EXPERIENTIA

Vol. 36 - Fasc. 11 Pag. 1243-1336 15.11.80

GENERALIA

Bioelectrochemistry and bioenergetics. An interdisciplinary survey

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Introduction[1]

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An appropriate first approach in trying to understand what is meant by bioelectrochemistry would have to start with its etymology. The 2 prefixes *bio* and *electro* already indicate that in this case we are dealing with an interdisciplinary branch of scientific research which investigates biological phenomena by using both experimental and theoretical electrochemical tools.

This definition, however, still seems to be too broad, and it would be worthwile to limit it further by an initial consideration of the whole series of biological phenomena. These can be roughly divided into 2 major groups. The 1st group considers life from an essentially morphological and functional point of view without neglecting, however, the environment which more or less conditions many biological phenomena. Examples of phenomena which can be classified in this group are cell subdivisions, morphological relationships, organization and differentiation of tissues and organs, etc. The 2nd group of phenomena includes physicochemical processes which develop mostly on a molecular basis, as for example, the respiratory chain (i.e. the combustion - at the expense of appropriately transferred atmospheric oxygen - of biological materials in the organisms through a series of redox reactions), heredity (transmission of typical properties through various combinations of genes), chemically induced cell proliferation, vision (photoelectric reactions), transmission of information by means of and through organized structures (conductance of electrical pulses through nerves, muscles, etc.), membrane phenomena which regulate the inflow and outflow of charged particles (ions) into and from living cells, the anabolic and catabolic processes through which the accumulation and consumption of the energy necessary for the vital processes are effected (photosynthesis, phosphorylation, amino acids and protein synthesis, catabolic reactions, etc.).

All the phenomena mentioned above as belonging to the 2nd group, are essentially of an electrochemical nature, and this justifies the definition of bioelectrochemistry as stated.

However, there is no precise demarcation line separating the 2 types of phenomena. As a matter of fact, between the two there is a slightly indefinite field of phenomena which could be studied using the principles and methods of either one of the 2 groups of phenomena: e.g. the phenomena caused by the behavior of biopolymers and macromolecules.

Thus bioelectrochemistry deals with electrical phenomena which are the result of the behavior of electrically charged particles belonging to biological systems; it also allows a large number of biological phenomena to be studied, including the energetics of living systems, by strict electrochemical methods (both experimental and theoretical) including theories on semiconductors and solid-state physics.

The above considerations led to the obvious conclusion that electrochemists, biophysicists, biologists and electrophysiologists must work in strict collaboration, without which any further development would remain just a dream that could not be translated into reality.

Perhaps unconsciously, but certainly in this spirit, scientists have been working in bioelectrochemistry for at least the past 2 centuries. It seems fitting to mention here the names of some biologists, physicians, physicochemists, chemists and physicists who may be considered the forerunners of the science of bioelectrochemistry.

First of all the physician Luigi Galvani (b. 1737), well known for his famous experiment on the frog (1792), can really be considered the founder of bioelectrochemistry. At about the same time the physicist Alessandro Volta (b. 1745), while taking a polemic attitude toward Galvani's theory, directed his attention to the electrical phenomena in living beings. A few years later, J. W. Ritter (b. 1776) started investigations in relation to electrophysiology. More recently, the physician Leonor Michaelis (b. 1875) and the 3 chemists René Bernard Wurmser (b. 1890), David Keilin (b. 1887) and Albert Szent György (b. 1893) brought decisive contributions to the development of bioelectrochemistry. The former devoted the greater part of his work to the study of biochemical methods and introduced for the first time in the study of biological processes the concept of quantitative redox reactions, with the participation of ionic species. Wurmser continuing in Michaelis' footsteps, found a way of studying potentiometrically - that is with strict electrochemical methods and techniques - many important biological redox reactions, thus finding a connection with the biological activity of living cells Keilin was the first to bring forth the model of the redox chains in respiratory processes. Szent György introduced the theories of semiconductors and solid-state physics into the investigation of the electric conductance through proteins and biological macromolecules, and thus through organized structures, and thereby made a considerable contribution to our understanding of the biological phenomena in this field.

Even more recently, the physico-chemist Ilia Prigogine (b. 1917) came forth with a detailed synthesis of the theoretical work done in the past and introduced some sophisticated original concepts (dissipative structures, local equilibrium, etc.). On the basis of a large mathematical framework, he was successful in obtaining results in agreement with thermodynamics for events not proceeding at equilibrium, and even developing far from equilibrium (among them the majority of biological phenomena at a molecular level) thus largely eliminating the apparent contradictions which existed between the second principle of thermodynamics and the energetics of living systems[2]. A few typical examples should be discussed in order to illustrate just how varied, and yet common, the fields involving electrochemistry in biological research really are.

Many, probably even the majority, of the chemical reactions in living things are reactions of the redox type in which an oxidizable biological system is oxidized, for a definite reason, by another biological system existing in the organism. The most characteristic example is the combustion (that is oxidation) of various substances, mainly glucose derivatives, in mammals by the oxygen carried by haemoglobin. In this case, the reducing system is most frequently a glucose derivative, while the oxidizing system is ultimately the oxygen carried by haemoglobin.

This global reaction actually takes place in a series of partial steps through a number of intermediates to the final products H_2O, CO_2 and eventually to nitrogen, sulphur and phosphorus derivatives. These are the reactions supplying the energy necessary for the preservation of life. Redox systems of this kind are very difficult to study in their entirety, although it is possible to expand the present state of knowledge by using electrochemical concepts and techniques which split the overall reaction into simpler one-step partial reactions. Practically all simple one-step redox systems can be electrochemically studied by following the laws of thermodynamics and kinetics. By means of the values of redox electric tensions, thermodynamics supplies the criteria for judging whether, and under what conditions, a given redox reaction may take place; while kinetics, by means of the information regarding the corresponding activation energies, and also using other considerations, e.g. the analogy of the Franck-Condon principle, makes it possible to ascertain the time evolution of the redox system under the specific conditions given. Using an appropriate conductor with electronic conduction (Pt, Au, W, graphite, etc.) and an appropriate reference electrode, each of these partial systems may become a galvanic cell which allows the measurement of the values of the standard electric tension and of the rest tension. Allowing then the galvanic element to work with the redox electrode under investigation first, under anodic and then under cathodic load, it is possible to gain the most important information regarding the activation energy and thus the reaction rate. In living things there are obviously no galvanic elements with Pt electrodes but, on the basis of the knowledge acquired under certain conditions, about the electric tension of the partial systems reacting with each other, it is possible to establish immediately which of the systems is the oxidizing one and which is the oxidized one. It is also possible to evaluate, even if only approximately, whether the reaction will be a fast or a slow one.

Further energetic contributions from superficial adsorption phenomena, e.g. at phase boundaries like membranes, should also be taken into account for a correct evaluation of the whole energetics involved. The data mentioned above become particularly important when the 2 systems reacting in a living organism with a specific biological aim show small differences in the corresponding redox tensions. In such a case, even small changes in the environmental conditions, and especially in the concentration, may even reverse the direction of the reaction. A typical example is the activity of hydrogen ions and of the reduced hydrogen (atomic and molecular) which appear in a large number of biological redox reactions. One can immediately assess the importance of the pH and realize the necessity of learning about the true activities of the different ionic species.

Continuing the discussion on the subject of biochemical reactions, it is possible to classify them into 2 large groups: the anabolic and the catabolic reactions. The former aim at the accumulation of the energy necessary for the performance of the functions of life in certain chemical products stored by living organisms; the energy thus stored is subsequently used by the catabolic processes. These processes, studied one by one, have led to those extremely complex and often clever representations, which are at times hard to understand. Among other things, these individual processes taken as a whole sometimes seem to be incompatible, mainly from the energetic point of view, and even sometimes appear to contradict the normal laws of chemistry and physical chemistry. For example, no chemist would even think of synthesizing glucose by direct hydration and by a simultaneous partial reduction of carbon dioxide, nor would he dream of building on ordered polypeptide-chain by simple dehydration of a mixture of amino acids; the oxidative decarboxylation of α-ketoacids is also hard to explain merely on a chemico-physical basis, etc. However, if one tries to split these overall processes into simpler ones which occur in steps, taking into account the thermodynamic quantities (redox electric tension, free enthalpy and entropy of the reaction), the kinetic ones (overpotentials and activation energy of each single process) and other possible coupling phenomena (with the involved energetics) almost all reactions presently known may be included in one of the 4 large classes of simpler reactions, i.e.: redox, condensation-hydrolysis, addition-elimination to or from double bonds, synthesis-degradation of skeletal C-atoms, without contradicting the laws of the thermodynamics, including the thermodynamics of the phenomena outside or even far from equilibrium. The sequence of biological events at the molecular level thus becomes more understandable and logical.

The application of the concepts of the theories of solid-state physics and semiconductors to the solution of biological problems is relatively recent (Szent György, 1941). Since then, a remarkable amount of research has been carried out which has led to electrical conduction in macromolecules being interpreted on the basis of such concepts. Many such macromolecules, proteins, phthalocyanines, haemoglobins, nucleic acids, etc. showed characteristics of semiconductors at the solid state, even if not dry. For example, for many proteins 3 closely lying energy bands exist, which was proven experimentally as well as by theoretical calculations. 2 of these bands are occupied by electrons, and the 3rd is empty, hardly 3 eV higher, and can therefore be used as a semiconductor conduction-band. On the basis of such a theory, excellent results were obtained for a number of biological phenomena, such as the activity of mitochondria, the behavior of chlorophyll, the mechanisms of photosynthesis, the respiratory chain, vision, etc.

Among these phenomena, particularly worth mentioning, is the mechanism of electric conductance through organized structures, like nerves or muscles, on which depend the transmission of information, and therefore the coordination, of a number of functions of life.

Another extremely broad field is that of membranes. A biological membrane is a barrier used by nature to maintain in every cell the internal conditions essential for the performance of its function, that is for life itself.

In living organisms the membrane fulfills the role of separating 2 regions and of acting as a barrier that must be able to close and to open. The transport of matter across the membrane takes place essentially on the basis of 2 mechanisms: one is nonselective, across more or less wide pores and channels, the other is specific by way of a selective permeability which can be changed according to the needs of the cell, e.g. by means of carriers which can specifically transport one kind of particle. In the case of specific and selective transport, if the particles are electrically charged, a number of electrical and electrochemical phenomena occur, which are consequential and in turn regulate the process. In view of the fact that the regions separated by a membrane are normally iso-osmotic, the transport of matter across the membrane must be governed by factors other than the difference in osmotic pressure. Therefore, the mechanism must be an electrochemical one; in fact it is possible to measure electric tensions that are different from zero across the biological membranes that separate the 2 iso-osmotic regions.

A large number of theoretical biologists are investigating these phenomena starting from the basic theories of electrolytic conduction and developing them by using the thermodynamics of irreversible processes. In fact the transport of matter and of electric charges is irreversible because of generalized forces consisting of gradients; therefore it becomes necessary to take into consideration the profiles of electrical potential (nonlinear gradients), the profiles of the reaction at the surface of the membrane (nonlinear gradients of chemical and electrochemical potentials), and coupling of the simultaneously occurring phenomena which could influence the phenomena of transport.

Unfortunately, the mathematical relationships needed to describe these phenomena are nonlinear and it is therefore necessary to use nonlinear differential equations in order to obtain results that can be compared with the experimental values.

In spite of this, it is possible to obtain results which indicate the possibility that under certain conditions negative conductance may exist for some ionic species, i.e. migration of charged species in a direction opposite to the one apparently predicted by the gradients of the electrochemical potential across the membrane. This gives a physical, not hypothetical, meaning to the concept of an ionic pump, sometimes suggested in biology in order to explain some matter-transport that would otherwise be difficult to explain. Together with the aforementioned development of the theory by Prigogine and co-workers in the field of energetics, this is another brilliant example of how the logical and rigorous use of new theoretical concepts can produce fruitful results. A further alteration in transmembrane potentials could also have macroscopical consequences. For example, on the basis of neurophysiological studies and from studies on various kinds of electroanesthesia, it appears that the drugs used substantially modify the ionic conductance of cellular membranes. Thus one can recognize not only from a biological but also from a physiological and pharmacological point of view the importance of the study of conductance and membrane-permeability for ionic species, that is for the whole field of electrochemistry of membranes.

The general concepts concerning the operation of membranes on an electrochemical basis were also used for explaining the characteristics and the functioning of organized biological structures: nerves, muscles, etc. For example, the transmembrane potentials could be applied in the interpretation of the concept of the action-potential which is transmitted at a speed of tens of meters per second.

Another point that should not be neglected in the interpretation of the behaviour of biopolymers and of macromolecules is the conformational analysis.

In this case as well, electrochemical methods, especially polarographical ones (Berg, Nürnberg, Elving, Palecek, etc.) achieved very valuable results by showing the changes in e.g. the shape of the helices constituting proteins and nucleic acids.

Another important field of bioelectrochemistry is photosynthesis which ultimately represents the source of energy required for the life processes of practically all species, either directly (green plants) or indirectly (organisms nourished more or less directly with products synthesized through photosynthesis from green plants). A translocation of protons and electrons takes place in photosynthesis with the help of the light energy absorbed, with the subsequent occurrence of transmembrane potentials, transport of matter, redox reactions, etc.

The last example (in sequence, not in importance) to be mentioned is given by the interactions between living cells and electric currents applied directly or generated inductively by applying a changing electromagnetic field. Our knowledge in this field is still extremely poor, but some facts have been incontestably proven and have very important clinical and therapeutical applications. In many cases of bone fracture, judged orthopaedically untreatable, the use of very small (some $\mu A/cm^2$) pulsing currents or of alternating electromagnetic fields of appropriate form and magnitude (which in turn generate the desired alternating currents), have produced a very high percentage of complete healing. It was initially thought that the use of such currents had a stimulating and organizing effect. At present, after a certain number of experiences in vivo and in vitro, we may think that both actions do exist. Furthermore, the abundant production of free Ca^{2+} ions by the bone cells of chicken embryos was experimentally established under the above mentioned electric stimulation. The same results were obtained in vivo and in vitro only when using the same energy values and the same time-distributions. On the other hand, the absence of clinical results was established to parallel the absence of production of free Ca^{2+} ions. The production of free Ca^{2+} ions is also connected with other biological phenomena like mitosis, transcription of genetic codes, calcification, etc. These observations lead also to the conclusion that the repair of damaged bone-tissue under electric stimulation can be of electrochemical origin as well.

Although necessarily incomplete, this brief survey clearly brings to light how broad the field of biological phenomena is and that it can and should be studied with electrochemical techniques if one wants to increase the knowledge of biological phenomena on even more rigorous foundations. Some (obviously not all) subfields mentioned are discussed in more detail in the following sections.

Bioelectrochemistry is at present in its very initial stages, mainly because of the difficulty of carrying out unambiguous and clear experiments in such complex systems as living systems. But it is incontestably a field of positive development in the way of electrochemical thinking and experimentation.

This is why the first international conference on bioelectrochemistry was organized in Rome in 1971, and it was so successful that it was repeated at Pont à

Mousson in 1973, at Jülich in 1975, at Woods Hole (Mass.) in 1977, and at Weimar in 1979; the next one is being scheduled to be held in Israel in 1981 and the one after that, in England in 1983.

A journal and a series of publications especially devoted to bioelectrochemistry have been founded; the journal *'Bioelectrochemistry and Bioenergetics'* is in its 7th year of publication (now as a section of the *'Journal of Electroanalytical Chemistry'*[3] and 3 volumes of the series *'Topics in Bioelectrochemistry and Bioenergetics'*[4] have already been published, the 4th being in press and the 5th in preparation.

Furthermore, the international Bio-Electrochemical Society (BES) was founded to provide scientists interested in the phenomena with an active forum so that they could meet and discuss their problems[5].

This article appears without bibliographical references; for each one of the fields mentioned a very long list of references should be compiled. Readers interested in one or more of the subjects mentioned will find extensive bibliographic lists at the end of each article published in the volumes of the serial *'Topics in Bioelectrochemistry and Bioenergetics'*, which has just been written with the scope of a first pedagogical introduction to the different fields of bioelectrochemistry.

[1] See J. electroanalyt. Chem. Interfacial Electrochem. *100,* 1 (1979).

[2] For this work Prof. Prigogine was awarded the Nobel prize for Chemistry in 1977.

[3] Elsevier Scientific Publishing Co. Amsterdam, Oxford, New York.

[4] John Wiley and Sons, Chichester, New York, Brisbane, Toronto.

[5] For information and application form write to the Secretary General, Prof. Dr. H.W. Nürnberg, Institut für Chemie IV Angewandte Physikalische Chemie, Kernforschungsanlage, D-517-Jülich, Federal Republic of Germany.

Historical roots of bioelectrochemistry

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The term 'bioelectrochemistry' was created at the 1st International Symposium Rome, 1971, although the first observations of galvanistic phenomena had already been described in the 18th century. In 1760 a Swiss physician, J.G. Sulzer (1720-1779) noticed that the combination of pieces of lead and silver caused a strange taste on his tongue, something like $FeSO_4$ solution. Connecting 2 different metals likewise with the nerves of frog legs, L. Galvani (1737-1798), anatomist at Bologna, discovered muscle convulsions in 1786. Some years before he had observed the same effects on frog legs, joined with one metal wire only, induced by an electrical machine or electrical discharges in the atmosphere. He published his results in a booklet with the title, 'de Viribus Electricitatis in Motu Musculari Commentarius' including 4 engravings of the experimental apparatus. He believed that muscles contained many little Leyden jars and produced electricity from inside, overlooking the prerequisite of the bimetallic connection. This explanation for the so-called 'animal electricity' might have been influenced by information from certain fish producing electricity, as discovered in 1774 by the physicist, J. Walsh (1725-1795). Therefore Galvani came to the false conclusion for the galvanic action, although his model fits fairly well with the electric organ of some fishes. This error of Galvani has been corrected by A. Volta (1745-1827), the famous physicist, who pointed out that the electricity comes from outside and the muscle works only as an indicator (electroscope), whereas nerves have the function of conductors. A prerequisite of galvanic action must be: 2 metals (conductors of the 1st class) and 1 electrolyte (conductor of the 2nd class). Despite his sophisticated experiments, especially with the voltaic battery, Volta's explanation for galvanism was erroneous, too. Volta postulated that electricity is produced only by the contact of both metals, in other words by a kind of perpetuum mobile[2]. Famous men, e.g. J.J. Berzelius (1779-1848), believed in this contact model, until in 1840 M. Faraday (1791-1867) finally refuted it[3]. Nevertheless, many people became excited by these phenomena between biological objects and electricity, and galvanism had an attraction comparable with that of molecular biology nowadays.

Quite another explanation was given by G.V.M. Fabbroni (1727-1822), an Italian chemist, in 1792, but published later[4]. He attributed galvanic actions, inclusive of taste sensitivity changes as in the case of the Sulzer experiment, to real chemical reactions without any generation of electricity present. However, the right interpretation[5-7] of galvanic action was presented by a 23-year-old student of Alma Mater Jenensis, Johann Wilhelm Ritter (1776-1810), who had worked in the field of galvanism since 1797, stimulated and encouraged by A. v. Humboldt (1769-1859), C.H. Pfaff (1773-1852), F.A.C. Gren (1760-1798) and others in Germany. First of all he found