. After the organ has been installed in the pump, a rubber stopper, containing tubes leading to the filter bulbs (1, 2), is placed in the organ chamber mouth. All stoppers and joints between glass and rubber on the sterile side of the filter bulbs are sealed with waterproof cement (DuPont clear dope No. 5332).

Starting Apparatus Preparatory to Installing Perfusion Pump.—Be sure that pinch-cocks are on all pump gas line tubes. Turn on the gas cylinder (58) valve and adjust the gas regulator to about 8 pounds outlet pressure. Open the clamp (39) which adjusts the flow of control gas into the oil flask inner chamber (25), until gas starts to bubble out into the outer chamber of the oil flask (27). Then close the clamp (39). If the oil level in the inner chamber of the oil flask (25) is too high when pulsation is started, the oil will overflow and close the oil check valve. Make sure that the clamp (37), on the air line leading to the pulsation valve (35), is closed. Set the air release valve (30) to release at about 8 pounds pressure, as indicated on the air reservoir tank gauge (32). Adjust the air line needle valve (29) until there is an appreciable leak through the release valve (30). Switch on the pulsation valve motor (34) and adjust the rheostat (33) to the desired pulsation rate. Open the clamp (37), on the air line leading to the pulsation valve (35), until the pressure gauge (47) indicates the maximum pressure desired in the reservoir chamber (18). Adjust the clamp (39), controlling the flow of control gas to the oil flask inner chamber (25), until the desired flow is obtained as indicated by the rate of bubbling through the saturation flask (54). Shut the clamp on the pressure gauge (47) gas line (to save wear on the gauge and to permit its removal and use on other systems).

To Install Perfusion Pump in Incubator.—Place the short rubber tube on the inner organ chamber filter bulb (2). Seal with waterproof cement. Place the pump in the incubator with the organ chamber mouth to the rear. Close the clamp (39) on the rubber gas line leading from the steel gas cylinder (58) to the oil flask inner chamber (25). Connect the capillary glass tube (52) to the rubber tube on the organ chamber inner filter bulb (2); and allow gas to pass through the organ chamber by removing the pinch-cock (51) from the capillary tube (52) gas line. After 2 or 3 minutes replace the pinch-cock (51) on the capillary tube gas line and disconnect the capillary glass tube from the organ chamber filter bulb rubber tube. Close the organ chamber inner filter bulb (2) rubber tube with a pinch-cock (50). Close the organ chamber outer filter bulb (1) with a rubber stopper. Seal with waterproof cement. Connect the gas tube from the six-way glass fitting (46), above the one-way valve (44), to the reservoir chamber filter bulb (22). Connect the gas tube from the six-way glass fitting (43), below the one-way valve (44), to the equalization chamber filter bulb (12). Connection of tubing to filter bulbs is simplified if the glass is moistened with water. Remove the pinch-cock from the gas line leading to the reservoir chamber (18). Remove the pinch-cock from the gas line leading to the equalization chamber (13). Make sure there is a slight leak past the air line relief valve (30). Set the maximum pressure by adjusting the clamp (37) on the air line leading to the pulsation valve (35). Set the minimum pressure by adjusting the clamp (45) which controls the constricted opening bypassing the one-way valve (44). Set the fluid level in the equalization chamber (13) by adjusting the clamp (48) on the gas line leading to the equalization chamber filter bulb (12). This level should be from 5 to 8 cm. above the holes (17) at the top of the lower floating valve reservoir (16). If the fluid level in the organ chamber is too high, insert the capillary glass tube (52) in the organ chamber inner filter bulb rubber tube. Remove the pinch-cock (50) from the inner filter bulb rubber tube, and allow gas to pass into the organ chamber by quickly opening and closing the pinch-cock (51) on the capillary tube (52) gas line, until the fluid level is satisfactory. Then replace the filter bulb pinch-cock (50) and disconnect. Readjust the clamp (39) on the rubber gas line leading from the gas cylinder (58) to the oil flask inner chamber (25).

The clamp (39) on the control gas line, leading from the gas cylinder (58) to the oil flask inner chamber (25), must be closed before gassing the organ chamber. Otherwise the fall in pressure in the control gas line during gassing would permit the escape of gas from the oil flask inner chamber (25), and consequent overflow of oil and closing of the oil check valve (41).

The pinch-cock on the reservoir chamber (18) gas line is removed before that on the equalization chamber (13) gas line, to avoid the violent bubbling which occurs if the reverse procedure is followed.

A slight leak past the air line release valve (30) is desirable to compensate for possible variations of pressure in the air line.

When minimum pressure is adjusted it is usually necessary to readjust the maximum pressure. A satisfactory procedure is, first, to set the maximum pressure; then to set the minimum pressure to the desired pressure difference between maximum and minimum, rather than to the actual minimum desired; then to make a final adjustment of maximum pressure. The pressure difference will remain approximately the same with minor adjustments of maximum pressure.

For a time after a pump has been installed, it is desirable to increase the flow of control gas into the oil flask inner chamber (25), in order to replace the air in the pump. After sufficient time has elapsed for the gas composition to become constant, the rate of bubbling may be decreased.

To Remove Perfusion Pump from Incubator.—Place a pinch-cock on the rubber gas line leading to the equalization chamber filter bulb (12). Place a pinch-cock on the rubber gas line leading to the reservoir chamber filter bulb (22). Disconnect the rubber gas line from the reservoir chamber filter bulb (22). If other pulsation pumps are on the system, readjust the pulsation pressure and the fluid level in their equalization chambers. Disconnect the rubber gas line from the equalization chamber filter bulb (12). Remove the pump from the incubator.

A pinch-cock should always be placed on the equalization chamber rubber gas line before one is placed on the reservoir chamber line. The reservoir chamber should be disconnected immediately after the two pinch-cocks are in place, and before the equalization chamber gas line is disconnected. The reverse procedure will cause bubbling.

Rate of Flow Measurement.—Rate of flow through the cannula leading to the organ is measured with the organ chamber open to atmospheric pressure. Therefore, there is no back pressure in the organ chamber, and the effective pressure within the organ rises an amount equal to the previous back pressure in the organ chamber. Consequently, to measure the rate of flow at normal operating pressures, it is necessary to reduce the pressure in the reservoir chamber an amount equal to the back pressure in the organ chamber.

Measure the back pressure in the organ chamber (4) by connecting the pressure gauge to the rubber tube, which is on the organ chamber inner filter bulb (2). Reduce the pressure on the reservoir chamber (18) an amount equal to the back pressure in the organ chamber, by adjusting the clamp (37) on the air line leading to the pulsation valve (35). Place a pinch-cock on the gas line leading to the equalization chamber filter bulb (12). Open the organ chamber to atmospheric pressure by removing the pinch-cock (50) from the rubber tube on the inner filter bulb (2). Readjust the pressure in the reservoir chamber (18). Time either the rate of rise of fluid in the organ chamber or the rate of fall of fluid in the reservoir chamber. If the rate of flow was measured by the fluid rise in the organ chamber, check the fluid level in the upper floating valve reservoir (14). If the rate of flow was measured by the rate of fall in the reservoir chamber, check the fluid level in the lower floating valve reservoir (16). If the fluid level has fallen in the floating valve reservoir, due to leakage past the valve seat, it is necessary to make a corresponding correction in computing the rate of flow through the organ cannula. Replace the pinch-cock (50) on the inner filter bulb (2) rubber tube. Remove the pinch-cock from the rubber tube leading to the equalization chamber filter bulb (12). Readjust the pressure on the reservoir chamber (18) to the original amount. Adjust the level of the fluid in the organ chamber (4) by letting in control gas.

Daily Inspection during Operation.—Check the pulsation pressures on the pressure gauge (47). Check the fluid level in the organ chamber (4). Check the fluid level in the equalization chamber (13). Check the flow of control gas to the oil flask inner chamber (25). Check the oil level in the oil flask inner chamber (25), to be sure the flow of control gas is sufficient to maintain a slow bubbling of gas from the inner to the outer chamber (27) of the oil flask. One bubble in several pulsations is sufficient. Check the amount of gas in gas cylinder (58). Check the outlet pressure on the gas regulator valve outlet gauge (55). Check the air line relief valve (30) pressure. Make sure there is an appreciable air leak past the relief valve (30). Check the reservoir and equalization chamber filter bulbs (12, 22) for condensation water. Condensation water may accumulate in these bulbs if the incubator door is opened frequently. If necessary, flame the bulbs sufficiently to remove the water. Grease the pulsation valve (35) by turning down the pulsation valve grease cup. Keep the pulsation valve motor (34) properly oiled and the reduction gear box packed with grease.

Stopping Apparatus after Removal of Perfusion Pumps.—Shut the clamp (39) on the control gas line leading to the oil flask inner chamber (25). Gradually close the clamp (37) on the air pressure line leading to the pulsation valve (35).

Switch off the pulsation valve motor (34). Close the air pressure line needle valve (29). Turn off the steel gas cylinder (58) valve.

Computation of Reservoir Chamber Pressure.—The pulsating pressure in the reservoir chamber (18) must be set above the pressure desired on the organ, an amount equal to the pressure required to lift the column of perfusion fluid in the feed tube (20) to the cannula (3), plus the back pressure generated in the organ chamber during operation.

Constant Pressure.—A comparatively constant pressure can be exerted on the organ, if desired, by closing the clamp (45) governing the constricted opening which bypasses the one-way gas valve (44).

Floating the Organ.—The organ can be floated in the perfusion fluid by removing the pinch-cock (50) from the rubber tube on the organ chamber inner filter bulb (2), and allowing the fluid level to rise in the organ chamber until the organ is covered, before replacing the pinch-cock (50). A twisted artery can sometimes be straightened out this way.

Stopping Pulsation Valve.—If it is necessary to stop the pulsation valve (35), temporarily, in order to adjust some part of the pulsation system, the following procedure is satisfactory. Gradually shut the clamp (37) on the air pressure line leading to the pulsation valve (35). Switch off the pulsation valve motor (34). Make the adjustment desired. Switch on the pulsation valve motor (34). Gradually open the clamp (37) on the air pressure line leading to the pulsation valve (35), until the desired pressure is reached on the organ. While opening this clamp (37), care must be taken that the oil level in the oil flask inner chamber (25) does not overflow and close the oil check valve (41). If necessary, let additional control gas into the oil flask inner chamber (25) to prevent overflow. Open clamp on the control gas line and let the control gas flow into the oil flask inner chamber (25), until the gas starts to bubble into the oil flask outer chamber (27). Then adjust the flow of control gas to the rate desired.

Filming in Reservoir Chamber.—Bubbling and filming in the main reservoir chamber (18) are caused by lack of proper adjustment of the fluid level in the equalization chamber (13), and may be stopped by raising the fluid in the equalization chamber to the proper level.

Collapse of Artery.—A collapse of the artery, leading from the cannula to the organ, during the release portion of the pulsation cycle, is caused if the clamp (48) is closed on the gas line leading to the equalization chamber (13). Adjustment of the clamp (48) will remedy this situation.

Pulsation Pressures after Installation of Perfusion Pump.—The pulsation pressures will not be properly transmitted to the organ when the pump is first installed in the incubator, unless there is sufficient fluid in the upper floating valve reservoir (14) to float the valve (9). This reservoir (14) should be filled (before the organ is attached to the cannula (3), and after the perfusion fluid has been inserted in the pump) by connecting a rubber tube to the reservoir chamber filter bulb (22), and blowing through the tube until sufficient perfusion fluid has passed through the cannula (3) to wet the sand filter (6) and fill the upper floating valve reservoir (14).

Fluid Rise in Organ Chamber.—If the perfusion fluid level rises in the organ chamber, it is an indication of a leak past one of the glass and rubber connections, or past the pinch-cock (50) closing the inner filter bulb (2) rubber tube. The organ chamber should be opened to atmospheric pressure, and another coat of waterproof cement applied to all joints between glass and rubber. The rubber tube leading to the inner filter bulb (2) should be coated with vaseline on the inner surface where it is closed by the pinch-cock (50).

Overflow of Oil Flask.—Any large leak of the control gas will cause the oil to overflow the oil flask inner chamber (25) and close the oil check valve (41). If this happens, the pulsating pressure in the gas line to the pulsation pumps is shut off and the circulation of perfusion fluid ceases.

The circulation should be started again by the following procedure. Close the clamp (37) on the air pressure line leading to the pulsation valve (35). Open the clamp (39) on the control gas line leading to the oil flask inner chamber (25), until the gas bubbles into the outer chamber (27). Then close the clamp (39). The pulsation valve (35) should be running while the control gas is being let into the inner chamber (25), so that the replaced air in the outer chamber (27) may escape. Disconnect the oil check valve (41) and all parts of the gas line which contain oil. Remove the oil and reconnect. Repair the leak which caused the overflow. If much oil has been lost, refill the oil flask to the proper level. Gradually open the clamp (37) on the air pressure line leading to the pulsation valve (35), until the desired pulsating pressure is reached. Care must be taken that oil does not again overflow the oil flask inner chamber while the clamp (37) is being opened. If the oil level becomes too high in the inner chamber (25), more control gas should be let into the chamber. Adjust the flow of control gas into the oil flask inner chamber (25).

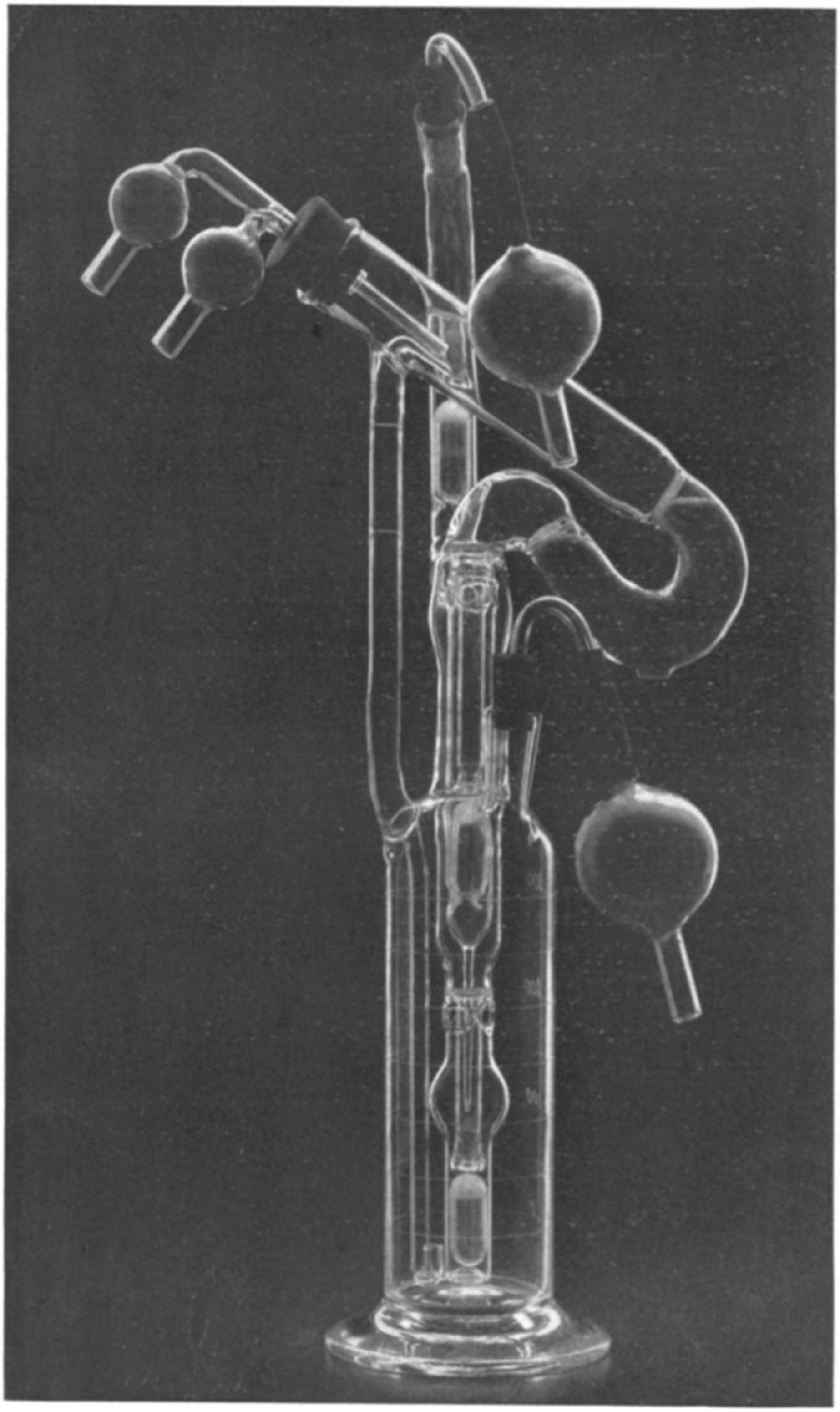
Inspection of Rubber Tubing.—Oil and grease will cause rubber tubing to swell in time. Inspection of rubber tubing should be made at intervals to make sure that it is still strong and that its inner diameter is not obstructed. This is particularly necessary in regard to rubber tubing leading to and from the pulsation valve (35).

SUMMARY

An apparatus has been developed which maintains, under controllable conditions, a pulsating circulation of sterile fluid through organs for a length of time limited only by the changes in the organ and in the perfusion fluid.

EXPLANATION OF PLATE 15

FIG. 1. Perfusion pump.



Photographed by Louis Schmidt and Joseph B. Haulenbeek

FIG. 1

(Lindbergh: Apparatus for culture of whole organs)