The Isolated Perfused Heart and Its Pioneers

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In 1866, Carl Ludwig together with Elias Cyon created the first isolated perfused frog heart preparation. Perfusion systems for the isolated mammalian heart were developed by H. Newell Martin in 1883 and by Oscar Langendorff in 1895. In its working mode, the isolated perfused rat heart was established in the 1960s.

The development of the isolated perfused heart was a process that extended over more than 100 years. Hardly any other methodological procedure has either taken such a long time to evolve or has resulted in such a wealth of fundamental knowledge about myocardial function, regulation of coronary blood flow, and cardiac metabolism. Several scientists of different character and idiosyncrasy were involved in this fascinating development. Their life stories and professional accomplishments in regard to the isolated heart have been compiled and are reported.

The beginning: Carl Ludwig

The inciting and driving force in the development of isolated perfused organs was Carl Ludwig. He was born on December 29, 1816, in Witzenhausen in the north of the current federal state of Hessen, Germany, at that time Kurhessen. In 1834, he commenced his medical studies at the University of Marburg and completed his MD degree in 1840. Until 1841, he worked in the laboratory of Robert Bunsen; then, he was made the Prosector at the Department of Anatomy of the University of Marburg and was appointed Extraordinary for Comparative Anatomy in 1846. In 1849, he was appointed Professor and Chairman of Anatomy and Physiology at the University of Zurich, Switzerland; in 1855, he became Professor of Physiology and Zoology at the medical-surgical military academy, the so-called Josephs Academy (Josephinum), in Vienna. However, he was not comfortable with the military atmosphere at Josephs Academy, with its bureaucracy and regimentations. When Carl Ludwig was offered a position as Chairman of the Department of Physiology at the University of Leipzig, he immediately accepted and took over this position on May 1, 1865. On April 26, 1869, the new Institute of Physiology that he had designed was opened. This Institute, in its design, reflected his integrated methodological approach and included morphology, biological chemistry, and physiology. He worked at the Institute for more than 25 years and died on April 23, 1895 (16).

Carl Ludwig was involved in the foundation of a new direction in physiological research. A small group of young, highly talented and dynamic physiologists fought against natural philosophy and vitalism as the determining factor in physiology. These young energetic physiologists were determined to establish that only physical and chemical forces were responsible for physiological processes and, ultimately, to describe nature in a mathematical manner. They named themselves ‘organic physicists.’”

“...and the future experiments, using both single and multiple channel knockouts, as well as other disease models, may further refine our views.”

References


The isolated frog heart: Carl Ludwig and Elias Cyon

The next step was to excise a heart and to keep it in this completely isolated state for a long period of time. The ideal species was the frog, since its heart has a spongy structure without coronary arteries. Exchange of respiratory gases and metabolites occurs entirely by diffusion processes. The pioneer in this heart preparation was Elias Cyon, who developed this procedure while working in Carl Ludwig’s Leipzig Physiological Institute. He published the first report on the effects of temperature changes on the function of the isolated frog heart, alluding to preceding experiments done by Calliburces and Schelske (cited in Ref. 3). “Cyon’s life remains shrouded in obscurity and can be reconstructed only uncertainly, from circumstantial rather than direct evidence” (6). He was born Ilya Fadeyevich Tsion in 1842 (or 1843) in a small Jewish community in Lithuania near the German border. He studied medicine at the Medical-Surgical Academy in Warsaw, at the medical faculty of the University of Kiev, and completed his medical studies with a doctorate in medicine and surgery from the University of Berlin, Germany. He then moved to the Medical-Surgical Academy in St. Petersburg and obtained the doctorate in medicine in 1865. He was sent by the Russian ministry of education to Paris to pursue his studies in physiology, probably under the auspices of Claude Bernard, and moved to Leipzig to work with Carl Ludwig. In 1867, Cyon returned to St. Petersburg and became director of the Physiological Laboratory at the University in 1868. Two years later, he became professor of anatomy. He was a brilliant lecturer and did vivisection demonstrations. One of his students was Ivan Petrovich Pavlov, who became the first physiologist to win the Nobel Prize in Physiology or Medicine in 1904.

Cyon was appointed Professor of Physiology and Chairman at the Medical-Surgical Academy of St. Petersburg against the decision of the faculty but on the orders of the Minister of War, Dmitry Milyutin; Cyon’s inaugural lecture was January 21, 1873 (6). However, by 1874, students demanded his removal. Disorder broke out, troops were called, and the Academy was closed. The termination of Cyon’s lectures was announced, and Cyon submitted to the Minister of Education a request to be sent abroad on an official scholarly mission to Leipzig to prepare and publish a work on the methodology of physiological research. He was dismissed from Russian governmental service in 1875, emigrated to Paris, France, called himself Élie de Cyon, and never resumed residence in Russia. He became an unofficial political agent, newspaper correspondent and editor, self-made diplomat, and cosmopolitan gentleman. He was involved at a very high social level in several activities to establish a Franco-Russian alliance against Germany in both military and financial terms. In his final years, he returned to physiological and scientific-philosophical topics. He died in Paris in 1910.

Cyon was an unfortunate and tragic character but a talented and productive physiologist. The work he did during his brief tenure at the Leipzig Physiological Institute resulted in four papers. All were communicated and submitted by Carl Ludwig at the Royal-Saxonian Society of Sciences at Leipzig and published in the Proceedings of this Society, most with Cyon as the only author, as was the custom with Carl Ludwig.

The first of these contributions concerned the isolated heart (Fig. 1). The heart was excised from a frog, and the aorta and vena cava were cannulated and filled with serum obtained from rabbit blood. This was circulated from the aorta through the glass tube pwzo to the vena cava (Fig. 1). When pressure
was to be measured, a stopcock was switched at \( l \), where a rubber tube was inserted (\( f \)) that was connected to a mercury manometer (right). The temperature of the serum in the system could be measured with a thermometer inserted into the oblique glass tube \( srq \). This circulatory system was surrounded by glass cylinders \( ABCD \) and \( JKLM \) in which the fluid could be adjusted to any desired temperature. Low temperatures were obtained by filling a mixture of ice and sodium chloride into \( ABCD \) and water with ice cubes into \( JKLM \) so that the glass tube \( pwzo \) was in close contact with this temperature. Higher temperatures were induced by a water flow from the glass cylinder \( JKLM \) through the connection \( GNFD \) to the double-jacketed \( ABCD \) container with the outflow at \( E \) (Fig. 1).

When medium temperatures were to be tested, two water containers were filled with ice-cold and hot water, respectively, and placed above the apparatus. The fluids were mixed until the desired temperature was achieved before they entered the \( JKLM \) container (3).

With this experimental design, it was found that the heart must be filled with a certain amount of serum to induce diastolic filling pressure so that the ventricle can eject fluid. Moreover, heart rate increased with increasing temperature, reaching a maximum that was different in individual hearts. When the temperature exceeded the maximum, heart rate declined precipitously. Heart rate was observed to increase within the temperature range that occurs in fever. On the
other hand, pressure increased already at temperatures slightly above 0°C to reach a maximum between 15 and 19°C. Beyond 20°C, there was a decline. It was concluded that there is a dissociation between heart rate and contraction amplitude over a wide temperature range. Given this discrepancy, it was further concluded that each individual heart has its own optimal temperature to pump most efficiently. In the frog heart, the optimal temperature range is between 18 and 26°C. Another aspect concerns the change in elasticity of the heart with temperature. Finally, a hypothesis was presented that explains the observations. It was speculated that there are two components of the “excitation apparatus” of the heart: the first produces the stimuli, and the second, a regulating or inhibiting system, modulates the rhythmic transition of the stimuli to the motor nerves of the heart (3). This is a very early version of cardiac “excitation-contraction-coupling” that was explored long before the roles of sodium and calcium ions in these processes were known.

The isolated frog heart preparation was modified in several studies performed in Carl Ludwig’s Leipzig Physiological Institute. To investigate the effects of the stimulation of the vagus, a preparation was designed in which this nerve was exposed from the spinal chord to the heart. The aorta was cannulated and connected with a mercury manometer, and another cannula was inserted into the atrium and connected to a reservoir containing rabbit serum. In contrast to Cyon’s heart preparation, this one was kept in a nonrecirculating mode (2). In another modification (1), a glass cannula was inserted into the atrium of an excised frog heart, advanced into the upper third of the ventricle, and fixed there. Another cannula was inserted into the ventricle and connected to a mercury manometer. The apex of the heart thus isolated and perfused with rabbit serum was electrically stimulated, and the frequency and amplitude of the contractions were recorded on a kymograph. In this way, the famous treppe phenomenon was discovered by Henry Pickering Bowditch: the amplitude of contractions increased until a plateau was reached when electrical stimulation of the apex of the heart was initiated and maintained after a resting period of several minutes. Furthermore, from the experiments in this study, three important phenomena were observed: the all-or-none-law, the absolute refractory period, and the origin of cardiac automaticity, which is located in the atrium and the atrioventricular area. During his stay at the Leipzig Physiological Institute, Bowditch (1840–1911) fell in love with the daughter of a Leipzig banker; they married and moved to Boston. He established the first university laboratory of physiology in the United States at Harvard Medical School (Boston, MA, USA). He was a founding member and the first President of the American Physiological Society, a position he held for two terms (1888 and 1891–1895).

Many important discoveries in cardiovascular research were made on the isolated rat heart. Although the animal species was not explicitly mentioned in his paper, Sidney Ringer most certainly used the isolated frog heart in the experiments in which he elucidated the role of calcium ions for heart contraction (13). In a modification of the isolated perfused frog heart, Otto Frank discovered the famous law of the heart (4). Otto Loewi demonstrated in the frog heart the chemical mode of transmission of the vagus effect (9). He was awarded the Nobel Prize in Physiology or Medicine in 1936 together with Henry Hallett Dale.

The mammalian heart-lung preparation: Henry Newell Martin

The problems involved with the mammalian heart had been recognized as early as 1846 by Wild and Ludwig. Most studies before 1880 were therefore done on the isolated frog heart, which has the advantage of having no coronary circulation and only one ventricle. This preparation was well known to all visitors of Ludwig’s Leipzig Physiological Institute. There were many, among them also some English visitors, including in 1870 Michael Foster who had established practical physiology and histology courses at University College, London, in 1867. He became familiar with Coats’ isolated frog heart preparation (2). When he returned to London, Foster invited H. N. Martin (born in 1848 in Ireland) to serve as demonstrator for him. Martin also became assistant to Thomas Huxley, Britain’s leading biologist, in his course on elementary biology at the Royal College of Science in London. Martin also helped in the preparation of Huxley’s popular textbook of elementary biology, which first appeared in 1875. Martin visited Ludwig’s Leipzig Physiological Institute and worked there in the summer of 1875. When Huxley was asked by Daniel Gilman, president of the Johns Hopkins University in Baltimore, MD, to suggest candidates for the Chair of Biology at this institution, he recommended Martin.

Martin obtained this position at Johns Hopkins in 1876 and concentrated his experimental efforts on the development of the isolated perfused mammalian heart in 1880, but soon his health and energy started to decline. After 1891, and more seriously after the death of his wife in 1892, his physical condition deteriorated and failed due to uncontrolled alcoholism, complicated by transient and possibly iatrogenic morphine addiction.

“...there is a dissociation between heart rate and contraction amplitude. . .”
and by intermittent painful and disabling polyneuropathy. He was treated by William Osler in Baltimore and by the leading neurologist S. Weir Mitchell in Philadelphia. The latter was one of the cofounders of the American Physiological Society in 1887, together with Martin and Bowditch. This attentive care resulted in several improvements but relapses followed. Finally, Martin's incapacitation increased so that he had to submit his resignation in 1893. He returned to England and died there on October 27, 1896 (5).

The major progress achieved by Martin was that he used the mammalian heart. His experimental model was basically a heart-lung preparation. In a first attempt, an anesthetized and curarized cat or dog was artificially ventilated and the systemic circulation was excluded, except that a cannula in the left subclavian artery was connected with a manometer. All blood ejected by the left ventricle was pumped into the coronary arteries. The coronary circulation emptied into the right atrium, flowed through the right ventricle, was pumped via the pulmonary artery to one lung for oxygenation, and returned to the left heart. Thus the only section of the systemic circulation was the coronary circulation. In an extension of this preparation, an artificial peripheral circulation was established (Fig. 2). Defibrinated calf’s blood from alternately used Mariotte flasks (C and D) flowed via the jugular vein into the right atrium and ventricle of the heart of an artificially respirated dog that was placed in a warm, moist chamber. Blood then passed through the lungs to the left heart and was ejected into the aorta where tube t was placed, which emptied into the funnel x. Aortic pressure was varied by sliding the support Q carrying tube t up or down the vertical rod R. From funnel x, the blood flowed through tube L to flask D for filling the right heart via tube n, which ended in tube y inserted into the superior vena cava. The Mariotte flasks C and D were suspended by the cords r and r' and could be raised and lowered to obtain the desired venous pressure. In this way, an artificial circulation with the option to change independently pre- and afterload was maintained in this heart-lung preparation. Cannula M was placed in the right carotid artery and cannula N in the left carotid artery. They were connected to tubes O and P, which passed through the wooden left side of the

FIGURE 2. Experimental setup for the mammalian heart-lung preparation developed by H. M. Martin. The dog, from which the systemic circulation was cut off and replaced by an artificial system, is not shown, only the tubes to and from the heart. Details of this preparation are given in the text. [From Martin (11).]
chamber to be connected with manometers for the recording of pressure and pulse rate on a large kymograph. The entire chamber shown in Fig. 2 rested on a trough that could be kept at the desired temperature by Bunsen gas burners (11).

The isolated mammalian heart: Oscar Langendorff

Langendorff did the next step to excise a mammalian heart, to perfuse it and keep it alive for several hours. He decisively and emphatically made a distinction between his and Martin’s isolated mammalian heart preparation and claimed priority for his method (8). Langendorff was born on February 1, 1853, in Breslau, Germany and studied medicine at the Universities of Breslau, Berlin, Freiburg, and Königsberg. He received his MD degree in 1875 and became assistant at the Physiological Institute of the University of Königsberg. He obtained his license to lecture (habilitation) in 1879 and was promoted to associate professor in 1884. In 1892, he moved to the University of Rostock, Germany, as Professor and Chairman of Physiology. He died there on May 10, 1909. As was characteristic for physiologists of that period of time, his interest covered a wide area of research. He concentrated mainly on the heart, but he also did research on the innervation of the respiratory tract, reflexes, the physiology of digestion, and the sympathetic and peripheral nervous system. He was described by one of his fellow colleagues “to have been not a conqueror, who discovered new and unimaginable areas, but a brave comrade-in-arms who served physiology, our wonderful science, well with diligence and conscientiousness in teaching and research” (14). It seems that his work on the heart was not overly appreciated at the time and that his discoveries, although less numerous and less spectacular than those of Ludwig, suffered a similar fate. They very soon became part of the anonymous body of knowledge that are essential and integral components of textbooks, without reference to the original work. Nevertheless, the term “Langendorff heart” has survived in laboratory jargon for more than 100 years, whereas no physiological phenomenon, invention, discovery, or method is explicitly associated with Ludwig’s name.

Langendorff’s experiments were carried out mostly on cats but also on rabbits and dogs. For perfusion, defibrinated blood from the respective species was used (7). The key element is the injection cannula M (Fig. 1 and Fig. 3 in Fig. 3), which is inserted into the aorta of the heart placed in a small container. The cannula M is connected via a tube to the blood bottle B, which can be filled...
from the reservoir K (Fig. 1 in Fig. 3). The small container and the blood bottle are immersed in the water tub N, which can be heated by the Bunsen burner O. The outflow from the heart drips into the beaker J. The perfusion pressure is regulated by the manometer C (Fig. 1 in Fig. 3), which is automatically controlled by a refined device that is connected via the valve H (Fig. 2 in Fig. 3) to the pressure air bottle A (Fig. 1 in Fig. 3). The shortening of the isolated heart is recorded by attaching a string from the apex of the heart via a wheel to the membrane of a Marey capsule (Fig. 3 in Fig. 3). The displacement of the membrane is transmitted to a lever and recorded on a kymograph (10). Also, a more sophisticated double-membrane system was used for recording (Fig. 1, left, in Fig. 3).

The description of the experimental setup and the preparation procedure is very clear and precise (7). Langendorff's first and surprising observation was that the excised, obviously dead or dying heart could be resuscitated on perfusion. The heart resumed automaticity and maintained it for hours. This was taken as proof that perfusion of the coronary arteries with blood as a nutrient is sufficient to induce the normal heartbeat, which is an intrinsic property of this organ. Because there was no blood in the ventricular cavities in his preparation, Langendorff concluded that in the mammalian heart the blood in the ventricular cavities has no effect on excitability. Experiments done with this isolated perfused heart demonstrated that brief stimulation of the vagus as well as the application of potassium chloride resulted in immediate cardiac arrest. Muscarine induced a negative chronotropic and inotropic effect and ultimately diastolic arrest, which was very similar to vagus stimulation. Atropine had an antagonistic influence. High temperature led to tachycardia and low tempera-

FIGURE 4. The isolated perfused working rat heart preparation as established by Neely and Morgan (12). [With permission from H. E. Morgan and the American Physiological Society.]

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ture to bradycardia. A single electrical stimulus induced postextrasystolic potentiation of contraction, and stimuli of higher frequency and greater strength led to fibrillation. Coronary artery ligation induced contractile failure and cardiac arrest, which was reversed with restoration of coronary blood supply (7).

The isolated working rat heart: Howard E. Morgan and James R. Neely

The last chapter of the isolated perfused heart story was completed in the middle of this century. The Langendorff rat heart was converted into the working mode. Neely and Morgan accomplished this at the Department of Physiology, Vanderbilt University School of Medicine (Nashville, TN). Morgan moved to Hershey, PA, as Chairman of the Department of Physiology, Milton S. Hershey Medical Center, Pennsylvania State University, where the studies on the isolated perfused working rat heart were continued. James Neely was born in 1935 and died in 1988.

Completely isolated rat hearts were first retrogradely perfused down the aorta through a cannula from a reservoir located 70 cm above the heart for 10 min to remove blood and to allow recovery from anoxia during excision and initiation of perfusion. During this time, the left atrium was cannulated and secured with a ligature. Heart work was begun by starting flow into the left ventricle, and ventricular contraction forced it into a pressure chamber partly filled with air to provide some elasticity (“windkessel”) to the otherwise rigid system. Pressure development in this chamber forced the fluid out through a tubing into an aortic bubble trap that 70 cm above the heart. Overflow from the aortic bubble trap was returned inside the long atrial bubble trap relative to the heart (12). The perfusate was a modified Krebs-Henseleit bicarbonate buffer equilibrated with a 95% O₂-5% CO₂ mixture.

This working heart preparation has been modified and adjusted to specific needs of several other laboratories throughout the world. For instance, it has been used for studies on cardiac metabolism and coronary regulation, applying conventional chemical methods and nuclear magnetic resonance techniques. The perfusion medium has been modified to achieve a greater functional and metabolic stability, and pressure in the left ventricle has been measured continuously with an ultraminiature catheter-tip manometer. It will be interesting to follow the new designs, improvements, and applications that may be developed in the future.

References

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