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FEATURE ARTICLE

BIOELECTROCHEMISTRY

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ABSTRACT. Electrochemistry was linked to biology from its very beginning at the end of the 18th century. The term, bioelectrochemistry, only appeared for the first time in 1964. Various topics of fundamental as well as of applied bioelectrochemistry are listed. As two examples, the generation of the electrical impulse in nerve cells and the electrochemical aspects of cell breathing are described.

INTRODUCTION

Electric fish (electric ray, eel and catfish) were known already in the ancient time. The ancient Greeks and Romans could well recongnize the electric ray which is quite common in the Mediterrean. Scribonius Largus, the personal physician to the Emperor Claudius, was well aware of its supposed electrotherapeutic effects, and, already much earlier, Aristotle described its ability to numb smaller fish. They ascribed these effects to a kind of cold emanating from Torpedo's body or to an unknown poison (Fig. 1,2).

Late Renaissance savants Redi and Lorenzini dissected the electric ray and found its electric organ. However, they thought that its shocks are caused by rapid expulsion of microscopic corpuscles called effluvia which act as arrows. Other authors like Borell (1680) or de Reaumur (1714) suggested a mechanical shock through the medium as the cause of Torpedo's action.

However, in the second half of the 18th century the inventor of the Leyden jar, Pieter van Musschenbrek, pointed out the similarity of the effects observed with the Leyden jar and with the electric ray. The main objection of the supporters of the mechanical theory to the electric explanation was that no electric spark could be observed during Torpedo's activity. The cause of this was a low voltage by the electric ray (as we know, it is 45 V only) as pointed out by the famous physicist, Henry Cavendish. At that time, however, the electric eel was brought to Europe producing shocks with voltage about 600 V. With this animal John Walsh, English scientist and politician, succeeded in obtaining luminous electric sparks (Fig. 3).

Next stage of investigations of "animal electricity" were the famous Galvani's experiments. In the first place Galvani showed that the muslces of frog's legs were contracted under stimulation from an electric machine. To his surprise a contraction was observed when, simultaneously, a muscle and a nerve of the leg were touched with a "metal arc" formed of two metals in series. According to Galvani the metal arc transfers electricity originally formed in the brain through the nerve into the muscle. Subsequently, however, Volta disclosed that the genuine source of excitation was the electricity formed at the contact of two different metals (according to our present knowledge the electric current is produced in the short-circuited galvanic cell where both the metals represent

Fig. 1. An ancient mosaic from Pompei showing the fight of an octopus with a lobster amongst other Mediterranian fish. The electric ray (Torpedo) is at the top of the figure.

Fig. 2. The effects of electricity were known to the ancient Egyptians through the Nile catfish (Malopterurus electricus) shown at the centre of a bas relief from the tomb of Ti (the Fifth Dynasty, about 2750 B.C.).

the electrodes). In order to show that his contact theory is valid also for other kinds of animal electricity he designed his famous pile. It had to be a sort of an "artificial electrical organ" simulating thus "the natural electric organ of the torpedo or of the electric eel". This was the first case where an

Fig. 3. The electric eel (Electrophorus electricu). A drawing by E. Opatrny.

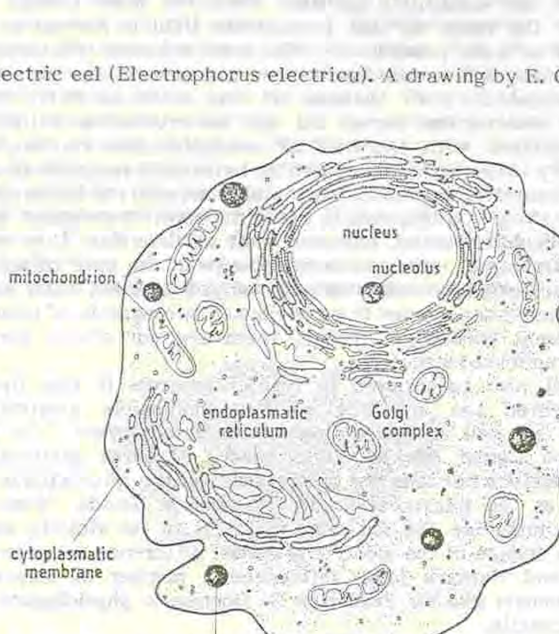

Fig. 4. An animal cell with various organelles inside.

electrochemical phenomenon was detected when a biological phenomenon was modelled (1).

By this fundamental discovery the contacts between biology and electrochemistry were terminated for many decades. It is of interest to note that the term "electrochemistry" first appeared in the book Elements of Electricity and Electro-chemistry by George John Singer only in 1814. The elements from which a new synthesis could be formed were of course built during the 19th century. Thus, the cell theory was originated in the work of M. Schleiden, Th. Schwann and R. Virchow. The physiologist, E. Du Bois Reymond, found that the surface of a wounded tissue behaved as an electrode of a galvanic cell. The famous scientist, H.V. Helmholtz, compared the nerve fibre to a telegraph cable.

The main problem, which was already pending from Volta's and Galvani's times, and to which the science of the second half of the 19th century was coming back, was: Why do the bioelectric phenomena exist at all when there are neither metallic electrodes nor wires in organisms? (2).

A certain reply to this question was given by W. Nernst and M. Planck who worked out the theory of diffusion potential. This phenomenon appears during diffusion in an electrolyte solution when the more mobile ionic component "overtakes" the other so that an electric field is formed in the solution (3). This effect is more pronounced in the semipermeable membrane which permits a definite ionic sort to pass easier than the other ones. Membranes of this kind were investigated by W. Ostwald (4) who wrote in 1891: "Not only electric currents in muscles and nerves but also the mysterious actions of electric fish will be explained with the help of semipermeable membranes". This was a revolutionary idea which did not find an immediate response among the biologists. Thus, for example, in the monumental compendium Elektrophysiologie by W. Biedermann (Vol. I, 1895, Vol. II, 1898) the term "membrane" was not mentioned at all! It should be noted, however, that at that time this term was not used for cell membranes - they were not known in the case of animal cells-but for multicellular formations like the wall of gall bladder, skin, etc. On the other hand the membranes seemed to be obvious in micrographs of plant cells. According to the present knowledge, though, these are not simple membranes but cell walls of complicated structure.

W. Nernst also contributed to further progress in this field. Around 1900 he investigated the interface of two immiscible electrolyte solutions at equilibrium as well as under flow of electric current. On the basis of this investigation Nernst worked out a theory of nerve excitability. He thought that the interface between the protoplasm and the intercellular liquid has similar properties as the interface of two immiscible liquids. When electric current passes this interface the ion concentrations in its vicinity are changed which results in a change of the electric potential difference between these two media. Ostwald's and Nernst's ideas influenced a number of investigators including physico-chemists like Sir Frederick G. Donnan or physiologists like J. Bernstain and L. Michaelis.

In the search for biological membrane models the botanist, M. Cremer, found in 1906 that a glass bubble showed a membrane potential dependent on the acidity of the solution into which it was immersed. On the basis of this finding F. Haber and A. Klemensiewicz designed a functioning glass electrode (5).

In 1925 E. Gorter and E. Grendel hemolysed red blood cells, i.e. by decreasing the osmotic pressure of the bathing solution the protoplasm was separated from the rest, the so called erythrocyte ghosts. When analysing the latter, they found that it consisted mainly of phospholipids. The principal component of a typical phospholipid is a polar head consisting of the phosphoric acid unit esterified by a substituted ethanolamin or aminoacid on one side and by glycerol on the other side. The non-polar tail of the phospholipid is formed by long alkyl chains of the fatty acids which are also linked to the glycerol.

When they spread the isolated phospholipids at the water/air interface Gorter and Grendel found that the compressed monolayer occupies roughly double of the area of the original surface of the erythrocytes. They concluded that (i) the cells are surrounded by a membrane several nanometers thick based on phospholipids, and (ii) the phospholipids are arranged in a bilayer where the non-polar tails are in the inside of the membrane stretching perpendicularly to it while the polar heads contact the adjacent aqueous solution (6).

This bilayer lipid membrane (BLM) was artificially built later, in 1962, by P. Mueller, D.O. Rudin, H. Ti Tien and W.C. Westcott (7). Together with A.D. Bangham's liposome they serve as a favourite model of a biological membrane, which is by number of publications a bioelectrochemical counterpart of the dropping mercury electrode in the flourishing period of polarography.

Good luck of Gorter and Grendel was in their choice of erythrocyte membrane where the content of phospholipids is quite high. The enormous variety of biological membranes encircles not only cells but also various tiny bodies inside the cells (Fig. 4). These membranes consist not only of phospholipids but also of proteins, lipoproteins and glycoproteins (compounds of sugars and proteins). According to J.F. Danielli and H. Davson the lipid bilayer is covered by adsorbed protein molecules but a number of various other models was later proposed. Fig. 5 shows the liquid-mosaic structure according to S.J. Singer and G.L. Nicholson which is compared with the structure of the mitochondrial membrane according to K. Dose. The proteins have many functions: they strengthen the membrane structure, mediate transport of various substances and enable various redox reactions, energy transduction, etc. to proceed. Many of the phenomena connected with this complicated structure of the membrane, such as the membrane potential, the surface potential, the transfer of electricially charged particles, ions and electrons, across the membrane, etc. belong into the realm of electrochemistry although, at the same time, other branches of science (electrophysiology, biophysics, membrane biology, bioenergetics, etc.) are justified to lay claims to them. In spite of the fact that the interpretation of many of these phenomena makes use of electrochemical concepts the corresponding section of electrochemistry received its proper name rather recently.

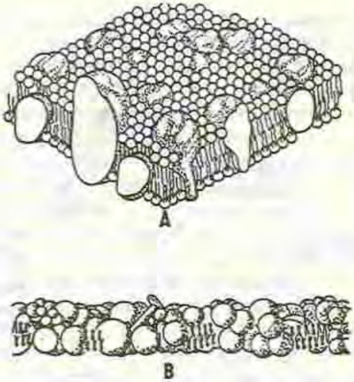

Fig. 5. Typical structures of biological membranes.
A - the liquid-mosaic structure according to S.J. Singer and G.L. Nicholson.
B - the structure of the mitochondrial membrane according to K. Dose.

The term, bioelectrochemistry, was probably used first in the Encyclopedia of Electrochemistry (ed. by C.A. Hampel), Reindhold, New York, 1964.

At present bioelectrochemistry comprises three sections, one concerned with basic knowledge, the other with applications of electrochemical methods in biology and medicine, and the last with various devices built on bioelectrochemical principles (in particular, with biosensors).

Basic bioelectrochemistry comprises (i) electrochemistry of redox transformations of components of biological systems, (ii) studies of electrical double-layer and interfacial tension at membrane surface, which are also connected with cell movements, cell fusion, etc., (iii) charge separation and transport in natural and model membrane systems (active and passive transport, proton and ion transport in photosynthesis, channel-mediated transport in membranes of excitable cells, etc.).

There is a number of applications of electrochemical methods in biological systems. Here I should like to mention the electrochemical analysis *in vivo*. After some preliminary investigations using voltammetric electrodes of conventional size, microelectrodes are mainly used at present, particularly for determination of neurotransmitters. Ion-selective microelectrodes are applied to analysis of potassium, sodium, chloride, choline, acetylcholine, etc. in single cells as well as in neural or muscular tissues (8,9).

Bioelectrochemical principles are used in the design of various enzyme electrodes. In spite of great effort in this fields only the glucose electrode based usually on β-glucose oxidase, has been introduced to clinical application. There has been a considerable interest in the field of tissue and bacterium electrodes. However, considerable difficulties with standardization of such systems strongly hinders their application to routine analysis.

Biological membrane electrochemistry comprises the most up-to-date topics in the field of bioelectrochemistry. The transduction of energy, coming from the solar energy to that of various useful structures in living organisms, is mainly based on membrane systems, as shown in Fig. 6.

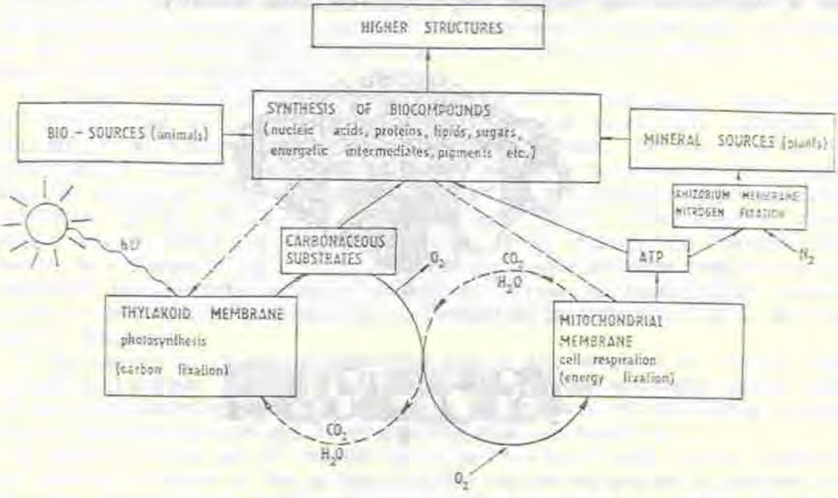

Fig. 6. Biological membranes and energy transduction in organisms.

All organisms use chemical energy in the form of an "energy-rich" polyphosphate bond of the adenosinetriphosphoric acid (ATP). The energy for ATP synthesis is gained by "burning" of carbonaceous fuels (sugars, low-molecular organic acids, etc.) in the process called oxidative phosphorylation. It occurs in tiny organelles termed mitochondria which are surrounded by a double membrane (Fig. 7). For disentangling the net of enzymatic reactions proceeding in this process P. Mitchell was awarded the Nobel Prize in chemistry. Mitchell's idea seems quite simple: the overall reaction of oxidation of the fuel by oxygen is separated in a large number of small steps which cause an almost reversible course of this process. In these processes the transfer of electron gained by oxidation of the substrate from the internal region of the mitochondrion (matrix space) to the region situated between the mitochondrial membranes (intracrystal space) is accompaied by transfer of a proton. In the chain of enzymatic electron donors and acceptors the electron is transported in the opposite direction but the proton remains accumulated in the intracristal space (see Fig. 8). This procedure is then repeated as long as the electrons reach the oxygen molecule while simultaneously the electrochemical potential of protons (due to increase of their activity in the intracristal space and the membrane potential) increases. The energy accumulated in this way is then transformed to the chemical energy of ATP in the re-transport of protons through the transport protein, proton-ATPase. This theory could explain a number of characterstics of oxidative phosphorylation including the uncoupling of this process. If charge could be transferred across the mitochondrial membrane outside the proton-ATPase then the energy accumulated would be wasted although oxidation of the substrate could proceed. This is, in fact, achieved by various uncouplers which are able to carry protons or alkali metal ions across the membrane.

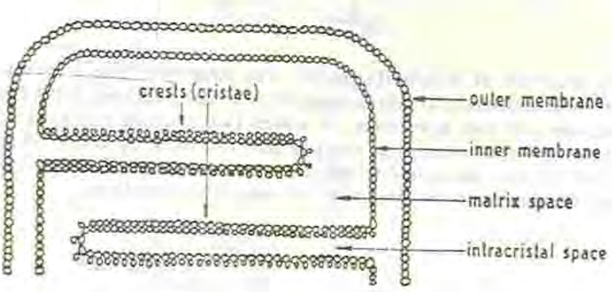

Fig. 7. Double membrane system of a mitochondrion.

The second fundamental phenomenon elucidated with the help of the electrochemical membrane theory is nerve excitation. In the study of excitation of the membrane of the axon, a long outlet of the nerve cell, (Fig. 9) A.L. Hodgkin and A.F. Huxley used various electrical impulses which well resembled the galvanostatic (Fig. 10, 11), and potentiostatic approaches in electrochemical kinetics. The Hodgkin-Huxley theory assumes the presence of ion-selective channels in the axon membrane, the opening of which depends on the membrane porosity. A direct proof of the existence of the ion-specific channels is supplied by Neher's patch-clamp method where a tiny portion of a nerve cell membrane containing only one channel is sucked into a glass capillary

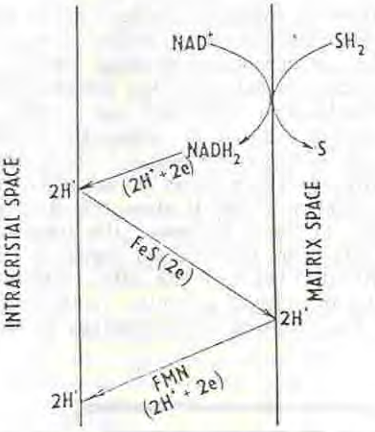

Fig. 8. An example of Mitchell's loops. The substrate SH_2 is oxidized by NAD^+ (nicotinamideadeninedinucleotide) and the reduced form transports two protons and two electrons, of which two protons remain in the intracristal space and two electrons are transported back by the Fe-S protein to reduce FMN (flavinmononucleotide), the reduced form of which transports two protons and two electrons in the opposite direction.

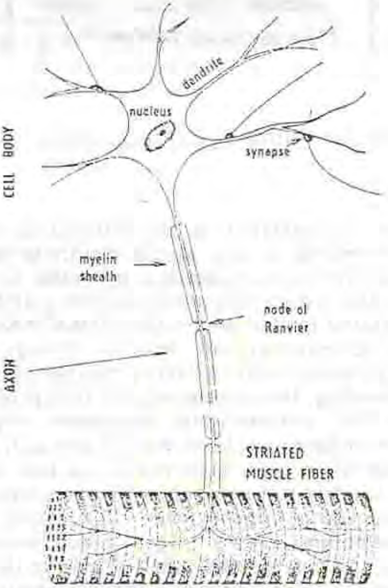

Fig. 9. A striated muscle cell.

and then investigated by means of the controlled membrane potential method (voltage-clamp). Discontinuous changes of the current are significant for opening and closing of the channel. These channels belong to the family of glycoproteins. The best known channel is the nicotine acetylcholine receptor (Fig. 12) which functions in connections of two nerve cells (synapses) or in nerve-muscle connections.

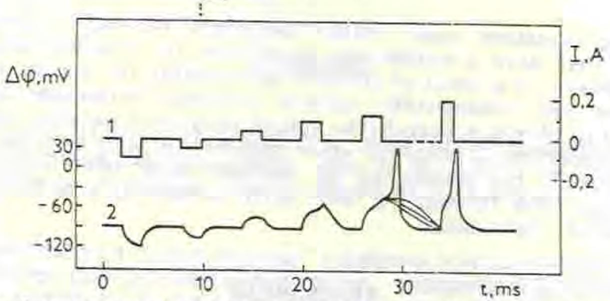

Fig. 10. Time dependence of excitation current pulses (1) and membrane potential Δφ (2). The abrupt peak is the spike. According to B. Katz.

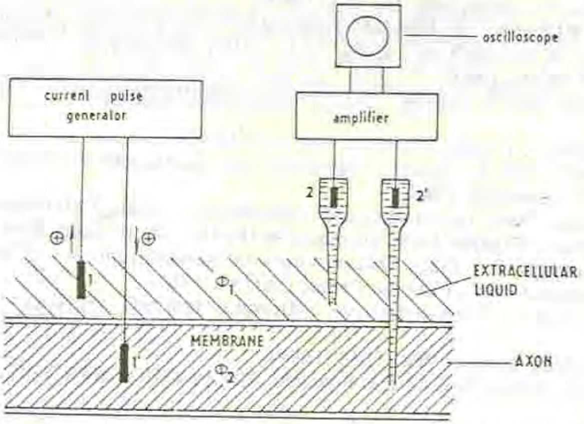

Fig. 11. Experimental arrangement for measurement of the membrane potential of an axon (muscle fibre) excited by means of current pulses: (1) excitation, (2) potential probes. According to B. Katz.

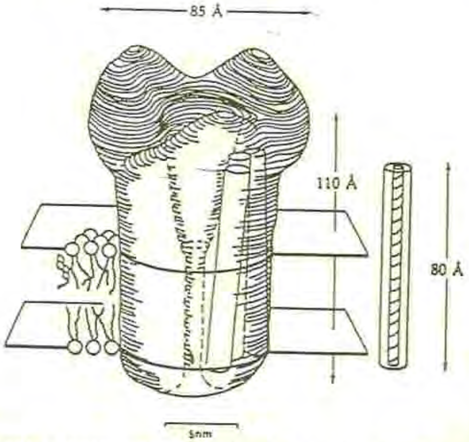

Fig. 12. Nicotine acetylcholine receptor in a membrane. The structure is based on measurements using X-ray diffraction and electron microscopy. According to Kistler and coworkers.

Thus living organisms need neither electrodes nor wires for generating electricity. They have a satisfactory substitution in Ostwald's semipermeable membranes where, as a result of different permeability for various ions electric double layers and, consequently, electric potential differences are formed. The transport of charge is mediated by various enzymatic systems, redox proteins or proton-transporting ATPases and other transport proteins or proceeds through specific channels. The opening of the channels along nerve fibres results in electric current flow through the fibre which behaves as a telegraph cable as predicted by H.V. Helmholtz.

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