ELECTROCHEMISTRY IN BIOLOGY AND MEDICINE

Edited by

Theodore Shedlovsky

ELECTROCHEMISTRY
in
BIOLOGY and MEDICINE

Edited by
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Sponsored by
THE ELECTROCHEMICAL SOCIETY, INC.
New York, N. Y.

JOHN WILEY & SONS, INC., NEW YORK
CHAPMAN & HALL, LIMITED, LONDON
1955

Library of Congress Catalog Card Number: 55-8561

PRINTED IN THE UNITED STATES OF AMERICA

ELECTROCHEMISTRY
in
BIOLOGY and MEDICINE

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PREFACE

This book grew out of the Symposium on Electrochemistry in Biology and Medicine held in New York during the One Hundred and Third Meeting of the Electrochemical Society in April of 1953. Although some of the chapters are essentially like the papers that were presented at the Symposium, others are the result of further thought and deliberation. The subjects covered include discussions, from several points of view, of membranes, nerve and plant cells, biologically important ions, and applications of polarography, electrocardiography, and electroencephalography in medicine.

The contributing authors are active scientific investigators whose chapters in this volume are based largely on their own experimental work in the fields they discuss. Among them are physicists, chemists, biologists, and medical men, yet they all share an interest in the difficult fundamental electrochemical problems of living processes.

My thanks are due to the members of the Editorial Advisory Board for their service and to all the authors, whose contributions and helpful cooperation with the editor have made this book possible.

THEODORE SHEDLOVSKY
Editor

New York, N. Y.
April, 1955

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1.

Introduction

THEODORE SHEDLOVSKY *

Electrochemistry is concerned with the electrical properties and behavior of substances and with the transformation of chemical energy into electrical energy or vice versa. It is related to biology and medicine in two ways. First, it provides powerful laboratory methods and tools for the study of biologically important substances, such as viruses, hormones, enzymes and other proteins, and also for the determination in biological environments of such factors as acidity, oxidation-reduction, ionic mobility, activity and diffusion, dielectric constant and dipole moments. Second, living organisms and in fact all living cells are complicated electrochemical systems capable of transforming chemical energy and ionic transport into electrical signals. With appropriate apparatus the neurophysiologist may examine such electrical signals to learn what he can about the functions of nerve and muscle. The medical clinician, armed with a substantial background of correlated, empirical knowledge, observes the electrical signals from the heart or from the brain and thus is aided in arriving at a diagnosis.

It is appropriate, I think, to introduce this symposium by recalling briefly the early history of electrochemistry.

In the eighteenth century, frictional electricity stored in Leyden jars, an invention of von Kleist in 1745, aroused considerable interest in electrical experimentation besides providing occasional entertainment for the laity. Benjamin Franklin's well-known contributions to electrical science belong to this period; but it remained for Luigi Galvani of Bologna, the anatomist, surgeon, and obstetrician who experimented with the effect of Leyden jar discharges upon frogs, to discover the intimate relationship between physiology and electricity. He reported this discovery in 1791 in his two classic papers on the subject. Its importance in the history of electrochemistry and biology warrants the following quotation from his first paper in the *Transactions of the Science Institute of Bologna.*[1]

* From the Rockefeller Institute for Medical Research, New York, N. Y.

I had dissected a frog . . . and had placed it upon a table on which there was an electric machine, while I set about doing certain other things. The frog was entirely separated from the conductor of the machine and indeed was at no small distance away from it. While one of those who were assisting me touched lightly and by chance the point of his scalpel to the internal crural nerves of the frog, suddenly all the muscles of its limbs were seen to be so contracted that they seemed to have fallen into tonic convulsions. Another of my assistants, who was making ready to take up certain experiments in electricity with me, seemed to notice that this happened only at the moment when a spark came from the conductor of the machine. He was struck with the novelty of the phenomenon, and immediately spoke to me about it, for I was at the moment occupied with other things and mentally preoccupied. I was at once tempted to repeat the experiment so as to make clear whatever might be obscure in it. For this purpose I took up the scalpel and moved its point close to one or the other of the crural nerves of the frog while at the same time one of my assistants elicited sparks from the electric machine. The phenomenon happened exactly as before. Strong contractions took place in every muscle of the limb, and at the very moment when the sparks appeared the animal was seized as it were with tetanus.

During the next 5 years Galvani occupied himself with other experiments that provided the basis for his theory of "animal electricity," which he formulated into the five following postulated principles. (1) Animals have an electricity peculiar to themselves. (2) The organs to which this animal electricity has the greatest affinity, and in which it is distributed, are the nerves, and the most important organ of its secretion is the brain. (3) The inner substance of the nerve is specialized for conducting electricity, whereas the outer oily layer prevents its dispersal and permits its accumulation. (4) The receivers of the animal electricity are the muscles, and they are, like a Leyden jar, negative on the outside and positive on the inside. (5) The mechanism of the motion consists in the discharge of the electric fluid from the inside of the muscle by way of the nerve to the outside, and this discharge of the muscular Leyden jar furnishes an electrical stimulus to the irritable muscle fibers, which therefore contract. In formulating these principles Galvani was, of course, influenced by the current ideas of his time, which held that animal spirits arose from the blood in the brain.

Galvani's work soon attracted the attention of Alessandro Volta, professor of Natural Philosophy at the University of Pavia. In 1800, two years after Galvani's death, he wrote a memorandum to Sir Joseph Banks in England, "On the Electricity Excited by the Mere Contact of Conducting Substances of Different Kinds." It was read before the Royal Society and published in the *Philosophical Magazine* within a few months. The fact that Volta was completely aware of a profound relationship between electricity and biology is evident in the following quotation from the first and last part of his paper.[2]

I have the pleasure of communicating to you, and through you to the Royal Society, some striking results I have obtained in pursuing my experiments on electricity excited by the mere mutual contact of different kinds of metal, and even by that of other conductors, also different from each other, either liquid or containing some liquid, to which they are properly indebted for their conducting power. The principle of these results is the construction of an apparatus having a resemblance in its effects to the Leyden flask. The apparatus to which I allude, and which will, no doubt, astonish you, is only the assemblage of a number of good conductors of different kinds arranged in a certain manner. Thirty or more pieces of copper, or, better, silver, applied each to a piece of tin or zinc, which is much better, and as many strata of salt water or any other conducting liquid, or pasteboard, skin, etc., well soaked in these liquids; such strata interposed between every pair of two different metals and always in the same order are all that is necessary for constructing my new instrument.

To this apparatus, much more similar to the natural electric organ of the torpedo or the electric eel, etc., than to the Leyden flask, I would wish to give the name of the "Artificial Electric Organ." . . .

All the facts which I have related in this long paper in regard to the action which the electric fluid excited and, when moved by my apparatus, exercises on the different parts of our body which the current attacks and passes through; an action which is not instantaneous, but which lasts, and is maintained during the whole time that this current can follow the chain not interrupted in its communications; in a word, an action the effects of which vary according to the different degrees of excitability in the parts, as has been seen; all these facts, sufficiently numerous, and others which may still be discovered by multiplying and varying the experiments of this kind, will open a very wide field for reflection, and of views, not only curious, but particularly interesting to Medicine. There will be a great deal to occupy the anatomist, the physiologist, and the practitioner.

Volta, the physicist, believed that the electricity in his pile, or artificial electric organ, arose from the contact of metals. Sir Humphry Davy, the chemist who experimented with the pile and observed that the zinc became more and more corroded, believed that it came from a chemical change. This controversy lasted for many decades and need not be reviewed here. Both views were, of course, partly right since the location of an electromotive force at any particular site in a circuit is not operational. It arises from the summation of all the metal-metal, metal-liquid, and liquid-liquid junctions.

Perhaps Davy's greatest contribution to electrochemistry was his assistant at the Royal Institution, Michael Faraday, who was born in the year Galvani published his work and a year after Franklin's death. Faraday's contributions, which established the modern science of electrochemistry, are so well known as to require no discussion beyond stating his law: The magnitude of the chemical effect, in chemical equivalents, is the same at each of the metallic-electrolytic boundaries in an electric circuit and is determined solely by the amount of electricity passed.[3]

In the chapters that follow the authors discuss a wide variety of topics that fall under one or the other of the two categories that have been indicated at the beginning of this introduction relating electrochemistry to biology and medicine. These papers are individual contributions to a symposium which indicate the trend of present work and thinking in a very wide field, some of it highly controversial. They can make no pretense of a glib, exhaustive treatise on the subject but, rather, serve the purpose of highlighting certain spots in the fascinating dark area between physical science and life.

Living matter or a living cell is not a mere assembly of chemical compounds. It is an oriented, dynamic system of complex materials in constant interaction with its environment, a complex chemical laboratory manufacturing many compounds no chemist has yet been able to synthesize, and electrochemical in many if not perhaps all of its functions. Between the inside and the outside of a living cell there exists normally an electrical potential usually of about a tenth of a volt. It is true of plant cells as well as cells of mammals, birds, or fishes. This is the so-called "resting" potential. In certain cells like nerve cells, this potential may be quickly altered and restored again, giving rise to electric "action potentials" in response to various stimuli. In nerve, this happens within a few milliseconds and is in the nature of electric transients. The theory of the fundamental electrochemical mechanism underlying these bio-electric phenomena is now an active and controversial subject of research.

A symposium has the character of a forum, and, of course, no author of the following chapters assumes any responsibility for any others. If the book stimulates thought, discussion, and research, it should also stimulate constructive controversy in a field that was, perhaps, too dormant until relatively recently.

In conclusion I should like to quote a passage that shows remarkable speculative foresight from Felice Fontana's "Treatise on the Venom of the Viper."[4,5] He was a Florentine who published this work a decade before Galvani's papers.

The considerable size of the nervous cylinders and blood vessels, when compared with the primitive fleshy threads, leads me to suspect that these threads are not put in motion, in any immediate way however, either by the blood or by the nerves. In a word, we are not only ignorant of muscular motion, but we cannot even imagine any way to explain it, and we shall apparently be driven to have recourse to some other principle; that principle, if it be not common electricity, may be something, however, very analogous to it. The electrical gymnotus and torpedo, if they do not render the thing very probable, made it at least possible, and this principle may be believed to follow the most common laws of electricity. It may likewise be more modified in the nerves than in the torpedo or gymnotus. The nerves should be the organs destined to conduct the fluid, and perhaps also to excite it. But here everything yet remains to be done. We must first assure ourselves by certain experiments whether there is really an electrical principle in the contracting muscles; we must determine the laws that this fluid observes in the human body; and after all it will yet remain to be known what it is that excites this principle, and how it is excited. How many things are left in an uncertain state to posterity!

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Membrane Potentials in the Donnan Equilibrium

DAVID I. HITCHCOCK *,[1]

HISTORICAL INTRODUCTION

The thermodynamic relations that form the basis of the theory of membrane equilibria were stated by Gibbs [2] in 1875. Since Arrhenius had not yet formulated the theory of electrolytic dissociation, this part of Gibbs's paper contains no mention of ions or of an electric potential difference. Indeed, as Guggenheim [3] has inferred from a later remark of Gibbs, he would have recognized that the difference of electric potential between two phases of different composition "involves the consideration of quantities of which we have no apparent means of physical measurement." Any discussion of membrane potentials must therefore include the admission that it is not possible to measure such a single potential difference, or to calculate its value exactly, without relying on some arbitrary, non-thermodynamic assumption.

The theory of ionic membrane equilibrium was developed by Donnan [4] in 1911. He considered a system in which two solutions containing electrolytes are separated by a membrane freely permeable to most of the ionic species but impermeable to at least one of them. A system in which the non-permeating ion is a protein cation, while the diffusible ions are those of hydrochloric acid, may be represented by the following diagram:

$$\begin{array}{rl|ll} z & R^{n+} & & \\ y & H^+ & H^+ & x \\ y+z & Cl^- & Cl^- & x \end{array}$$

The vertical line represents a membrane impermeable to the cation R^{n+}, and the lower-case letters x, y, and z denote the normalities or equivalent concentrations of the ions at equilibrium. This notation is

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consistent with the rule of electroneutrality for each solution. By the application of thermodynamics and the laws of dilute solutions, Donnan obtained a simple equation showing an unequal distribution of the diffusible ions at equilibrium. This may be written in the form

$$x^2 = y(y + z)$$

from which it may be seen that x is greater than y and that $y + z$ is greater than x. The presence of the non-diffusible ion on one side causes the diffusible electrolyte (in this case, hydrochloric acid) to become more concentrated on the other side. Donnan's equation may also be written as an equality of the ionic ratios,

$$r = \frac{x}{y} = \frac{y + z}{x} \qquad (1)$$

and here the ratio r is greater than unity. Donnan also obtained an expression for the electric potential difference commonly known as the membrane potential between the two phases. This expression may be written in the form

$$E_M = \frac{2.303RT}{F} \log r \qquad (2)$$

for univalent diffusible ions. The value of 2.303 RT/F is 59.15 mv. for 25° C. A positive value of E_M means that the solution containing the non-permeating cation is at the higher (i.e., more positive) electric potential.

Both Donnan [4] and Gibbs [2] pointed out that in such equilibria the two parts of the system will, in general, be at unequal pressures. Osmotic or membrane equilibria have been called by Guggenheim [3,5] partial equilibria, to emphasize the fact that the two phases are not in equilibrium with respect to the non-permeating substance.

The membrane potential is commonly taken to be the electromotive force measured between identical reference electrodes (e.g., calomel half-cells), connected by salt bridges with the two solutions on opposite sides of the membrane. This would be a measurement of the true membrane potential only if the liquid junction potentials at the ends of the salt bridges were equal and opposite. It is assumed that this is true when the salt bridge is a saturated potassium chloride solution, although no way to prove this assumption is known. The same assumption is made in estimating the activities of single ionic species (cf. Scatchard [6]). The results reported by Loeb [7] for membrane potentials in the equilibrium of proteins with electrolytes are all based on this assumption.

If a Donnan system at equilibrium is converted into a galvanic cell by inserting into the two solutions identical electrodes, reversible to one of the diffusible ion species, the resulting electromotive force must be zero. This conclusion, based on thermodynamic reasoning, was stated by Donnan and Allmand,[8] Michaelis,[9] and Hill.[10] As a result of this property of the system, it is possible to obtain a rough estimate of the membrane potential by making a measurement in the absence of the membrane, provided the solutions have first come to equilibrium across the membrane. The electromotive force between such reversible electrodes, in direct contact with the two solutions, is measured after the solutions have been connected by a salt bridge. This is essentially what Loeb [7] did in obtaining the figures reported as "calculated P.D." or "hydrogen electrode potentials." The solutions were placed in turn in a vessel provided with a hydrogen electrode and a salt bridge leading to a calomel half-cell; the difference between the e.m.f. readings obtained with the two equilibrated solutions was numerically equal to the e.m.f. between two calomel half-cells, each containing saturated potassium chloride, which made contact with the experimental solutions on opposite sides of the membrane. Similar results may be obtained with a glass electrode in place of the hydrogen electrode. Scatchard, Batchelder, and Brown [11] used two silver-silver chloride electrodes dipping directly into the two solutions, which were connected by a salt bridge. The rational basis for this procedure may be understood by considering the following galvanic cells:

$$\underset{e_1}{\text{Ag, AgCl;}} \quad \text{HCl, membrane:} \underset{E_M}{} \ \text{HCl + protein;} \quad \underset{e_2}{\text{AgCl, Ag}} \qquad \text{(I)}$$

$$E_{\text{I}} = e_1 + E_M + e_2 = 0$$

$$\underset{e_3}{\text{Hg, HgCl;}} \quad \underset{e_4}{\text{KCl(sat.):}} \quad \text{HCl, membrane:} \underset{E_M}{} \ \underset{e_5}{\text{HCl + protein:}} \ \text{KCl(sat.);} \quad \underset{-e_3}{\text{HgCl, Hg}} \qquad \text{(II)}$$

$$E_{\text{II}} = e_3 + e_4 + E_M + e_5 - e_3 = E_M + E_J$$

Here E_J is written for the net liquid junction potential, the algebraic sum of e_4 and e_5.

$$\underset{e_1}{\text{Ag, AgCl;}} \quad \underset{-e_4}{\text{HCl:}} \quad \underset{-e_5}{\text{KCl(sat.):}} \quad \text{HCl + protein;} \quad \underset{e_2}{\text{AgCl, Ag}} \qquad \text{(III)}$$

$$E_{\text{III}} = e_1 - e_4 - e_5 + e_2 = E_{\text{I}} - E_{\text{II}}$$

$$-E_{\text{III}} = E_{\text{II}} = E_M + E_J$$