A Method of Perfusing an Isolated Rabbit-heart, with Simultaneous Pressure-recordings of the Aorta, Vena Cava, Pulmonary Artery and Pulmonary Vein

Sakuzi Kodama

The First Department of Physiology, Kumamoto University School of Medicine, Kumamoto

For the analytical investigation on an isolated mammalian heart, a method of perfusing the heart was devised, with which the heart could manifest its activities sufficiently as in the body.

The method furnishes the heart with 2 systems, these being connected with each other in series as in the body: One system corresponds to the pulmonary circulation and the other to the systemic circulation. For the perfusion, one perfusion generator and three pulse-generator arrangements are devised. The perfusion generator serves to flow the perfusate through the whole perfusion systems — aortic—cava and pulmonary passages, some part of the perfusate being introduced directly into the coronary circulation system. Two pulse-generators are used to give pulsatory changes to the perfusing stream in the aortic and the pulmonary venous passages, if necessary, and one is used chiefly for coronary flow measurement.

The method presented here may be regarded as a beginning for the analytical investigation on the heart with regard not only to its physical properties, but also to its metabolic and pharmacological characteristics.

In a previous report, a device for perfusing an isolated rabbit-heart was published, in which the isolated heart was provided with similar circulation systems as are found in the body, that is, the pulmonary perfusing system and the systemic perfusing one, the former being inserted between the pulmonary artery and vein, and the latter between the aorta and vena cava.

Principle of the method to be reported here is quite the same as in the former device. Some modifications and improvements have been made for a practical use of the method. These will be described here in detail.

**Perfusion Apparatus**

*Heart container (A)* (chest flask in the previous report) is provided with 6 openings at its wide airtight cap, of which 4 openings are used for connecting the heart with the respective vessel cannulae, pulmonary arterial (*pa*), pulmonary venous (*pv*), aortic (*ao*), and vena cava (*vc*), and 2 for connecting the
air spaces in the arrangement with each other \((rh_1, rh_2)\).

Perfusion reservoir \((C)\) is a flask (about 300 ml) with a wide airtight glass cap, through which 8 openings are provided, respectively, for pulmonary arterial and venous passages \((pa, pv)\), for a side channel to artificial perfusate-inflow \((Pis_1)\), for a siphon tube \((s)\) to the venous reservoir, for a perfusate transporter \((Pt)\), and for aerating of the perfusate in the reservoir \((ri, rx, rx_1)\).

In the beginning of perfusion, 260 ml perfusate is kept in the perfusate reservoir.

Pulmonary flow-meter \((B)\) is a graduated vessel (about 30 ml), making a side access to the pulmonary arterial passage \((pa)\). It has 3 openings through its airtight stopper, one for a pulmonary side flow \((sa_1)\), one for the outflow \((sa_2)\) and one for aerating tube \((rp)\), which also serves to remove any leaked fluid in the heart container into the meter-vessel.

Venous reservoir \((D)\) is a vessel (about 20 ml) having 4 openings at its airtight stopper, respectively for the aortic passage \((ao)\), for the vena cava passage \((vc)\), for a siphon \((s)\) to the perfusate reservoir \((C)\), serving to prevent overfilling of the perfusate in the venous reservoir, and for the second side channel of the perfusion inflow \((Pis_2)\).

Artificial perfusion generator \((APG)\) has been designed for artificial perfusion through two systems, pulmonary and systemic.

The reservoir consists of a circular silicon tube, provided with 2 pairs of rotating rollers \((a-a', b-b')\). One end of the circular tube is connected with the perfusate transporter \((Pt)\) in the perfusate reservoir, and the other end with a perfusate inflow tube \((Pi)\). This inflow tube ends in a thin tubule \((cp)\), which is inserted in the beginning of the aortic passage \((ao)\) and ends near the aortic origin. At the working of the perfusion generator \((APG)\), the perfusate in the reservoir is sucked up into the transporter \((Pt)\) and sent toward the perfusate inflow tube \((Pi)\) and into the coronary tubule \((cp)\). Some of the perfusate may flow under adjustable pressure into the coronary circulation system and some into the aortic outflow tube \((ao)\).

General adjustment of the artificial inflow can be attained by controlling the positions of the rotating pressors \((a-a', b-b')\), the motor cycles and adequate shunting of the flow into the perfusate reservoir through a side channel \((Pis_1)\). This side channel has another outlet on the side \((Pis_2)\), leading to the venous reservoir \((D)\), serving to keep the perfusate in the reservoir adequately.

We had hoped to obtain a quite smooth artificial inflow, without oscillatory variations of the inflow, but did not succeed with this perfusion arrangement, though we were able to decrease the oscillations by inserting tambours with
thick rubber membranes in the inflow passage.

**Pulse-generator (PG):** In order to give pulsatory changes to the perfusing stream, if necessary, a pulse-generator arrangement has been devised. In the side channels (sa, spv, sao) of the pulmonary arterial and venous passages, and of the aortic passages, specially constructed vessels (pg1-3) are inserted. Each vessel has a hollow watertight stopper, at the lower part of which a rubber sack is attached, and the upper part is connected with a rubber bulb (p1-3) of the pulse-generator (PG). The rubber sack, the bulb and the connecting tube are all filled with water.

At both sides of each vessel (pg), two glass valves are provided (in case of the pulmonary artery, one of the two valves is at the bottom of the flow-meter vessel (B)), each leading the flow in one direction. The bulbs of the PG are rhythmically pressed by a motor system specially devised. Thus the pulsating stream in channels occurs in one direction.

The phases of pulsation are the same at the aortic and the pulmonary channels, and the phase at the pulmonary venous channel is made to be reversed.

**Aerating system.** The gas phase distributed among the perfusion arrangements (A, B, C, D) is kept under a condition of continuous flow in the following way. By pressing the rubber bulb (p4) kept at the pulse generator (PG), the air in the bulb is driven by ventile arrangement (riv) through the tube (ri) into the perfusate-reservoir. The perfusate is aerated with the driven gas, which escapes through a tube (rx) to a specially arranged bulge (rxb), bubble-remover. The bubble-remover has a side tube (rx1) at the lower portion, which enables the bubbled perfusate to flow back into the reservoir. The driven gas flows through a tube (rxp), some part toward the pulmonary flow-meter (B) through rxp, some through rh1 or through rh2 into the heart container (A). The gas flows out from there through rh2 into the air.

The respiratory bulb (p4), when freed from the pressing, sucks the outer air through the ventile arrangement (riv).

By repeating the pressing and releasing of the bulb (p4), the perfusate can be sufficiently aerated.

**Perfusion System**

**Pulmonary perfusion system**

The perfusate in the right atrium sent from the venous reservoir through the vena cava passage (vc) flows into the right ventricle, and then into the pulmonary arterial passage (pa). This perfusate can flow directly, or indirectly through one side channel (sa1) and the pulmonary flow-meter (B), to the perfusate reservoir (C).
At the measuring of flow through the right ventricle, the perfusate moving through the pulmonary arterial passage is introduced to the inflow-meter (B), closing the direct flow to the perfusate reservoir. At the coronary flow measuring, the venous flow from the cava passage (vc) into the right ventricle is interrupted too, the volume collected in the flow-meter being regarded as that of the coronary flow. The perfusate collected in the meter vessel can be sent to the perfusate reservoir through the side channel (sa) by operating the PG-arrangement (pg1-p1).

The perfusate sent from the right ventricle is aerated in the perfusate-reservoir and then sent directly through the pulmonary venous passage (pv), or indirectly through the side channel (spv) to the left atrium and ventricle. The pulmonary venous pressure can be regulated by adjusting the flow through the side channel.

Aorta-cava system

The perfusate in the left ventricle is sent directly through the aortic passage (ao), or indirectly through a side channel (sao), to the venous reservoir (D). The perfusate flows then into the right ventricle through the cava passage (vc). Pressure in the passage can be regulated by changing the position of the venous reservoir, or by regulating the inflow from the side channel (Pis2) or the siphon flow (s).

Pressure recording of the perfusion system

In the present experimental procedure, the pressure in the perfusate inflow tube at the starting point of the coronary tubule was read by a Hg-manometer, without kymographic recording. This was usually much higher than that in the aortic passage. Pressures in the aortic, vena cava and pulmonary arterial and venous passages were recorded by tambours connected with the respective passages. Each passage was also arranged to be connected with Hg-manometers for easy gauging of the tambour recording. When air bubbles were present in the cava or pulmonary venous passage, they were removed by leading into the flow-meter vessel through the tube rp connected with the passage tube. (These arrangements are omitted in the figure to avoid confusion).

Preliminary Experiment

In a preliminary experiment, the perfusate was the blood-Ringer as previously described, the blood in the perfusate being diluted to about one-fifth. The heart which was in an inactive state before application to the apparatus began to pulsate when the blood-Ringer was artificially sent through the perfusion systems by working the motor arrangements (APG and PG).

During the artificial aortic perfusate inflow, the heart activities could not be demonstrated by the tambour recording, being perhaps overcome by the artificial pressure and confused with the artificial rhythms, though they could
Perfusion of an Isolated Rabbit-heart

Fig. 1. Schematic representation of rabbit-heart perfusion.

A: Heart container
- pa: Pulmonary arterial passage
- pv: Pulmonary venous passage
- ao: Aortic passage
- rh1, rh2: Air space connectors

B: Pulmonary flow meter
- sa1: Side flow tube of pa
- sa2: Outflow tube to pg
- rp: Aerating tube

C: Perfusate reservoir
- Pis1: Perfusion side channel
- Pt: Perfusate transporter
- rx: Air-outflow tube
- ri: Air-inflow tube
- s: Siphon tube to venous reservoir (D)

D: Venous reservoir
- Pis2: Side channel of Pis1

APG: Artificial perfusion generator
- a-a', b-b': Rotating rollers
- cp: Thin narrow tube for coronary perfusion

PG: Pulse generator
- sa, spv, sao: Side channels of respective passages
- pg1-3: Side channel vessels
- p1-3: Generator pulse bulbs

Aerating system
be observed distinctly with the naked eye.

When the working of the PG was ceased, the APG being kept working, both atria and ventricles could manifest their activities in various ways. However, when the artificial perfusate inflow was stopped, the heart activities decreased gradually, this perhaps being due to an inadequate composition of the perfusate for the heart activities. These observations could be repeated for at least 2 hours.

Two examples of the tracings obtained are presented in Fig. 2.

Fig. 2. Preliminary pressure tracings taken at 11h30' and 12h30'.

pa: Pressure (pulsatory) tracing in pulmonary artery.
vc: Pressure (pulsatory) tracing in vena cava.
pv: Pressure (pulsatory) tracing in pulmonary vein.
ao: Pressure (pulsatory) tracing in aorta.
t: Time in 3 sec.
a-part: Tracing during applications of APG and PG.
b-part: Tracing during application of APG (PG omitted).
c-part: Tracing during self pulsations.
In the b-part of pa-tracing, pulsations are those generated by the right ventricle mixed with slight oscillations due to APG.

COMMENT

From a physical, not physiological point of view, the present perfusion systems are similar to the circulation systems in the body, so that it would be possible to analyze the activities of the isolated heart under a similar condition to
that where the heart is active in the body. It would be expected that if a much more adequate perfusate is used, the isolated heart would manifest its full activities for a long period of time.

Thus, the present perfusion method would enable us to make analytical investigations on the heart activities with regard not only to its physical properties, but also to its metabolic or pharmacological characteristics.

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Reference