Were the first ones really the first ones?

Jaakko Malmivuo
Department of Electrical Engineering and Automation
Aalto University
Espoo, Finland
jaakko.malmivuo@aalto.fi

Jussi Honkonen
Idman Airfield Lighting
Vantaa, Finland

Katrina Wendel-Mitoraj
BrainCare
Pirkkala, Finland

***Abstract*— In the history of science it is usually listed who were the scientists who first made certain important contributions to the science. So it is in the field of bioelectromagnetism as well. A typical example is Luigi Galvani as the first to do bimetallic stimulation of the frog muscle. Without attempting to take from Galvani the honor as famous scientist I will critically consider his contributions to bioelectromagnetism and introduce another person to be the first to do bimetallic stimulation. He was Jan Swammerdam, whose experiment we considered theoretically and repeated experimentally to prove him being the first to do bimetallic stimulation over 100 years before Galvani.**

Keywords—bimetallic stimulation, frog muscle, frog nerve

I. Introduction

Luigi Galvani (Italian, 1745–1827), who was professor of anatomy at the University of Bologna, produced electric stimulation of the frog leg in 1781 with electricity generated with electric machine [1]. In September 1786, Galvani was trying to obtain frog leg contractions from atmospheric electricity during calm weather when no thunderstorm and lightning existed. He suspended frog preparations from an iron railing in his garden by brass hooks inserted through the spinal cord of the frogs. The hook was hanging on the iron railing and the frog leg happened to contact the iron structure. Due to this contact the frog leg muscle was stimulated and contracted [2].

Galvani continued his experiments systematically and found that when the frog nerve and muscle were touched with a bimetallic arch of copper and zinc, similar stimulation of the muscle was produced. This experiment is popularly known and it is usually cited as the classic study to demonstrate the existence of bioelectricity [3 p. 39].

But was Luigi Galvani certainly the first to do and document this kind of experiment of bimetallic stimulation? It has been speculated that the first one actually was Jan Swammerdam who conducted and documented similar experiment over 100 years before Galvani in 1664 [3, 4]. Jan Swammerdam documented his experiment very accurately which gives the possibility to reproduce it. We considered his experiment theoretically and repeated the experiment. We succeeded to demonstrate both theoretically and experimentally that Jan Swammerdam made the first experiment of bimetallic stimulation. However, apparently he did not understand the mechanism of his experiment, but he was nevertheless the first.

Even though Swammerdam was the first to do well documented bimetallic stimulation experiment, we do not want to take the honor away from Galvani. Firstly, Galvani made his work without knowing about Swammerdam. Secondly, for his merit we have to count that he continued to do his experiments very systematically and he attempted to understand the mechanism in the experiment. In addition, he published his work in detail [2], and he got wide publicity for it and thus started a new discipline.

For Galvani's benefit I would also like to mention that he actually invented another very important physical phenomenon which was reinvented one hundred years later. Similarly as Swammerdam did not understand what was the mechanism in his experiment, Galvani did not understand this new phenomenon. I will reveal this invention in the oral presentation.

II. Swammerdam's Experiment

The first carefully documented scientific experiments in neuromuscular physiology were conducted by Jan Swammerdam (Dutch, 1637-80). At that time it was believed that the contraction of a muscle was caused by the flow of "animal spirits" or "nervous fluid" along the nerve to the muscle making it thicker and shorter. In 1664, Swammerdam conducted experiments to study the muscle volume changes during contraction, Fig. 1. The experiment was described so detailed in his book which was published posthumously in 1738 [5] that it is possible to reproduce it.

Swammerdam placed a frog muscle (b) into a glass vessel (a). When contraction of the muscle was initiated by stimulation of its motor nerve, a water droplet (e) in a narrow tube, projecting from the vessel, did not move, indicating that the muscle did not expand. Thus, the contraction could not be a consequence of inflow of nervous fluid. Actually in this experimental arrangement no fluid could flow along the nerve because it was cut off and isolated from the body.

In this experiment stimulation was achieved by pulling the nerve with a wire (c) made of silver (*filium argenteum*) against a loop (d) made of brass (*filium aeneum*). According to the principles of electrochemistry, the dissimilar metals in this experiment, which are embedded in the electrolyte provided by the tissue, are the origin of an electromotive force (emf) and an associated electric current. The latter flows through the metals and the tissue, and is responsible for the stimulation (activation) of the nerve in this tissue preparation. In other similar experiments, Swammerdam stimulated the motor nerve by mechanically pinching it.

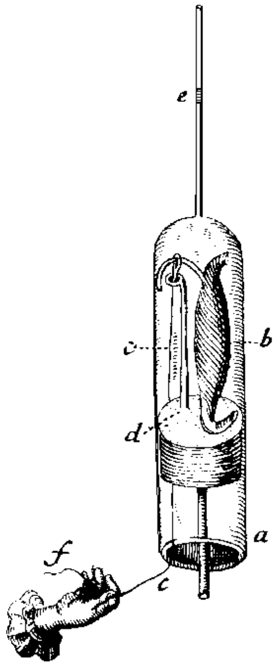

Fig. 1. Swammerdam's experiment

It has been speculated that this was the first documented experiment of motor nerve stimulation resulting from an emf generated at a bimetallic junction [4]. Swammerdam apparently did not understand that neuromuscular excitation is an electric phenomenon. Some authors interpret the aforementioned stimulation to have resulted actually from the mechanical stretching of the nerve which we doubt.

III. Theoretical Background

A. Metals used in this Work

Swammerdam used brass and silver to stimulate frog's muscle. Brass is an alloy of copper and zinc. In the late 17th century calamine brass was still used where zinc-percentage was about 15 % to 30 %. New smelting methods were developed during the 18th century and nowadays the zinc-percentage for normal common brass is about 37 % [6, 7]. Dezincification occurs in brass when it is in electrolyte with another metal. Zinc is selectively leached from brass alloy and copper remains. This changes the mechanical properties of brass [8]. Theoretical half-cell potential for brass is from -400 mV to -260 mV referenced to Saturated Calomel Electrode, SCE. This range is for all brasses. There is no reference of half-cell potential for calamine brass, but it is pure zinc-copper alloy so it must be within the aforementioned voltage range [9].

Silver is precious metal which has the highest electrical and thermal conductivity of any metal. Processes of making silver and the purity of silver have not drastically changed after 17th century. Theoretical half-cell potential for silver is from -150 mV to -100 mV referenced to SCE [9]. Voltage difference between brass and silver is then from 110 mV to 300 mV. Temperature affects on reaction speed. In higher temperatures the reaction speed rises and therefore the volt-age between the metals rises [10].

Theoretical voltage differences for different metal pairs are shown in Table 1. These metals are iron, copper, silver and brass. Cathodic metal is on the left and anodic metal is on the right. These values are calculated from the maximum and minimum half-cell potentials for each metal. [9, 11, 12]

Table 1 Calculated theoretical voltage between different metal pairs in electrolyte [9, 11, 12]

Metal Pair	Voltage [mV]
Copper – Brass	***-110…100***
Silver – Copper	***150…270***
Silver – Brass	***110…300***
Copper – Iron	***250…420***
Brass – Iron	***200…450***
Silver – Iron	***460…600***

Voltage differences were calculated with different metal pairs for comparing them to the later measurements. Later on the stimulation is tested with different metal pairs to achieve different voltages for stimulation. Two of these metal pairs have lower voltage difference than brass and silver and three of them have higher voltage difference.

B. Frog's Muscle

Jan Swammerdam probably used common frog, *Rana temporaria* in his experiment. In our experiment oriental fire-bellied toad, *Bombina orientalis* is used instead. They are from different families and they differ from each other at size [13]. It is assumed, that between these frog species threshold voltages of muscles and electrical properties do not differ from each other radically.

Rheobase is the minimum strength of an electrical stimulus that is able to cause contraction of a muscle. The average rheobase for frog's sartorius muscle occurs at -51.9 mV when the average resting potential is -92.9 mV [14]. The voltage needed to raise the membrane potential from resting potential to the rheobase is called threshold voltage. The calculated threshold voltage is then 41 mV. These measurements were made with frog's sartorius muscle.

Voltage clamp method was used in the experiment by Adrian et al [14]. In voltage clamp method measuring electrode is put inside the nerve [1]. In this work the measuring of the threshold voltage is however achieved by stimulating the surface of the nerve. This is done with two silver electrodes and altering the voltage between them. This measurement can be done because the bimetallic stimulations are all made on the surface of the nerve. In Swammerdam's experiment brass and silver wires were also touching only the surface of the nerve.

The electrical properties of frog skin and blood are similar to human skin and blood [15]. To approximate the voltages between different metal pairs in frog's nerve before the actual measurements with the frog are conducted, we can measure voltages between different metal pairs in physiological saline solution. This is because the physiological saline solution has the same electrical properties as bodily fluids.

IV. Measurements with Metals

Voltage measurements between different metals in physiological saline solution were made to compare them to the theoretical values. Fifty milliliters of physiological saline solution was poured in to a glass beaker. Then the silver wire and the brass wire were placed in to the solution. Voltage meter was connected to the wires with alligator clips, silver to + and brass to -. Five voltage readings between the metal pairs were taken. Between each measurement wires were cleaned, dried and put back in the solution. Physiological saline solution was in room temperature in each of the measurements.

Results from the measurements between different metal pairs are shown in Table 2. These are average values from five different measurements with each of the metal pairs and they are in the range of theoretical values which are shown in Table 1.

Table 2 Voltage between different metal pairs in physiological saline solution.

Metal Pair	Voltage [mV]
Copper – Brass	***52***
Silver – Copper	***176***
Silver – Brass	***242***
Copper – Iron	***416***
Brass – Iron	***423***
Silver – Iron	***616***

Voltage between brass and silver seems to be high enough to activate frog's muscle, because the theoretical threshold voltage for the frog's muscle is 41 mV [14]. Cal-culated voltage difference from measured half-cell values was 223 mV and the measured voltage in physiological saline solution at room temperature was 242 mV. Both of these voltages are over five times the theoretical threshold voltage necessary to stimulate the frog's sartorius muscle.

V. Measurements with Frog's Nerve and Muscle

A. Preparation

Preparation of the frog was done just before the measurements. First the frog's right leg was prepared and experiments were made with it. Then the left leg was prepared and experiments were repeated.

There is no specific information on which muscle Jan Swammerdam used in his experiment. It is only mentioned that he used thigh muscle, but not exactly which one of the thigh muscles [16]. The theoretical threshold voltage used in this work was obtained with frog's sartorius muscle, which is one of the thigh muscles [14]. In this experiment gastrocnemius muscle was used because the sciatic nerve connected to the muscle was more easily isolated from the leg than the nerve connected to the sartorius muscle. The threshold voltage should be the same for these nerves and muscles.

B. Testing of Bimetallic Stimulation

Bimetallic stimulation with frog's muscle was tested with different metal pairs. Nerve was put over the metal wire and it was stimulated with the other metal wire. Both wires were constantly in contact with the nerve and stimulation was achieved by connecting the metal wires together.

All metal pairs were able to stimulate the frog's muscle except one, brass and copper. The measured voltage between brass and copper in physiological saline solution was 52 mV and the theoretical voltage was from -110 mV to 100 mV. This was the lowest voltage between the different metal pairs used in this work so it can be assumed that the stimulation measurements were correct.

The most important metal pair in this work, silver and brass worked fine. Also the voltage between silver and copper was high enough to stimulate frog's muscle though that voltage is lower than that between silver and brass. It seems that the threshold voltage for frog's muscle is then something between 52 mV and 176 mV. These voltages are measured in physiological saline solution, so the real voltages in nerve between different metal pairs have to be measured before any further conclusions about the threshold voltage can be made.

C. Voltage Measurements with different Metal Pairs in Nerve

Voltages between different metal pairs on frog's nerve were measured with a voltage meter which was connected to different metal wires. Metal wires were then put on the nerve without touching each other. Voltage was then measured between the metals three times with each of the metal pairs. Other metal wire was taken off from the nerve between the measurements. These measurements were repeated with frog's different nerve similarly.

The results from the measurements for frog's both muscles are shown in Table 3. Average voltage values are calculated from the measured ones. These voltages are lower than the measured voltages in physiological saline solution. The electrical properties of frog's nerve and physiological saline solution are not quite the same. These lower voltages may be also due to the impurities on the surfaces of the metals. Metal-chloride ions on metal wires can effect on voltage. Nevertheless these voltages between different metal pairs excluding copper and brass are still high enough to stimulate the frog's muscle. After these measurements with different metal pairs on frog's nerve it seems that the threshold voltage for frog's muscle is somewhere between 48 mV and 106 mV.

Table 3 Success of stimulation and average voltages between different metal pairs in nerve.

Metal Pair	Stimulation	Voltage [mV]
Copper – Brass	***No***	***48***
Silver – Copper	***Yes***	***106***
Silver – Brass	***Yes***	***147***
Copper – Iron	***Yes***	
Brass – Iron	***Yes***	
Silver – Iron	***Yes***	

D. Threshold Voltage for Frog's Nerve and Muscle

Real threshold voltage for frog's nerve and muscle was measured by connecting the nerve to DC-voltage supply. Silver wires were used as electrodes. One electrode was constantly connected to the nerve and the nerve was stimulated with the other electrode by touching the nerve with it.

The measured threshold voltage for the frog's muscle was 50 mV ± 1 mV. The voltage between silver and brass in nerve was 147 mV. That is almost three times the measured threshold voltage.

VI. Results and Conclusion

Voltages between different metal pairs and stimulation occurrence are shown in Table 3. The threshold voltage for frog's muscle was about 50 mV. The voltage between brass and silver in nerve was 147 mV. That is almost three times the voltage needed to stimulate the frog's muscle.

The stimulation also worked with silver and copper which have smaller voltage difference than silver and brass. In minimum the theoretical voltage between silver and brass is 110 mV. That is still over twice as much as the measured threshold voltage. The bimetallic stimulation was easy to achieve comparing it to the mechanical stimulation. [17, 18]

After these results we can say that it is highly probable that Jan Swammerdam did the first reported bimetallic stimulation over one hundred years before Luigi Galvani.

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