RETROSPECTROSCOPE

Ludwig: The Physiologist

By Max E. Valentinuzzi, Klaus Beneke, and Germán E. González

Thinking something that no one before ever thought whilst seeing something which everybody sees.

The thought reproduced in the above epigraph is taken from an article by Thurau et al. [1], who attribute it to Arthur Schopenhauer (1788–1860), an outstanding philosopher and author of the far-reaching piece *Die Welt als Wille und Vorstellung* (*The World as Will and Representation*). In German, it would perhaps read as "etwas denken, das niemand vorher gedacht hat, während etwas sehen, was jeder sieht." We could not assert whether Schopenhauer really said that, but it should not be at all surprising if it were, because it sounds simple, perhaps even naïve, and very deep, indeed. It fits perfectly to Carl Friedrich Wilhelm Ludwig's personality (1816–1895), whom we will look at as physiologist in this second note. Yes, second note—because in the first one [2], we looked at him as bioengineer. A third and last "Retrospectroscope" column completing this series will deal with his wonderful and always humble and generous activities as teacher.

Ludwig was, by training, a physician, although he never practiced the profession; however, very early in his academic life, he developed a wide vision of medicine probably influenced by the rather long sojourns in Marburg, Vienna, and Zurich and his scientific contacts with Robert Bunsen and, obviously, with his three lifelong friends Helmholtz, Brücke, and Du Bois Reymond. Ernst Heinrich Weber also played a significant role in his scientific career. There is no doubt that an open-minded attitude and brilliant mind must have been essential factors as well.

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First, he clearly realized that measurements were mandatory if one wanted to quantify physiology (a basic medical discipline along with anatomy), which was his central objective of study, and measurements led him to the kymograph [2]. This instrument brought to the world a strong, long-lasting breath of fresh air and with it, many new contributions were born, especially in the Leipzig Institute inaugurated in 1869 [3], several of which stemmed from his own ideas and were developed further by his students from Germany and abroad. Except for his periods of time spent in Switzerland and Austria, Ludwig never traveled beyond other frontiers in Europe or America, where his links **<AU: can "links" be clarified? Do you mean his contacts or connections?>** were close and more in number.

Heinz G. Zimmer, who was a relatively recent former director of the Leipzig Institute of Physiology, listed several scientific subjects dealt with by Ludwig and his coworkers [4], [5]. Another invaluable source, probably the best for its completeness, belongs to Heinz Schröer [6]. Much of this firsthand knowledge was also partially collected and updated (until 1861) in the different editions of his *Physiology Textbook* [7]–[9]. Let us list the scientific subjects and proceed thereafter, trying to offer a brief review for each:

- kymograph
- flow meter (*stromuhr)*
- mercury blood gas pump
- injection methods for vessel visualization
- filtration theory of urine formation
- localization of the medullary vasomotor center
- depressor reflex
- viability of isolated perfused organs
- function of the cardiac valves
- gland secretion and secretory nerves
- cardiac physiology (several subjects)
- a variety of other physiology subjects.

The first four scientific subjects listed should be classified as technological in nature, acting as tools for further advancement in knowledge, while the rest are clearly physiological. Let us go somewhat deeper, underlining those that are more relevant to physiology as such and leaving out the first four, particularly the kymograph, for they were examined in this magazine's previous "Retrospectroscope" column as technical contributions serving physiology [2]. To begin with, it must be emphasized that a general feature of Carl Ludwig's research was set on rigorous scientific thinking, way of working, perseverance, and self-criticism. With regard to all his contributions in different areas of physiology, he showed three stages, i.e., a theory, a development, and a concrete finding. **<AU: Please note that this was broken into two sentences for clarity. Kindly check whether the edits are OK.>** Besides, it is difficult to determine how much his own contribution was to each published article since his name was not often included as an author, since his students were listed first or even as the only authors; as Schröer states [6], "die Veröffentlichungen Ludwigs nur in den wenigsten Fällen seinen Namen tragen." Translated, this means "Ludwig's publications in very few cases carried his name." **<AU: please check that edited sentence is OK as given.>** Often, Ludwig wrote the whole article because the student did not handle **<AU: Can "handle" be replaced with "understand" here for clarity?>** German well (and such a fact was recognized by the student himself).

Filtration Theory of Urine Formation

In 1842, when Ludwig was 26, he submitted his habilitation thesis (written in Latin [10]) while he was in Marburg, embodying a revolutionary new scientific philosophy that he was to follow and develop further during his entire academic career, i.e., physics, mathematics, and chemistry were to explain physiological problems. Specifically, he also launched the modern vision of renal function, also to be revisited several times for years to come. Sir Thomas Lauder Brunton (1844–1916), a former student of Ludwig, participant in many experimental sessions in Leipzig and strong supporter of his, wrote in 1912 [11]:

> Bowman's theory forms the basis of the knowledge we now possess regarding the secretion of urine, but it is Ludwig's experiment on the relation between blood-pressure and secretion that practically enables us to explain the use of diuretics in cardiac disease, and as I have already said, Ludwig's theory, as modified by him in 1870, closely resembles Bowman's though supplemented in some very important respects.

Brunton also recalls that several students collaborated on the subject. As Thurau et al. say [1], the formation of urine was experimentally examined in Ludwig's habilitation thesis and explained based on physic-chemical laws. This was a daring and revolutionary new framework, which, not surprisingly, faced strong opposition from many respected scientists for many years. Supported by such an intellectual background, Ludwig developed his concept of renal function, specifically glomerular filtration and subsequent tubular reabsorption. Three observations were essential.

1) From model experiments, he recognized that the fluid blood components are able to get through a membrane without carrying proteins, being driven only by a hydraulic pressure gradient. His is the ultrafiltrate concept.
2) Again, based on model experiments, Ludwig came out with the concept of a hydrostatic pressure profile in the glomerular capillaries.
3) The similarity between blood and urine composition, aside from concentration differences, gave Ludwig the belief that urine was mainly derived from blood, and it was not a product of kidney synthesis, as accepted by others.

Hence, Ludwig correctly derived what glomerular filtration is. The reader should consider that such conclusions were arrived at more than 160 years ago—weigh the technology that was available during those days and the surrounding and prevailing scientific knowledge, and ponder the magnitude of Ludwig's accomplishment.

The story and further details over the years were wonderfully described and interpreted in 1996 by Thurau, Davis, and Häberle (all three from the Physiological Institute, University of Munich, Germany) [1]. Schröer devoted several pages in his book to this subject and also reproduced a paragraph in Latin [6].

Localization of the Medullary Vasomotor Center

In 1996, Prof. H. Seller was with the Physiological Institute of Heidelberg University, Germany, and he told the story of this medullary center and even added results from his own research **<AU: Kindly check whether the edit made here is OK.>** [12]. Ludwig's interest in the subject started during his long stay in Vienna (1855–1865), where he carried out experiments with Ludwig Thiry (1817–1897) [13]. They saw that electrical stimulation of the cervical cord increased blood pressure. In 1871 and 1873, two students from Leipzig, Philipp Owsjannikow (1827–1906) and C. Dittmar, proceeded further with a better localization of the vasomotor center in the medulla oblongata [14], [15]. As Prof. Seller points out, it is surprising how this concept stood the proof of time, for they nicely located the center within a small ventrolateral area of the medulla. However, such knowledge was ignored for more than 100 years until about 1980, which led to many confusing reports. Currently, the term *rostro-ventrolateral medulla (RVLM)* is generally used.

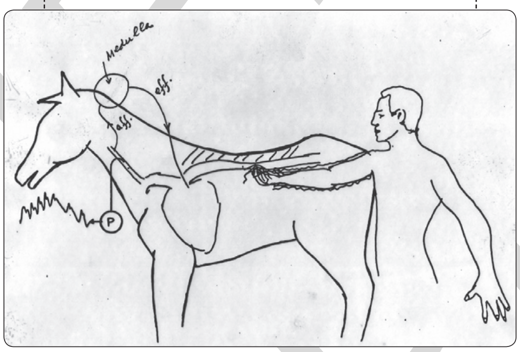

FIGURE 1 The Chauveau–Marey maneuver as performed in the unanesthetized horse (1863). Chaveau, a veterinarian, and Marey, a physician, were superb experimenters who also acted as engineers. The operator introduced one of his arms in the animal's rectum, felt the abdominal descending aorta through the large intestinal walls, and strongly pushed upward against the vertebral column so as to suddenly compress the artery (electronic engineers would call it a *step function*). Afferent vagal fibers sent pulses to the medulla oblongata from where, reflexly, vagal efferent pathways produced the typical negative chronotropic effect. A beautiful and well-thought demonstration, but they were not yet aware of its explanation [19]–[20]. **<AU: Please confirm that permission has been obtained to reprint this image and also provide the proper credit line.>**

Depressor Reflex

The vagal (or parasympathetic) action on the heart is common physiological knowledge today, with its decreased cardiac rate and arterial blood pressure, which are also involved in the baroreceptors' reflex. Medical students usually have hands-on laboratory exercises to see those effects for themselves; however, long and slow was its full elucidation. This column is not the place to review it, since there are other reports that well accounted for its development, for example, one by Erwin H. Ackerknecht published in 1974 [16]. Ludwig and some of his students were part of these events. Let us briefly go over them.

Brothers Ernst Heinrich Weber (1795–1878), Wilhelm Eduard Weber (1804–1891), and Eduard Friedrich Weber (1806–1871) were three outstanding scientists. Surprisingly, one of these brothers **<AU: Please clarify which brother is mentioned here.>** stimulated the vagus nerve in 1845, finding an inhibitory effect over the heart and even bringing it to a standstill [17]. According to Eric Neil [18], there was also a report that appeared in *Annali Universali di Medicina*, of

Milan, Italy (vol. 116, pp. 227–228). The latter **<AU: can you clarify which the "latter" and "other" refer to? It is unclear since there is one reference mentioned here.>**, obviously, must have been a preliminary short communication published in 1845, while the other was a longer article reporting the new finding the year after. Ernst and Eduard Weber discovered not only an important phenomenon but also a whole new notion in neurophysiology, that of inhibition. Their explanation was violently opposed by some while it was similarly defended by others. Étienne-Jules Marey (1830–1904) was the first researcher in 1859 to recognize the inverse relationship between arterial pressure and heart rate, which was later known as Marey's law of the heart [19], [20]. Around 1861, Jean-Baptiste Auguste Chauveau (1827–1917), a veterinarian and close collaborator of Marey, developed a procedure (now known as the Chauveau—Marey maneuver; see Figures 1 and 2) by which that law was verified in the horse and, obviously, can also be tested in other mammals [21], [22]. The latter two papers described a more sophisticated maneuver performed in the dog with a catheter and a balloon to obtain some evaluating parameters as, for example, the sensitivity of the system (see Figure 3 for the definition of the latter parameter). Johannes Nepomuk Czermak (1828–1873), who also worked in Vienna with Ludwig, came up with the "Vagusdruckversuch" concept in 1866, which, translated, meant something like "pressure search by the vagus nerve" [23]. Thereafter, in 1886, Elie de Cyon (1843–1912) and Carl Ludwig performed a notable experiment by stimulation of the central end of the depressor nerve, ensuing a fall in blood pressure and bradycardia that were recognized to be reflex in nature. Ludwig thought that the nerve endings of the depressor lay in the ventricles but correctly argued that the afferent depressor nerves served to brake the cardiac rate and lower the peripheral resistance when the blood pressure was too high [24]. This can be considered the starting point of what is currently known about the baroreflex. **<AU: Kindly check that the edited sentence retains original meaning. Please note that "baroreflex reflex" was changed to just "baroreflex," OK?>** Thereafter, several other important contributions were added.

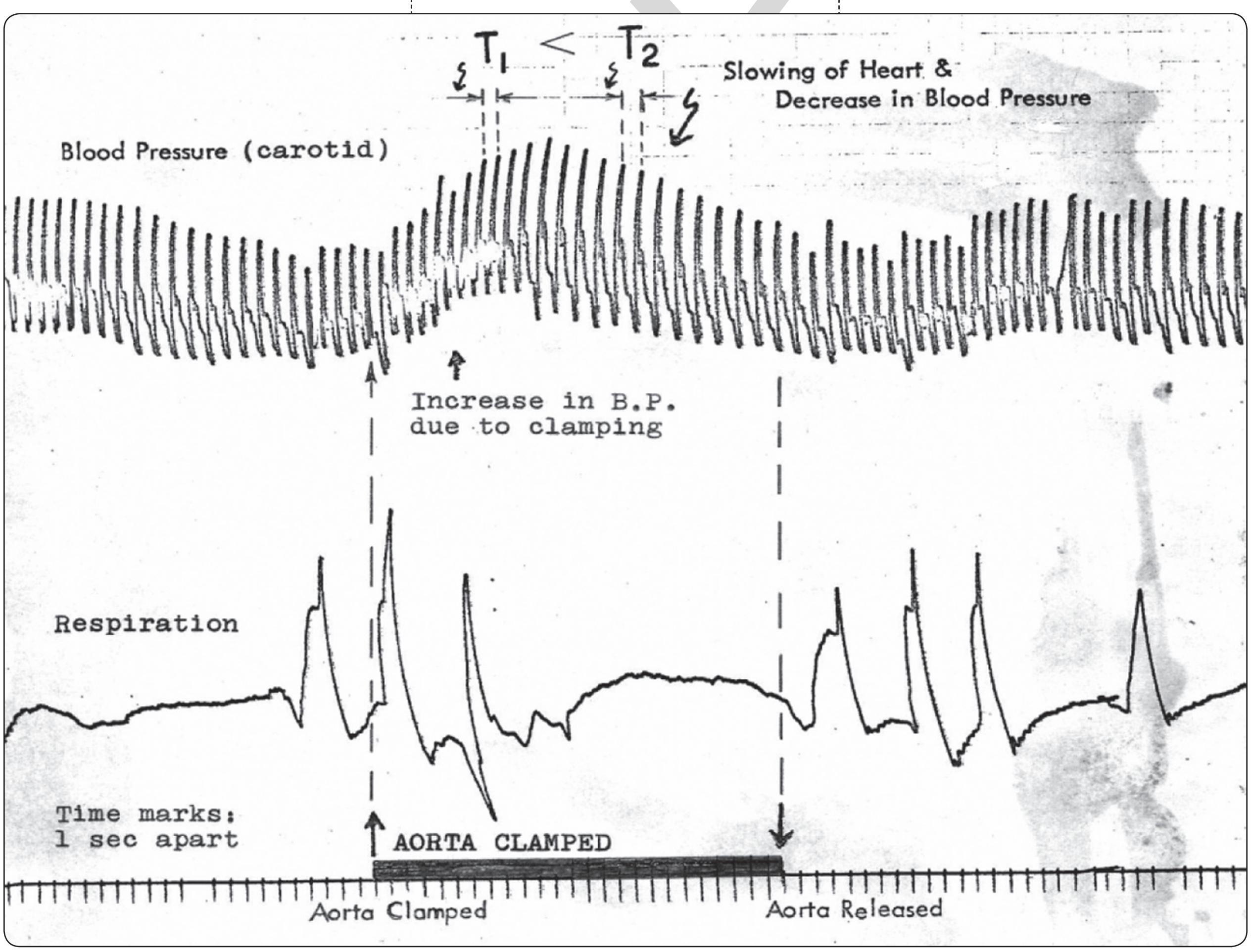

FIGURE 2 The Chaveau–Marey maneuver in the anesthetized horse, essentially the same procedure as depicted in Figure 1. Blood pressure first increases after the hand pushing the abdominal aorta (a step function) against the vertebral column followed by a reflex decrease in rate and blood pressure. Usually, respiration in the horse is rather aperiodic; however, because of the vagal action, there was a period of apnea during clamping **<AU: please check that edits here are OK>** followed by a few breaths after release. (Records were obtained from Texas A&M Veterinary School, College Station, in 1962. Clamping was performed by Max E. Valentinuzzi.) **<AU: Is this figure courtesy of Max E. Valentinuzzi? Please clarify, otherwise, please confirm that permission has been obtained to reprint this image and also provide the proper credit line.>**

Viability of Isolated Perfused Organs

In 1866, Carl Ludwig and Elie de Cyon came up with the first isolated perfused frog heart preparation, although the report appeared the year after and was signed only by Cyon, typical and generous of Ludwig, for the latter appears just as communicator [25]. After this, perfusion systems for the isolated mammalian heart were developed by Henry Newell Martin (1848–1896), in 1883, but were published much later and in collaboration with Edward Carey Applegarth (1866–? **<AU: Please update year of death if possible.>**) [26] and Oskar Langendorff (1853–1908) in 1895 [27]. The observations made using both methods at the end of the 19th and the beginning of the 20th centuries led to important discoveries, such as the role of temperature, oxygen, and calcium ions for heart contractile function, the origin of cardiac electrical activity in the atrium, the negative chronotropic effect of vagal stimulation, the now well-known law of the heart, (thanks to Otto Frank (1865–1944) [28]), and the chemical transmission of impulses in the vagus nerves by acetylcholine demonstrated by Otto Loewi (1873–1961) [29]. There were many modifications, but its general principle remains the same today [30]. In 1998, Zimmer published an excellent and detailed account of the perfused heart development, a subject that no doubt projected significantly over time in physiology and medicine at large [31].

Function of the Cardiac Valves

By means of an excised pig heart, a manometer, and a visualizing apparatus, Giulio Ceradini (1844–1894), an Italian physiologist who worked with Ludwig in 1871–1872, showed the closure mechanism of the cardiac semilunar valves,

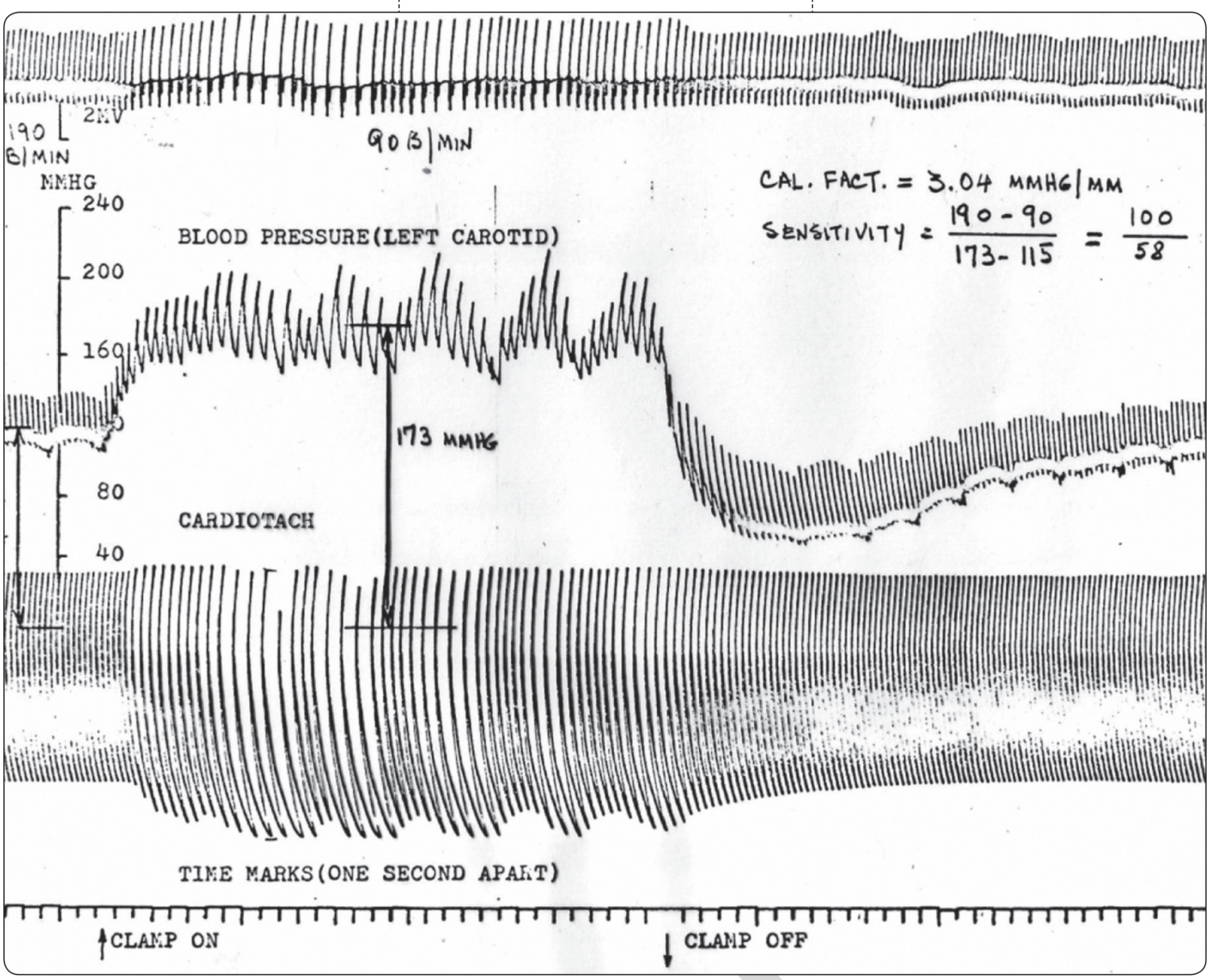

FIGURE 3 The Chauveau–Marey maneuver in the dog by direct manual compression of the descending abdominal aorta through a lateral abdominal incision. The animal was a 13-kg male under pentobarbital anesthesia. Upper channel: Electrocardiography (ECG) (lead II), where a 2-mV calibration is shown on the left. By it, the preclamp cardiac frequency is also indicated (190 beats/min). In the middle of the clamp, that frequency dropped very significantly to 90 beats/min. The second channel displays the carotid blood pressure with its calibration on the left side. The third channel shows a cardiotach output, which increased the trace amplitude when the rate decreased (without calibration, it was simply qualitative). The fourth channel shows time marks 1 s apart, besides also the moments when the clamp was manually applied and thereafter released. Increase in blood pressure is clearly displayed when the clamped was on, soon followed by a reflex heart rate decrease. The average blood pressure step was about 58 mm Hg with an average heart rate decrease of roughly 100 beats/min, leading to a sensitivity of 1.73 beats/min $\times$ mm Hg. During the clamp duration, a clear variation in frequency is observed, i.e., the respiratory heart-rate response. Figure obtained by Max E. Valentinuzzi in 1970 at the Department of Physiology, Baylor College of Medicine, Houston, Texas. **<AU: Please confirm that permission has been obtained to reprint this image and also provide the proper credit line.>**

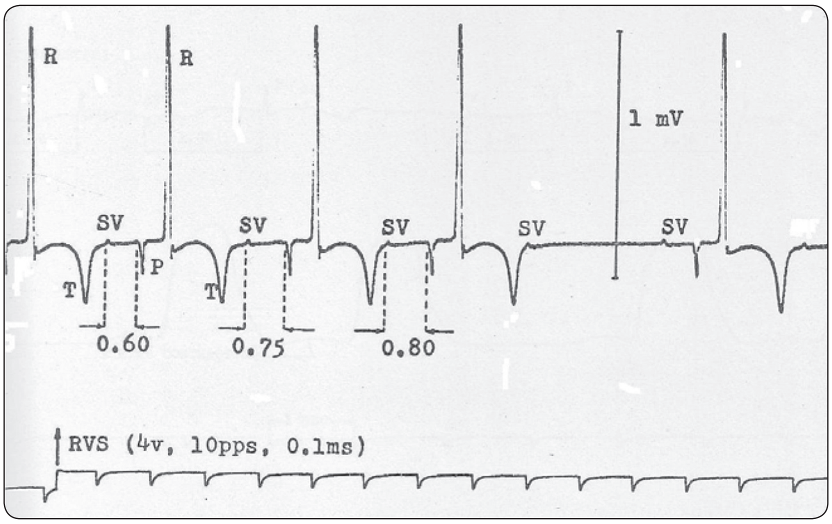

FIGURE 4 Sinus venosus-atrial Wenckebach-Luciani progressive block. Obtained from an anesthetized snake, *Elaphe obsoleta*, by right vagal stimulation. The ECG was recorded with subcutaneous electrodes. The electrocardiographic four components are well depicted: SV, P, QRS-complex, and T-wave. Observe the sequence of increasing SV-P intervals until the SV-P block takes place. The beat after the latter resumed its initial duration. (Figure used courtesy of Max E. Valentinuzzi, copyright 1969, *Electrophysiology and Mechanics of the Snake Heart Beat*.)

says Diana Troiani and Ermanno Manni from the Medical School Catholic University, Rome [32]. There are three reports produced by the original author: one published in Germany in 1872 [33] and a second, longer one appeared in Italy in three versions, in 1873 and 1906 [34]–[36].

The closure semilunar valves' mechanism was not well understood and still remains in the current 21st century as a matter of research for the

FIGURE 5 A beautiful specimen of a male *Bufo arenarum*, a common frog species found in the Argentine Northwest that is unfortunately in danger of disappearing. It is absolutely harmless and useful, for it feeds itself with a variety of insects. It has glowing eyes, a bright green color, and emits soft and cradling **<AU: "Cradling" does not seem to fit here. Can it be replaced with "soothing" or "relaxing" here?>** croaking songs during rainy nights. All physiology labs should have a sculpture in its honor; thanks to this species, much knowledge was produced. **<AU: please check that edits are OK>** (Photo courtesy of Max E. Valentinuzzi.)

achievement of an ideal valve prostheses. Ceradini conceived a simple technique for observing the behavior of these valves during the cardiac cycle. In an excised pig's heart, he introduced a special speculum into the pulmonary artery. The right ventricle was connected to a pump so that different pressures could be applied to simulate systole and diastole. A manometer and a speculum connected to the pulmonary artery monitored pressure values. The same procedure was applied to the left ventricle to visualize the aortic valve closure. Such a preparation was so successful that it was described in textbooks and routinely carried out in Italian medical schools into the 1960s. Ceradini's observations clearly indicated that the closure of the valves was not the effect of the beginning of diastole but of the end of systole.

Essentially, Ceradini's description of valve function has been validated by 20th-century investigators. In the last few decades, computational and geometric models of valve function have led to highly attractive models; however, a full dynamic simulation has not been reached due to the complexity of valve anatomy, geometry, motion, deformation and flow, and their interactions. Although the present understanding of valve function has definitely increased, to date, an ideal nonthrombogenic, noncalcific prosthetic device able to maintain normal valve properties and hemodynamic flow has yet not been designed [32].

Gland Secretion and Secretory Nerves

Carl Ludwig, as early as 1850 when still in Zurich, was aware of the gross anatomy of the nerves to submandibular glands (he had been a prosector before in Marburg) and found that the electrical stimulation of the chord-lingual nerve causes the flow of saliva in dogs. He also observed that the secretory pressure could surpass the level of blood pressure [37]. In 1999, Garrett et al. published an excellent review where its historical development is well told **<AU: Can "well told" be replaced with "told in detail" for clarity?>** [38]. Schröer also devoted a few pages to this concept [6, pp. 115–120].

Cardiac Physiology

Cardiac physiology represents a wide area, much more in our days than during Ludwig's. His contributions, either directly from him or his students, were outstanding and always the first ball kick or very close to that, so in a way, starting the game we can say. **<AU: The meaning of this sentence is unclear as given. Please clarify.>** His model was Ernst Heinrich Weber's *Wellenlehre*, which was published in Leipzig [39], along other contributions in the same field made later on by this visionary researcher. Besides, Ludwig also had personal contacts who respected him so highly that it can be asserted that his own contributions followed Weber's spirit. On the other hand, Ludwig's findings in Marburg had always attracted Weber.

During the period that was spent in Marburg, due to some discrepancies with Poiseuille's blood pressure findings and those obtained by one of Ludwig's students, Ludwig Spengler (1818–1866), the concept of arterial side and head pressures and the slightly different methods to measure them were clarified [6], [40], [41]. The former requires a tubing simply facing the blood stream sideways, while the latter needs a tubing bent at a right angle so that its opening looks at the

blood flow head-on, thus giving a slightly higher pressure value. Between 1848 and 1850, Ludwig worked with Alfred Wilhelm Volkmann (1800–1877), a collaboration that led to Volkmanns's monograph titled *Hämodynamik nach Versuchen* (or *Hemodynamics After Test*) in 1850, where a full chapter was devoted to blood pressure [42]. As typical and usual of Ludwig's personality, his name did not appear **<AU: Ok to add "as an author" here?>**.

Belonging to his own harvest **<AU: can you please clarify this saying, as it is not commonly used>**, we find during the same period (i.e., Carl was rather young, about 32–33, spending his final time in Marburg, though he started in Zurich) that three papers were devoted to the heart [43]–[45]. We should point out that Ludwig had been promoted to the position as the first prosector in comparative anatomy in 1846. In the same year, he started his experiments on the physiology of circulation. In 1849, he was appointed professor and chair of the Department of Anatomy and Physiology at the University of Zürich, Switzerland. Thereafter, in 1850, in collaboration with Moritz Hoffa, another report dealing with the heart movement was produced [46]. In it, they described for the first time what is now called *ventricular fibrillation*, which was triggered by faradic stimulation (*alternate current* in present terminology). They called it *delirium cordis* or "Herzdelirium" (cardiac delirium). Long was the story **<AU: Do you mean "Long story short" here? Please clarify what is meant by "Long was the story to follow in that">** to follow in that specific subject, first picked up by John Alexander MacWilliam (1857–1937), another student of Ludwig, when he spoke of the fibrillar contractions of the heart [47], [48].

Ludwig's students dealt with other cardiac physiology subjects, such as the staircase or Treppe phenomenon [myocardial contractility increases with an increase in heart rate, studied by the American physiologist Henry Pickering Bowditch (1840–1911)]. In a modified version of the isolated heart, a cannula was inserted into the atrium of an excised frog heart and advanced into the ventricle. Another cannula, placed into the ventricle, was connected to a manometer. The heart, thereafter, isolated and perfused with rabbit serum, was electrically stimulated, recording the frequency and amplitude of the contractions with a kymograph. So the Treppe phenomenon was discovered. The amplitude of contractions increased until a plateau was reached when stimulation of the apex was initiated and maintained after a resting period of a few minutes. These experiments then led to the concept of the all-or-none-law, the absolute refractory period, and the origin of cardiac automaticity [49]. The Luciani progressive block [50], in the hands of the Italian physiologist Luigi Luciani (1840–1919), is a form of partial cardiac block characterized by a progressive prolongation of the P-R interval until a ventricular QRS **<AU: Kindly spell out QRS and P-R.>** complex is dropped. After such a failure in the atrioventricular conduction, the P-R interval shortens, only to progressively lengthen again and repeat the whole cycle as described earlier. A similar pattern may occur in lower species (fishes and herptiles) between the sinus venosus (SV-wave) and the atria (P-wave) or, in mammals, between the sinoatrial node and the atria, even though the latter is rare and difficult to demonstrate (Figure 4). A more adequate name, to be historically fair, should be the Wenckebach-Luciani block [51], [52], because of the contribution of the Dutch cardiologist and anatomist Karel Frederik Wenckebach (1864–1940).

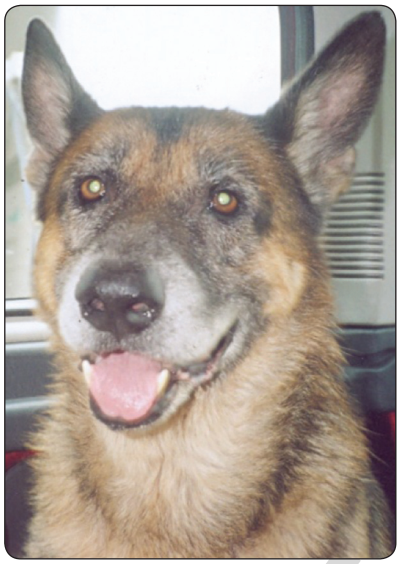

FIGURE 6 Apollo (1993–2005), a beautiful male German Shepherd. Look at his glowing eyes and the alert, upward ears. Even though his white snout shows his then 12 years of age, there is aesthetic content that brings pleasure to an observer. Do you know how many gave their lives to physiology? "Nur Gott, der Herr hat sie gezählt." Translated, this means "Only the great engineer has counted them." Physiology labs should also have a monument dedicated to this animal **<AU: Please check that edited sentence is OK>** (Photo courtesy of Max E. Valentinuzzi.)

It is difficult to separate exactly what all of Ludwig's contributions were to physiology at large, because he often mixed his contributions with those of his students, whom he closely supervised and followed. A long and detailed account can be found in Schröer's book [6]; this is not the place to go into further detail, but certainly Ludwig's influence, thought, and creative philosophy permeated subjects such as respiratory gas exchange; capillary permeability; absorption and metabolism of sugar, fat, and proteins; and the formation and movement of lymph. With respect to the latter, we refer to articles by Wladimir Tomsa (1831–1895), from Prague, and Theodor Zawarykin (1835–1905), from Russia [57]–[62].

Discussion

Some people erroneously believe that Ludwig's contributions in physiology were few and minor. Without a doubt, there is an overt lack of good information, due to the generosity and low personal profile displayed by this exemplary scientist. The answer is a loud "no," **<AU: Please note that there is no question asked here and "the answer is no" is given. Kindly clarify.>** for Ludwig was an accomplished physiologist standing on his own ideas and production. In this column, we partially describe some of the various physiological areas he covered, some showing outstanding discoveries, and others of less impact, but nonetheless not insignificant. **<AU: Kindly check that edited sentence retains original meaning.>** As mentioned before, it is difficult to determine how much he was involved, but there is no doubt that his supervision and touch was always present. As Spieckermann says, "physiology was Ludwig's obsession" [63], and he devoted his life to it.

However, something else should be added. Ludwig said in his habilitation

thesis [10]: “Thus, we observe in these relationships [referring to the kidneys] simplicity and a harmony not surpassed in beauty by any other natural process.” Once more, the aesthetic side of science comes up, as long before advocated by Ludwig van Beethoven (1770–1827), in turn, and inspired by the poet and philosopher Johann Christoph Friedrich Schiller’s views (1759): “Isn’t that experiment, that surgical field, that graph, that formula a beauty?” To pursue and find the aesthetic content that leads to true freedom, as they proposed, the former in *An die Freude*, to joy, even though he meant “freiheit,” or “liberty,” and the latter in his ninth symphony represent an essential mission of a human being, perhaps what makes him/her human and different from animals. No wonder that the “Ode to Joy” has become the European anthem **<AU: please note that “hymn” was changed to “anthem” here for clarity. OK?>.** Theirs was an eternal message to all mankind. We may slightly modify these thoughts as “the aesthetic content leads to true freedom and richness of spirit” [64]. Look at the frog shown in Figure 5, for example, look at its glowing eyes. Isn’t it a beauty, isn’t it aesthetic? It can catch a mosquito in a jiffy; surely, you can’t do that. See the dog in Figure 6, alert, watching you.

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References <AU: As many of these references are from older texts, please supply as much information as possible. Otherwise, please ignore query.>

[1] K. Thurau, J. M. Davies, and D. A. Häberle, “Carl Friedrich Wilhelm Ludwig: The founder of modern renal physiology,” *Pflügers Arch. Eur. J. Physiol.*, vol. 432, pp. R68–R72, 1996. **<AU: Kindly provide the issue no.>**

[2] M. E. Valentinuzzi, K. Beneke, and G. E. González, “Ludwig: The bioengineer,” *IEEE Pulse*, vol. 3, no. 4, pp. 68–78, 2012.

[3] C. Ludwig, *Rede beim Beginn der Vorlesungen in der Neuen Physiologischen Anstalt zu Leipzig* (in German, *Address on the Occasion of the Beginning of Classes in the New Physiology Building of Leipzig*). Leipzig, S. Hirzel Publisher, 1869. **<AU: Kindly provide the location of the publisher.>**

[4] H. G. Zimmer, “Carl Ludwig: The man, his time, his influence,” *Pflügers Arch. Eur. J. Physiol.*, vol. 432, pp. R9–R22, 1996. **<AU: Kindly provide the issue no.>**

[5] H. G. Zimmer. (2007). Carl Ludwig. Internal Report of the Carl-Ludwig-Institute of Physiology, Univ. Leipzig, Germany [Online]. Available: http://www.feps.org/downloads/CarlLudwig.pdf

[6] H. Schröer, *Carl Ludwig: Begründer der messenden Experimentalphysiologie*. Stuttgart, Deutschland: Wissenschaftliche Verlagsgesellschaft M.B.H., 1967.

[7] C. Ludwig, *Lehrbuch der Physiologie der Menschen* (in German, *Textbook of Human Physiology*), erster band (vol. 1), *Physiologie der Atome, der Aggregatzustände, der Nerven und Muskeln* (*Physiology of the Atom, Physical States, Nerves and Muscles*). Heidelberg: Winters’che Verlagsbuchhandlung, 1852.

[8] C. Ludwig, *Lehrbuch der Physiologie der Menschen* (in German, *Textbook of Human Physiology*), vol. 2, *Neubearbeitete Auflage* [revised edition]. Heidelberg: C.F. Winters’che Verlagsbuchhandlung, 1858.

[9] C. Ludwig. (1861). *Lehrbuch der Physiologie der Menschen* (in German, *Textbook of Human Physiology*), vol. 2, *Neubearbeitete Auflage* [revised ed], *Aufbau und Verfall der Säfte und Gewebe, Thierische Wärme* [*Structure and Decay of Fluids and Tissues, Animal Heat*]. Heidelberg: C. F. Winters´che Verlagsbuchhandlung [Online]. Available: http://books.google.de/books?id=CXmBNoziH8wC&printsec=frontcover&hl=de&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false

[10] C. Ludwig, “De viribus physicis secretionen urinae adjuvantibus,” (in Latin, “On the physical forces that promote the secretion of urine”), Marburge Cattorum, Habilitationsschrift, 1842.

[11] L. Brunton. (1912, Mar. 19). Ludwig’s and other theories of the secretion of urine and the action of diuretics. *Proc. Roy. Soc. Med.* (Therapeutical and Pharmacological Section) [Online]. pp. 133–151. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2005444/

[12] H. Seller, “Carl Ludwig and the localization of the medullary vasomotor center: Old and new concepts of the generation of sympathetic tone,” *Pflüger’s Arch. Eur. J. Physiol.*, vol. 432, pp. R94–R98, 1996. **<AU: Kindly provide the issue no.>**

[13] C. Ludwig and L. Thiry, “Über den Einfluß des Halsmarkes auf den Blutstrom,” (in German, “On the influence of cervical roots over the circulation”), *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften zu Wien. Mathematisch-Naturwissenschaftliche Classe*, vol. 49, pp. 421–454, 1864. **<AU: Kindly provide the issue no.>**

[14] P. Owsjannikow, “Die tonischen und reflectorischen Zentren der Gefäßnerven, ” (in German, “Tonic and reflex center of the vessel nerves”), *Berichte über die Verhandlungen der Königlich Sächsischen Gesellschaft der Wissenschaften zu Leipzig: Mathematisch-Physische Klasse*, vol. 23, pp. 135–147, 1871. **<AU: Kindly provide the issue no.>**

[15] C. Dittmar, “Über die Lage des sogenannten Gefäßzentrum in der Medulla Oblongata” (in German, “On the location of the so-called vascular center in the medulla oblongata”], *Berichte über die Verhandlungen der Königlich-Sächsischen-Gesellschaft der Wissenschaften zu Leipzig: Mathematisch-Physische Klasse*, vol. 25, pp. 449–469, 1873. **<AU: Kindly provide the issue no.>**

[16] E. H. Ackerknecht, “The history of the discovery of the vegetative (autonomic) nervous system,” *Med. Hist.*, vol. 18, pp. 1–8, 1974. **<AU: Kindly provide the issue no.>**

[17] E. Weber and E. H. Weber, “Expériences qui prouvent que les nerfs vagues peuvent retarder le mouvement,” *Arch. Gén. Méd.*, suppl. 12, 1846. **<AU: Kindly provide the vol. no., issue no., and page range.>**

[18] E. Neil. (1961). Carl Ludwig and his pupils. *Circulation Res.* [Online]. *9*, pp. 971–978. Available: http://circres.ahajournals.org/content/9/5/971.full.pdf

[19] E. J. Marey, "Recherches sur le pouls au moyen d'un nouvel appareil enregistreur: le sphygmographe," *Comptes Rendus des Séances et Mémoires de la Société de Biologie*, vol. 3, pp. 281–309, 1859. **<AU: Kindly provide the issue no.>**

[20] E. J. Marey, *Physiologie médicale de la circulation du sang, basée sur l'étude graphique des mouvements du coeur et du pouls arteriel avec application aux malades de l'appareil circulatoire*. Paris: Delahaye, 1863.

[21] M. E. Valentinuzzi, T. Powell, H. E. Hoff, and L. A. Geddes, "Control parameters of the blood pressure regulatory system (Part I): Heart rate sensitivity," *Med. Biol. Eng.*, vol. 10, pp. 584–595, 1972. **<AU: Kindly provide the issue no.>**

[22] M. E. Valentinuzzi, T. Powell, H. E. Hoff, L. A. Geddes, and J. A. Posey, "Control parameters of the blood pressure regulatory system (Part II): Open-loop gain, reference pressure and basal heart rate," *Med. Biol. Eng.*, vol. 10, pp. 596–608, 1972. **<AU: Kindly provide the issue no.>**

[23] J. N. Czermak, "Über mechanische Vagusreizung beim Menschen" (in German, "On the mechanical excitation of the vagus in the human"], *Jenaische Zeitschrift für Medizin und Naturwissenschaft*, vol. 2, pp. 384–386, 1866. **<AU: Kindly provide the issue no.>**

[24] E. Cyon and C. Ludwig, "Die Reflexe eines der sensiblen Nerven des Herzens auf die motorischen der Blutgefäße" (in German, "Reflex activity of one of the sensitive cardiac nerves on the circulatory motor response"), *Arbeiten aus der Physiologischen Anstalt zu Leipzig*, vol. 1, pp. 128–149, 1866. **<AU: Kindly provide the issue no.>**

[25] E. Cyon, "Über den Einfluß der Temperaturveränderungen auf Zahl, Dauer und Stärke der Herzschläge" (in German, "On the influence of the temperature changes over the number, duration and strength of the heart beat") in *Arbeiten aus der Physiologischen Anstalt zu Leipzig*, Hirzel, Leipzig, 1866, vol. 1, pp. 77–127, communicated by C. Ludwig. Also published in *Berichte über die Verhandlungen der Königlich-Sächsischen Gesellschaft der Wissenschaften zu Leipzig: Mathematisch-Physische Klasse*, vol. 18, pp. 256–306, 1866. **<AU: Kindly provide the location of the publisher.>**

[26] H. N. Martin and E. C. Applegarth, "On the temperature limits of the vitality of the mammalian heart," *Stud. Biol. Lab. Johns Hopkins Univ.*, vol. 4, p. 275, 1889. **<AU: Kindly provide the issue no. and complete page range.>**

[27] O. Langendorff, "Untersuchungen am überlebenden Säugetierherzen" (In German, "Investigations on the surviving mammalian heart"], *Pflügers Arch. Gesammte Physiologie des Menschen und der Thiere*, vol. 61, p. 291, 1895. **<AU: Kindly provide the issue no., page range>.**

[28] O. Frank, "Zur Dynamik des Herzmuskels" (in German, "About the dynamics of the myocardial muscle"], *Zeitschrift für Biologie*, vol. 32, pp. 370–437, 1895. **<AU: Kindly provide the issue no.>**

[29] O. Loewi, "Über humorale Übertragbarkeit der Herznervenwirkung. I. Mitteilung" (in German, "Transferability of cardiac nerve activity. I. Communication"), *Archiv Physiol.*, vol. 189, pp. 239–242, 1921. **<AU: Kindly provide the issue no.>**

[30] M. Skrzypiec-Spring, B. Grotthus, A. Szeląg, and R. Schulz, "Isolated heart perfusion according to Langendorff—Still viable in the new millennium," *J. Pharmacol. Toxicol. Methods***,** vol. 55, no. 2, pp. 113–126, Mar.–Apr. 2007.

[31] H. G. Zimmer, "The isolated perfused heart and its pioneers," *News Physiol Sci*, vol. 13, pp. 203–210, Aug. 1998.

[32] D. Troiani and E. Manni. (2011). The work by Giulio Ceradini in explaining the mechanism of semilunar cardiac valve function. *Adv. Physiol. Educ.*, [Online]. *35*, pp. 110–113. Available: http://advan.physiology.org/content/35/2/110.full.pdf

[33] G. Ceradini, *Der Mechanismus der halbmondförmigen Herzklappen. Mit einer lithographirten Tafel* (in German, *The Mechanism of the Semilunar Cardiac Valves. With a Litographed Table*). Leipzig: Verlag von S. Hirzel, 1872.

[34] G. Ceradini, *Il meccanismo delle valvole semilunari del cuore* (in Italian, *The Mechanism of the Semilunar Cardiac Valves*]. Firenze, Italy: Editoriale Cenniniana, no. 8, 1873.

[35] G. Ceradini, **"**Il meccanismo delle valvole semilunari del cuore" (in Italian, "The mechanism of the semilunar cardiac valves"), in *Opera Omnia*, vol. 1. Milano, Italy: Editoriale Hoepli, 1906, pp. 1–599. **<AU: Kindly provide the editor names.>**

[36] G. Ceradini, **"**Il meccanismo delle valvole semilunari del cuore" (in Italian, "The mechanism of the semilunar cardiac valves"), in *Opera Omnia*, vol. 2. Milano, Italy: Editoriale Hoepli, 1906, pp. 437–502.

[37] C. Ludwig, "Neue Versuche über die Beihilfe der Nerven zur Speichelabsonderung" (in German, "New investigations on the action of nerves over the salivary glands"), *Mittheilungen der Züricher Naturforschenden Gesellschaft*, vol. 50, pp. 210–239, 1850. **<AU: Kindly provide the issue no.>**

[38] J. R. Garrett, J. Ekström, and L. C. Anderson, "Neural mechanisms of salivary gland secretion," in *Frontiers of Oral Biology*, R. W. A. Linden, Ed. Basel, Switzerland: Krager, vol. 11, 1999. **<AU: Kindly provide the page range.>**

[39] E. H. Weber and W. Weber, *Wellenlehre auf Experimente gegründet oder über die Wellen tropfbarer Flüssigkeiten mit Anwendung auf die Schall-und Lichtwellen* (in German, *Wave Lessons Based on Experiments or on Waves of Dripping Fluids with Applications to Sound and Light Waves*). Leipzig: Gerhard Fleischer, 1825.

[40] L. Spengler, "Über die Stärke des arteriellen Blutstromes" (in German, "On the strength of the arterial bloodflow"), *Müllers Archiv für Anatomie, Physiologie und Wissenschaftliche Medicin*, pp. 49–69, 1844. **<AU: Kindly provide the vol. no., issue no. and the abbreviation of the journal.>**

[41] K. Beneke. (2005). Carl Friedrich Wilhelm Ludwig: Mitbegründer der messenden Experimentalphysiologie und Erfinder des Kymographen (in German, Cofounder of measurements in experimental physiology and inventor of the kymograph), Institut für Anorganische Chemie der Christian-Albrechts-Universität, Kiel, Germany [Online]. Available: http://www.uni-kiel.de/anorg/lagaly/group/klausSchiver/ludwig.pdf

[42] A. W. Volkmann. (1850). *Die Hämodynamik nach Versuchen*. von Breitkopf-Härtel: Leipzig [Online]. Available: http://books.google.de/books?id=VSNOAAAAcAAJ&printsec=fro

ntcover&hl=de&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false

[43] C. Ludwig, "Über die Herznerven des Frosches" (in German, "On the frog's cardiac nerves"). *Müllers Archiv Anatom. Physiol. Wissenschaft. Med.*, p. 139, 1848. **<AU: Kindly provide the vol. no., issue no., and complete page range.>**

[44] C. Ludwig, "Über den Herzspitzenstoß" (in German, "On the apical cardiac impulse"), *Henle und Pfeuffers Zeitschrift für rationelle Medicin*, vol. 6, p. 191, 1848. **<AU: Kindly provide the vol. no., issue no., complete page range>**

[45] C. Ludwig, "Über den Bau und die Bewegungen des Herzventrikel" (in German, "On the genesis and movements of the ventricle"], *Henle und Pfeuffers Zeitschrift für rationelle Medicin*, vol. 7, p. 189, 1849. **<AU: Kindly provide the issue no., complete page range.>**

[46] M. Hoffa und C. Ludwig, "Einige neue Versuche über Herzbewegung" (in German, "A new study on the cardiac movement"). *Henle und Pfeuffers Zeitschrift für rationelle Medicin*, vol. 9, pp. 107–144, 1850. **<AU: Kindly provide the vol. no., issue no. and the abbreviation of the journal.>**

[47] J. A. MacWilliam, "Fibrilar contraction of the heart," *J. Physiol.*, vol. 8, pp. 296–310, 1887. **<AU: Kindly provide the issue no.>**

[48] M. E. Valentinuzzi, *Fibrillation-Defibrillation: Clinical and Engineering Aspects* (Series on Bioengineering & Biomedical Engineering, vol. 6), Singapore: World Scientific, 2010.

[49] H. P. Bowditch, "Über die Eigenthümlichkeiten der Reizbarkeit, welche die Muskelfasern des Herzens zeigen" (in German**, "**About the peculiarities of irritability, showing the muscle fibers of the heart"), in *Berichte über die Verhandlungen der Königlich-Sächsischen Gesellschaft der Wissenschaften zu Leipzig: Mathematisch-Physische Klasse*, vol. 23, 1871, pp. 652–689. Also in *Arbeiten aus der Physiologischen Anstalt zu Leipzig* vol. 6, pp. 139–176, 1871. **<AU: Kindly provide the editor names and name and location of the publisher.>**

[50] L. Luciani, "Eine periodische Function des isolirten Froschherzens" (in German, "A periodical function of the frog's heart"). *Arbeiten aus der Physiologischen Anstalt zu Leipzig*, vol. 7, p. 113, 1872. **<AU: Kindly provide the issue no.>**

[51] M. E. Valentinuzzi and H. E. Hoff, "The sinus venosus-atrial Wenckebach-Luciani phenomenon," *J. Electrocardiol.*, vol. 5, pp. 1–14, 1972. **<AU: Kindly provide the issue no.>**

[52] C. B. Upshaw, Jr., and M. E. Silverman. (2000). Luigi Luciani and the earliest graphic demonstration of Wenckebach periodicity. *Circulation* [Online]. *101*, pp. 2662–2668,. Available: http://circ.ahajournals.org/content/101/22/2662.full

[57] C. Ludwig and W. Tomsa, "Die Anfänge der Lymphgefäße im Hoden" (in German, "The beginning of the lymphatic vessels in the testicle"). *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften zu Wien, Mathematisch, Naturwissenschaftliche Classe*, vol. 44, p. 155, 1861. **<AU: Kindly provide the issue no., complete page range.>**

[58] W. Tomsa, "Beitrage zur Lymphbildung" (in German, "Contributions to the generation of lymph"), *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften zu Wien, Mathematisch, Naturwissenschaftliche Classe*, vol. 46B, p. 185, 1863. **<AU: Kindly provide the issue no., complete page range, and the abbreviation of the journal.>**

[59] C. Ludwig and W. Tomsa, "Die Lymphgefäße des Hodens und ihr Verhältnis zu den Blut- und Samengefäßen" (in German, Lymph circulation in the testicles and its relationship to blood and seminiferous circulation"), *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften zu Wien, Mathematisch, Naturwissenschaftliche Classe*, vol. 46B, p. 221, 1863. **<AU: Kindly provide the issue nono., complete page range>.**

[60] W. Tomsa, "Beiträge zur Anatomie des Lymphgefäß-Ursprungs" (in German, "Contribution to the anatomy of the lymph circulation-Origin"), *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften zu Wien, Mathematisch, Naturwissenschaftliche Classe*, vol. 46, p. 324, 1863. **<AU: Kindly provide the issue no., complete page range.>**

[61] C. Ludwig and Th. Zawarykin, "Die Lymphwurzeln in der Niere des Säugethieres. Eine vorläufige Mittheilung" (in German, "The kidney lymphatic roots in mammals"), *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften zu Wien, Mathematisch, Naturwissenschaftliche Classe*, vol. 47B, p. 242, 1863. **<AU: Kindly provide the issue no, complete page range.>**

[62] W. Tomsa, "Die Lymphwege der Milz" (in German, "Lymph movement in the spleen"), *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften zu Wien, Mathematische, Naturwissenschaftliche Classe*, vol. 48B, p. 652, 1863. **<AU: Kindly provide the issue no., complete page range, and the abbreviation of the journal.>**

[63] P. G. Spieckermann, "Physiology with cool obsession: Carl Ludwig—His time in Vienna and his contributions to isolated organ methodology," *Pflügers Arch. Eur. J. Physiol.*, vol. 432, pp. R33–R41, 1996. **<AU: Kindly provide the issue no.>**

[64] M. E. Valentinuzzi. (2012). 50 years a biomedical engineer: Remembering a long and fascinating journey. *Biomed. Eng.* [Online]. Available: http://www.biomedical-engineering-online.com/content/11/1/1

A general feature of Carl Ludwig's research was set on rigorous scientific thinking, way of working, perseverance, and self-criticism.

Ceradini conceived a simple technique for observing the behavior of these valves during the cardiac cycle.

Ceradini's observations clearly indicated that the closure of the valves was not the effect of the beginning of diastole but of the end of systole.

The vagal (or parasympathetic) action on the heart is common physiological knowledge today, with its decreased cardiac rate and arterial blood pressure, which are also involved in the baroreceptors' reflex.