BRAIN TISSUE ELECTROLYTES

A. VAN HARREVELD

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PREFACE

An investigation of impedance changes in the central nervous system, which first directed my attention to the problem of the electrolyte and water distribution in this tissue, was carried out at the time that a lack of extracellular space in the central nervous tissue was being found in electron micrographic studies. Since then the conflicting conclusions based on various approaches to this problem have been an interesting and confusing subject of study. The present monograph is the result of the experimentations and reflections of many authors on this subject. An attempt has been made to quote the publications which are most relevant to the problem; however, no complete review of the literature is claimed. Considerable emphasis has been placed on the methods used in investigations on extracellular space, since the conclusions reached are usually determined to a great extent by the interpretation of the procedures used. If this monograph stimulates thoughts and experimentation which will lead to a more definitive conclusion on the subject of the electrolyte and water distribution in central nervous tissue than is possible at this time, then it will have realized its purpose.

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A. VAN HARREVELD

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Introduction

SINCE Bernstein's [2, 3] suggestion that the injury and action potentials of muscle and nerve are due to differences in ion concentrations across membranes with semipermeable properties, a great deal of attention has been paid to the electrolytes in the peripheral and central nervous system. The electrolytes and their concentrations are now known in considerable detail in favorable objects such as the giant nerve fiber of the squid. In this case it has been possible to analyze directly the chemical composition of the axoplasm squeezed out of the fiber [1, 4, 5, 6, 7, 12]. A detailed theoretical concept accounting for many of the electrophysiological observations on this object in particular, and on nervous tissues in general, has been built on these findings. Since the mechanism of the membrane potential and action potential is generally ascribed to differences in ionic concentrations inside and outside the nervous element, it is necessary to know not only the electrolytes and their concentrations in the cytoplasm, but also those in the surrounding medium. This does not provide great difficulties in the peripheral nervous system. In the central nervous system of the vertebrates, however, the composition of the extracellular fluid which bathes the nervous elements is poorly known. There are also widely differing opinions about the amount of extracellular fluid and its distribution in this tissue. The uncertainty is aggravated by the presence of an additional tissue, the glia, which is unique to the nervous system, and which like the neurons, is of ectodermal origin. Features have been ascribed to this tissue which would enable it to take over some of the functions performed by the extracellular fluid in the other organs. For instance, from the intimate connections of glia cells with

the capillaries on the one hand, and with nervous elements on the other, it has been suggested that the glia provides a pathway for transport of materials between these structures [8, 9, 11]. It is with the distribution of the water and the electrolytes among the main elements of the central nervous system, the neurons, the glia, and the extracellular space, that this monograph will be concerned.

The application of electron microscopy to the central nervous system has resulted in a drastic revision of the estimates of the extracellular space in this tissue. Before electron microscopy, estimates of an interstitial space of 30–35 per cent of the tissue volume were based on the concentrations of the traditionally extracellular ions, chloride and sodium, in the brain. The first electron micrographs published showed a surprising lack of space between the cellular elements. Measurements resulted in estimates of not more than a few per cent of the tissue volume. Since then a considerable number of investigations has dealt with the magnitude of the extracellular space in central nervous tissue. Four different approaches have been used:

(1) In many organs of the body a fair estimate of the extracellular space can be obtained by determining the distribution between plasma and the tissue of compounds, such as inulin or thiocyanate, which do not readily penetrate cell membranes. Such methods have been applied to the central nervous system.

(2) During the last decade physical methods for the determination of extracellular space were proposed. The electrical resistance of tissue is determined mainly by the interstitial electrolytes, since the intracellular electrolytes enclosed by high resistance cell membranes cannot readily participate in the transport of the measuring current. The specific impedance of a tissue can therefore be expected to give information on its extracellular space.

(3) It has been attempted to determine with histological and histochemical methods the position and movements of water and electrolytes in nervous tissue.

(4) Numerous electron microscopic investigations of central nervous tissue have been published which show the space between cellular elements.

Attempts were made to reconcile investigations suggesting the presence of a large extracellular space with the paucity of extracellular material shown in electron micrographs, which, by most authors, were regarded as fairly representing the living tissue. This view has become almost an article of faith with some investigators, as demonstrated by the following quotation [10], which paraphrases a saying of Thomas Huxley: "There is, a physiologic, an embryologic, a phylogenetic, and a pathological basis for believing that interstitial spaces do not exist in the brain. It will be sad if this hypothesis, which articulates so beautifully, is slain by some ugly fact."

All estimates of the water and electrolyte distribution between the neuronal, the glial and the extracellular compartments are based on certain assumptions, which, although they may seem fair and reasonable at the time and in the context of the investigation, may turn out to be unjustified. Even the seemingly most direct method, the observation of the extracellular compartment in electron micrographs, is based on the assumption that the micrograph faithfully represents the living tissue. It will be attempted to review and evaluate the results obtained by the four approaches to this problem. While no definitive conclusion can be reached at this time, the weight of the present evidence seems to favor a specific water and electrolyte distribution in the central nervous system.

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Contents

CHAPTER ONE

Chemical Aspects of Water and Electrolyte Distribution

THE oldest speculations about the water and electrolyte distribution in the central nervous system were based on the chemical analysis of the tissue. There exists an extensive literature on the electrolytes in the normal nervous system, as well as on the changes in electrolyte composition which can be caused by many procedures. Some of these publications will be reviewed in this chapter.

The electrolytes in tissues are present in compartments separated from each other by membranes with semipermeable properties, an arrangement which is closely related to the physico-chemical systems studied by Gibbs and by Donnan.[86] Indeed, the water and electrolyte distribution under normal and abnormal conditions can, to a great extent, be understood on the basis of the concepts laid down by these authors, if the additional restrictions and complications present in living tissue are taken into consideration. A short account of the properties of the system described by Donnan and of its application to some biological systems is therefore useful at this point.

THE DONNAN EQUILIBRIUM APPLIED TO BIOLOGICAL SYSTEMS

The Donnan concept

Donnan [86] considered the ion distribution in two electrolyte solutions separated by a membrane permeable for all the ion species but one. Such a situation is schematized

in *Figure 1A,* in which it is assumed that the membrane separating two compartments, *I* and *II,* of equal and constant volume, is permeable for Na^+ and Cl^- but not for the anion An^-. Let the concentration of these ions, indicated by the heights of the columns, in the initial state be as shown in *Figure 1B.* The Na^+ concentration

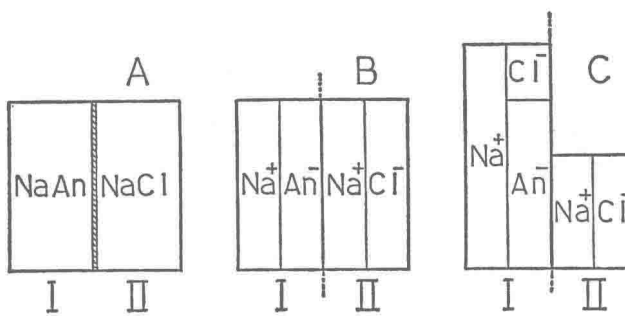

Figure 1.—A shows two electrolyte solutions NaAn and NaCl in compartments of equal, not expandable volume (*I* and *II*), separated by a membrane permeable for Na^+ and Cl^- but not for An^-. *B* and *C* show the initial and equilibrium concentrations of the ions respectively, indicated by the heights of the columns.

is the same in both compartments, and equal to the Cl^- concentration in *II* and the An^- concentration in *I.* The osmoconcentrations are thus the same in the two compartments. Chloride ions will now start to diffuse across the membrane from *II* into *I* where no Cl^- is present. However, since equality of the positive and negative charges has to be maintained in each of the two compartments, the chloride ions have to be accompanied by Na^+. The Na^+ concentration in compartment *I* thus becomes greater than that in *II.* Na^+, accompanied by Cl^- to maintain equality of charges, then starts to diffuse from compartment *I* into *II.* After some time the diffusion of NaCl from *I* into *II* equals the diffusion of salt

from *II* into *I*, and a Donnan equilibrium has been reached. The concentrations of the ions under these conditions are shown in *Figure 1C*. The Na^+ concentration has increased in compartment *I*, which now also contains Cl^-. The An^- concentration in *I* is obviously unchanged, and the Na^+ and Cl^- concentrations in *II* are diminished, but equal.

It has been shown that under equilibrium conditions the product of the concentrations of ions which can pass through the membrane in one compartment equals this product in the other compartment:

$$[Na^+]_I \times [Cl^-]_I = [Na^+]_{II} \times [Cl^-]_{II} = [Na^+]^2_{II} = [Cl^-]^2_{II}$$

or

$$\frac{[Na^+]_I}{[Na^+]_{II}} = \frac{[Cl^-]_{II}}{[Cl^-]_I}. \qquad (1)$$

Designating the original ion concentrations as C, and the concentration of the Na^+ and Cl^- ions which passed from compartment *II* into *I* as x, then the sodium concentration in compartment *I* has, under equilibrium conditions, become $(C+x)$ and the Cl^- concentration x. In compartment *II* the concentrations of Na^+ and Cl^- are $(C-x)$:

$$x(C+x) = (C-x)^2$$

and

$$x = \frac{1}{3} C$$

If for instance the two compartments contained initially equal amounts of a molar solution of NaAn and NaCl, then the Na^+ concentration in compartment *I* would have become 4/3 moles, the Cl^- concentration 1/3 mole, and in compartment *II* the Na^+ and Cl^- concentrations would have been 2/3 mole at equilibrium.

The osmoconcentration in compartment *I* has become considerably greater than that in *II*. The difference (in moles) can be expressed as

$$(2C+2x) - (2C-2x) = 4x$$

In the example described, the difference in osmoconcentration is then $4/3\,C$. This represents a difference in osmotic pressure (expressed in atmospheres) of

$$RT \cdot 4/3C$$

where R is the gas constant (0.08205 l-atm deg^{-1} $mole^{-1}$) and T the absolute temperature. Starting with molar solutions of NaAn and NaCl the osmotic pressure would at 25°C and at equilibrium be 32.6 atm higher in compartment *I* than in compartment *II*.

A Donnan equilibrium can be established only when the walls of the compartments are strong enough to resist the considerable pressures which build up in such systems. If the walls lack mechanical strength then the difference in osmotic pressure between the compartments results in a water movement from compartment *II* into *I*. This will tend to produce an increase in the Na^+ and Cl^- concentrations in *II*, and a decrease in the ion concentrations in *I*. A redistribution of the Na and Cl ions will then occur, according to the Donnan rule, resulting in an additional transport of NaCl into compartment *I*, increasing the osmoconcentration in that compartment again. This movement of electrolyte and water will continue until all the NaCl solution of compartment *II* is transported into compartment *I*.

A potential difference develops across the membrane of a Donnan system in equilibrium. Since Na^+ and Cl^- are free to move across the membrane, diffusion will tend to equalize the concentration of the ions in the two compartments. This will result in a small surplus of negative ions in compartment *I* and an excess of positive charges in compartment *II*. This potential difference between the compartments counteracts a movement of Cl^- to the left and of Na^+ to the right. As expressed by Davson,[28] the equilibrium can be regarded as the result of two mutually exclusive tendencies: one, to achieve equal concentrations of the species of diffusible ions in

the two compartments and two, to achieve electrical neutrality throughout the system. The result of the operation of these opposing tendencies is the unequal distribution of the various ion species and the potential across the membrane. Expressed differently, there is equilibrium between the forces of diffusion which tend to move ions from the compartment with the higher concentration to that with the lower concentration, and the potential which tends to move the ions in the opposite direction. The magnitude of the membrane potential in volts (E_v) is given by Nernst's equation which in the example used becomes

$$E_v = \frac{RT}{zF} \ln \frac{[Na^+]_{II}}{[Na^+]_I} = \frac{RT}{zF} \ln \frac{[Cl^-]_I}{[Cl^-]_{II}} \qquad (2)$$

in which R is the gas constant (expressed in electrical units, 8.314 joules deg^{-1} mole^{-1}), T the absolute temperature, F Faraday's constant (the charge on an equivalent of monovalent ions, 96,500 coul), and z the valency of the ion (in this case 1). Starting with molar concentrations in the compartments the potential in *I* will become about 0.018 v negative with respect to *II*.

Application of the Donnan concept to lymph formation

Donnan pointed out that the physico-chemical principles discussed above are applicable to biological systems, but it was Van Slyke,[105, 106] who introduced these concepts in biology. From the nature of the Donnan equilibrium, which results in differences in osmotic pressures between the compartments, balanced by considerable hydrostatic pressures, it can be inferred that such equilibria would be rare in biological systems. One instance may be the system formed by blood plasma and tissue fluid (lymph, edema fluid) separated by the capillary membrane. In this system the blood pressure can provide the necessary hydrostatic pressure. The capillary membrane is considered to be impermeable for protein