FROM LUIGI GALVANI UP TO NOW: BIOELECTROCHEMISTRY TWO CENTURIES LATER

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Introduction

In the decade of the eighties of two centuries ago, the so-called Animal electricity was considered a fascinating reality, stimulating the initiative of the physician Luigi Galvani, professor of anatomy at Bologna University and of gynaecology at the Arts and Sciences Institute, Bologna. For several years already, he had been doing fundamental research in anatomy and physiology; his thesis entitled De ossibus (concerning bones), considered very good and more than sufficient to obtain the appointment of lecturer De Rebus Medicis (concerning medical things), was presented in 1762. He continued very seriously his work when, almost exactly two centuries ago, in 1786, he observed a muscular contraction of the legs of a frog, dead and skinned, as a consequence of touching its nerves with scissors during a storm. His first paper on this theme was published several years later (1791) under the Latin title De Viribus Electricitatis in Motu Musculari, Commentarium (Concerning Electricity's forces in Muscular Motion) followed (in 1792) by Lettera al sig. Prof. Bassano Carminato: Sulla Sede della Electricità Animale (Letter to Prof.Bassano Carminato: On the origin of the Animal Electricity). and by another (1794) : Dell'uso dell'Attività dell'Arco

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Conduttore nella Contrazione dei Muscoli (Concerning the Activity of the Conducting Arc on Muscular Contraction).

The preceding observations, later condensed under the concise title "Galvani's frog experiments" apparently belong to a type of current experiments, as they are carried out by any scientist in order to solve a particular problem. They immediately excited a great attention beyond the limits of pure anatomy and physiology.

Galvani's interpretation of these experiments at that time was partly correct, but also partly wrong. He postulated the existence of a special fluid, animal electricity , that through metallic (or also non metallic) conductors originated the contraction of frogs' muscles. This interpretation was initially accepted by A.Volta, but, later, strongly criticized. Also Volta was partially correct and partially wrong, because denying the existence the animal electricity, he attributed the muscular contraction (observed by Galvani) to the contact potential difference between two different metals, that discharging through the biological medium (nerves, muscles and biological fluids acting as electric conductors) provokes the contraction.

Galvani's error was that no animal electricity does exist, while Volta's error was that he implicitly postulated some kind of perpetuum mobile . A somewhat more correct interpretation was given by a young German scientist, Johann Willhelm Ritter, who, although without any academic degree, had a natural, exceptional capacity of observation and also an exceptional capacity of inventing and correctly carrying out logically founded experiments. He drew attention to the fact that a difference of electric potential between two contacting metals could not act as an energy source, and that, therefore, the source of the energy required for the frog's muscular contraction should be found somewhere else, perhaps in a chemical reaction capable of releasing this energy.

In fact, a muscular contraction without any intervention of metallic electricity (according to Volta) had already been demonstrated by Galvani.

All these experiments, observations and controversies represented the starting point of a new branch of interdisciplinary scientific research: Bioelectrochemistry; but, chronologically, the very first unambiguous observation, and its tentative interpretation, was due to Galvani. He has, therefore, to be considered the true father and founder of bioelectrochemistry.

Development of Bioelectrochemistry during two centuries.

After this somewhat stormy start, bioelectrochemistry did develop rather slowly but continuously, widening its domain in various directions towards different biological phenomena, deepening our knowledge in biochemistry, photosynthesis, membrane phenomena, electrophysiology, cell fusion and gene transfer, medical diagnostics and therapeutic treatments,

etc, thus gradually revealing the electrochemical nature of many (perhaps the majority)of biological phenomena. It is not possible, in a short synthetic presentation like this one, to discuss and illustrate in detail the various fields branching out from the principal trunk of bioelectrochemistry and therefore, in order to show the variety of so many branchings it will be appropriate to discuss only some significant examples.

Chemical Metabolism

Chemical reactions occur in living bodies, *i.e.* chemical metabolism , first to store and subsequently to use materials and energy for vital requirements (nutrition, growth of tissues and organs, reproduction, elimination of waste material). They are usually redox reactions, capable of being monitored and explained by means of electrochemical techniques and theories. Let us consider, for example, the combustion of glucose and of its derivatives in mammals using the oxygen transported by hemoglobin . This combustion has as final products CO_2 and H_2O, but it occurs by steps through a series of intermediate reactions. Also other biological products, possibly containing S,P,N *etc.*, are burned in similar ways with production of the corresponding oxidized products. This reaction makes available the energy required by life. The investigation of such systems in their wholeness is very difficult, while it is easy to follow them after subdivision of the global reaction in simpler partial reactions. Practically all these simple reactions can be investigated electrochemically using the laws and rules of chemical thermodynamics and kinetics.

From the electric tension (usually, but wrongly, called electrode potential) thermodynamics gives the criterion to judge whether a certain reaction is possible and under what conditions it can occur. For example the first step of the glucose oxidation can be investigated using the following reactions (1) and (2)

$$C_6H_{12}O_6 + H_2O \rightarrow C_6H_{12}O_7 + 2H^+ + 2e^- \quad (1)$$

$$2H^+ + 2e^- + 1/2\,O_2 \rightarrow H_2O \quad (2)$$

$$C_6H_{12}O_6 + 1/2\,O_2 \rightarrow C_6H_{12}O_7 \quad (3)$$

which together give equation (3), *i.e.* glucose oxidation to gluconic acid. Reactions (1) and (2) can be easily followed by potentiometry, thus providing the value of the standard free enthalpy of reaction (3). Similar subdivisions of more complex biological systems occurring in subsequent steps, capable of being followed electrochemically in a complete and unequivocal way, are nearly always possible , thus providing the corresponding values of, for example, the free enthalpy of the direct oxidation of an oxidizable system by another reducible system, both simultaneously present in the given medium. The values obtained provide information on whether under the given conditions the first system can be oxidized by the second one. On the other hand, kinetic investigation, providing information on the activation energies, and the introduction of other considerations, as for example the analogy of the Franck and Condon principle enable us to follow the evolution of the observed system. Using appropriate electronic conductors (W, Au, C, *etc*) and appropriate reference electrodes, every half reaction, for example (1) and (2), becomes a galvanic element providing the standard electric tension and the electric tension under the experimental conditions required for the computation of the free enthalpy of the reaction (thermodynamic quantity). Subsequently by passing an anodic or cathodic current at the electrode being studied , the measured overtension provides the activation energy and the reaction rate (kinetic quantities). It is obvious that in living bodies no galvanic elements with metallic terminals are present, but on the basis of information on the free enthalpy of the half-reaction under given experimental conditions it is possible to establish which of those present in the living systems, is the oxidising one and which is the reducing one. In addition, the activation energy allows an evaluation, at least as a first approximation, of the reaction rate.

This is perhaps a somewhat oversimplified presentation of the biological importance of the redox reaction and the possibility of its electrochemical investigation . Of course other parameters must also be considered (presence of not entirely free reactants, energy contributions from adsorption phenomena, activities instead of concentrations *etc*.) in order to obtain more realistic results.

Photosynthesis

The primary event occurring for capture and storage of energy from the external medium through absorption of solar radiation, *i.e.* the photosynthesis, is a very complex

process, constituted globally by electronic excitation of certain absorbing molecular species, translocation of electrons and protons with subsequent creation of electric potential differences across separating walls (membranes) followed by several anabolic chemical reactions. Schematically the reaction can be symbolized as follows

$$A + H_2D + h\nu \longrightarrow H_2A + D$$

where A and D stand for electron acceptor and donor, respectively: this is, therefore, an endergonic reaction. The most simple example is the photosynthesis of carbohydrates carried out by the chlorophyll pigments present in green plants. Photosynthesis is, therefore, the primary source of the energy required by all living species directly (green plants, algae, various bacterial species), or indirectly (living bodies using for nutrition products photosynthesized by other living species). Small industries are working on the culture and subsequent growth of unicellular organisms to use them as biomass, mainly as fodder.

Membrane Phenomena

Membrane phenomena represent a very wide field. Membranes are organized tissues used in nature to accomplish several functions: to maintain, within the living cells, the conditions essential for their functioning , to selectively transport given species, including electrons, to carry out special surface reactions, *etc*. In living bodies a membrane acts as a separation barrier and must be capable of opening and closing channels to allow or stop, more or less selectively, transfer of species. In the case of ions crossing the membrane, several electrical and/or electrochemical phenomena occur, regulating in turn the further course of the process. The spaces separated by a membrane are generally iso-osmotic; therefore the transfer of species must be governed by non-osmotic forces, *i.e.* by an electrochemical mechanism. In fact, electric potential differences can be measured across biological membranes separating two iso-osmotic regions containing ions capable of being transported selectively. In some cases a coupling of transfer across a membrane with an exoenergetic chemical reaction makes possible the simultaneous transfer of both species and energy. Such couplings are often observed in the so-called active transport, where one, or more components migrate against the gradient of its electrochemical potential.

Such an event could not even be suspected as possible under ordinary conditions because it is in contradiction with the principles of thermodynamics. To get a possible interpretation two hypotheses have been developed and are still in conflict. The first one tries to connect (and to find connecting mechanisms) between the event producing free enthalpyh with the event requiring energy needed to transfer a component from a lower to a higher energy level (the ionic pumps or the thermodynamic coupling).The other tries to solve the problem on a purely theoretical basis considering all possible interactions between all

components of the system and solving the resulting differential equations. Some positive results have been obtained in certain cases as, for example, the case of negative conductance, *i.e.* ionic migration against the electric potential gradient, without any intervention of an additional hypothetical ionic pump.

An important medical application is represented by the modification of transmembrane potentials utilized in anaesthesia and electroanaesthesia. It is very likely that the drugs or the electrical pulses, modify the electric conductance of cell membranes toward electrically charged species, thus producing a lack of balance and the observed anaesthesia.

Information Transmission in Nervous System

The transmission of information in living bodies occurs through rather complicated mechanisms. One of the most important ones is constituted by electrical signals sent by the peripheral to the central nervous system and re-transmission by the latter, to the organs to accomplish the required actions. Various types of electrical conduction are operating in this process: ionic, electronic and semiconductor . This is the domain of electrophysiology, covering vision, motion, heart beat, pain, hunger, thirst *etc.*, directly deriving from Galvani's experiments, where an electrical pulse caused a muscular contraction.

Electrofusion and Gene Transfer

External electrical pulses can lead to the creation of membrane pores of relatively long lifetime, *i.e.* an increased membrane permeability. This phenomenon is called electroporation.

Areas of two membranes of two neighbouring cells both having an electrically induced layer porosity, can fuse into only one membrane, in the case of the two cells touching together: cell electrofusion. In such a way double helices of DNA can cross the porous membrane and become part of the cellular genoma: gene transfer.

Electroporation, electrofusion and gene transfer are more and more utilized in biotechnology, *i.e.* in new industries producing genetically modified organisms, which, in turn, are utilized to produce biologically important pharmaceutical products at lower costs, like insulin, various hormones, peptides etc.

Biomedical Applications

The last, but not least important example to be mentioned is medical application in diagnosis and therapy. Concerning diagnosis it is enough to remember the large number of electrodes (usual selective membrane, enzyme *etc*) by means of which a large number of chemical components (both physiological and pathological) of living bodies can be identified and quantified. The number and variety of these new electrodes has grown in an almost explosive way during the last 20 years. They are based either on direct measurement, for example O_2 in blood, or indirect of an

electrochemically inert component of interest, that catalyzed by an appropriate enzyme reacts quantitatively with another component present in excess in the system , giving an electroactive compound. For example, urea catalyzed by urease produces ammonia, that, in turn, modifies proportionally the local pH of the fluid contacting the electrode. Complex electrodes are nearly always membrane electrodes, once more underlining the importance of membranes, physiological, natural and also artificial, in investigating systems in vivo both in physiological and/or pathological conditions. During recent years processes and techniques were set up in order to obtain quantitative data on antibodies and antigens in living organisms.

In therapy several applications are already in regular use: tissue stimulation for better cicatrization, regulation of cardiac arithmies, pacemakers, psoriasis, treatment of bone fractures, even in very bad conditions. Statistics of some years ago gave for this last application positive results of recovery of about 75 % of the cases treated.

Probably today this value is even better. Some significant examples are collected in the following table:

Extreme cases of bone fractures treated by means of electromagnetic alternating pulsating fields with complete recovery.

Patient	Age	Bone	Type	Surg.	Infect	Amput	Treatment
	years	fract		operation		recomm.	months
1	3	tibia	congen	4	no	yes	2
2	12	tibia	congen	12	no	yes	3
3	19	tibia	congen	3	no	no	4
4	34	tibia	acquir	4	yes	yes	7
5	38	shoulder	"	3	yes	no	4
6	47	splint bone	acquir	-	no	no	4
7	62	femur	acquir	1	yes	yes	6

The therapeutic treatment consists of the application in pulses of an alternating electromagnetic field of appropriate frequencies of both the field and its pulses.

The mechanism of recovery is still obscure but it is certain that it is electrochemical in nature.

What has been said is still an incomplete description of the extraordinary development of bioelectrochemistry in very different biological directions. But it is sufficient to demonstrate its importance and the requirement, because of its interdisciplinary character, of a strict cooperation amongst scientists of different basic education: biologists, chemists, physicists, physicians *etc.*

The development of bioelectrochemistry reminds us of the extraordinary development of another branch of science, apparently as routine research, also starting from Bologna University. At the time when Hertz, at the Technical

University of Karlsruhe and Righi at Bologna were investigating the propagation in space of electromagnetic waves, nobody could predict, perhaps not even suspect that another physicist, Guglielmo Marconi, developing ideas and experiments by Hertz and Righi, in 1901 could clearly receive, at Terranova, radio signals sent from Poldhu (Cornwall) across the Atlantic Ocean and that a few years later, in 1930, he could switch on the light at the International Exhibition at Sydney by means of a radio signal sent from his yacht in Genoa harbour, *i.e.* from one point to another point of the earth, at the antipodes, easily overcoming the curvature of the earth. The seed found by Hertz, sown by Righi and cultivated by Marconi developed into a variety of scientific and technical applications: radio-communications, radio-goniometry, guide to sea and air navigation, television, radar, cosmic exploration etc.

Conclusion

The conclusion to be drawn is that no scientific experiment or discovery, even if it is very small, can be neglected and that nobody can predict future developments of scientific research, even if apparently very restricted, provided it is carried out with maximum of rigour, honesty and humility - as Galvani always did in his scientific activity.