# Biochemistry of the Central Nervous System

*Edited by*

**F. BRÜCKE**

*General Editor*

**O. HOFFMANN-OSTENHOF**

Proceedings of the

FOURTH

INTERNATIONAL

CONGRESS OF

BIOCHEMISTRY

Vienna

**PERGAMON PRESS**

*London New York Paris Los Angeles*

Proceedings of the

FOURTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY

VIENNA, 1–6 September 1958

General Editor

O. HOFFMANN-OSTENHOF, *Vienna, Austria*

Secretary-General of the Congress

VOLUME III

SYMPOSIUM III

# BIOCHEMISTRY OF THE CENTRAL NERVOUS SYSTEM

EDITED BY

F. BRÜCKE, *Vienna, Austria*

Symposium Director

*Published for and on behalf of*

The Organizers and The International Union of Biochemistry

*by*

THE SYMPOSIUM PUBLICATIONS DIVISION

PERGAMON PRESS

LONDON · NEW YORK · PARIS · LOS ANGELES

PERGAMON PRESS LTD.
*4 and 5 Fitzroy Square, London, W.1*

PERGAMON PRESS INC.
*122 East 55th Street, New York 22, N.Y.*
*P.O. Box 47715, Los Angeles, California*

PERGAMON PRESS S.A.R.L.
*24 Rue des Écoles, Paris Ve*

First Published 1959



Library of Congress No. 59-8791.

Printed in Great Britain by Page Bros. (Norwich) Ltd.

PROCEEDINGS OF THE

# FOURTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY

VIENNA, 1958

VOLUME III

SYMPOSIUM III

I.U.B.
SYMPOSIUM SERIES
Volume 5

Veröffentlichungen des

VIERTEN INTERNATIONALEN KONGRESSES FÜR BIOCHEMIE

Wien, 1.–6. September 1958

*Gesamtherausgeber:*

O. Hoffmann-Ostenhof, *Wien, Österreich*

Generalsekretär des Kongresses

Band III

Symposium III

# BIOCHEMIE DES ZENTRALNERVENSYSTEMS

*Herausgegeben von*

F. Brücke, *Wien, Österreich*

DIREKTOR DES SYMPOSIUMS

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Comptes rendus des Travaux du

QUATRIÈME CONGRÈS INTERNATIONAL DE BIOCHIMIE

Vienne, 1–6 Septembre, 1958

*Publié par*

O. Hoffmann-Ostenhof, *Vienne, Autriche*

Secrétaire général du Congrès

Tome III

Symposium III

# BIOCHIMIE DU SYSTÈME NERVEUX CENTRAL

*Rédigé par*

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IN THE PROCEEDINGS OF THE

FOURTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY

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# VORWORT

Der Wunsch, auf dem IV. Internationalen Kongreß für Biochemie ein eigenes Symposium über die Biochemie des Zentralnervensystems zu veranstalten bedarf einer Begründung, weil in den letzten Jahren wiederholt Kongresse mit ähnlichem Thema abgehalten wurden. Zu derartigen Veranstaltungen werden jedoch neuerdings nur eingeladene Gäste zugelassen, so daß sich die Beratungen einer kleinen Gruppe von Spezialisten beinahe unter Ausschluß der Öffentlichkeit vollziehen.

Ein entschiedener Vorteil der grossen internationalen Kongresse besteht darin, daß einem weiten Kreis von Interessierten der neueste Stand auf einem Wissensgebiet vermittelt werden kann, welches sie vielleicht erst seit kurzem, oder nur nebenbei interessiert, aber doch für ihre Arbeit von Wert ist.

Aus diesen Gründen wurde auch das Symposium inhaltlich möglichst vielseitig gestaltet und z.B. am ersten Tag ausführlich über die Kationenverschiebungen im Zusammenhang mit dem Erregungsvorgang gesprochen, ohne daß diese Erörterungen direkt das ZNS berührten.

Um für das Programm möglichst viele Wünsche berücksichtigen zu können, wurden frühzeitig zahlreiche Fachleute in der ganzen Welt schriftlich darüber gefragt, welche Themen sie berücksichtigt haben wollten und welche Referenten sie für geeignet hielten. Allen diesen Kollegen sowie besonders den aktiven Teilnehmern sei für ihre Hilfe herzlich gedankt. Die Diskussion, die leider zeitlich zu beschränkt war, kann im Folgenden nur teilweise berücksichtigt werden.

Meinen besten Dank möchte ich noch dem Verlag "Pergamon-Press" aussprechen, der nicht nur die Ausführung des vorliegenden Buches, sondern auch die schon beim Kongreß vorliegenden Vorabdrucke mit größter Sorgfalt und in bester Form besorgt hat.

F. BRÜCKE

# CONTENTS

# IONIC MOVEMENTS IN CELL MEMBRANES IN RELATION TO THE ACTIVITY OF THE NERVOUS SYSTEM

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The sodium theory of nervous conduction, first proposed by Hodgkin *et al.*,[1] and later elaborated in detail by Hodgkin and Huxley[2], has made it possible to describe the electric events in the active giant nerve fibre in terms of a sequence of changes in permeability to sodium and potassium. This treatment is formally very satisfactory indeed. When supplemented with the concept of active sodium extrusion (possibly coupled with active potassium uptake) during recovery, a process for which notably Keynes has presented ample evidence, we have obtained a self-consistent picture of ionic shifts and electric phenomena in the giant fibre, and it seems that its main features can be found in many other excitable tissues as well.

The relative simplicity and consistency of the theory is, however, obtained by ascribing to the nerve fibre membrane a number of highly peculiar properties. It must possess certain elements or "patches" which are passively permeable to potassium but not to sodium, others which are permeable to sodium but not to potassium, and the effective number of both permeability elements must vary in a characteristic manner with the membrane potential. Moreover the membrane has a third kind of element which is capable of forcing an exchange of internal sodium against external potassium at the expense of energy derived from the metabolism. For none of these permeability elements has a striking analogue been found in model experiments. It may therefore be pertinent to devote a little time to discussing the soundness of the underlying concepts. The first requirement must be that the concepts are theoretically sound, but since inanimate models are missing, such a discussion must primarily be centred around the question whether a description of ionic and electric behaviour of living systems generally can be obtained in terms of the same or similar entities as those used in the description of excitable tissues. If, as the present author believes, the answer is affirmative, the inquiry may also point to experimental

objects where certain characteristics of the permeability elements can be studied in greater detail than is possible with nerve.

One point which has given rise to much controversy in the past, and which is still being discussed, is whether or not it is necessary to assume the existence of an active sodium transport out of and active potassium transport into living cells. Since high potassium and low sodium is a property of almost all cells, it is understandable that several workers have found it more attractive to consider the ionic distribution a consequence of the structure of the living cytoplasm (compare the fixed charge hypothesis of Ling[3]), so that in essence the cell would have the properties of a highly selective ion-exchange resin preferring potassium to sodium. It is true that none of the components of cells so far isolated (proteins, phosphate esters, nucleic acids, etc.) have the required binding ability for potassium, and even more mystical is the capacity to exclude sodium from cells into which, manifestly, this ion can be shown to penetrate, as evidenced by tracer experiments, but the advocates for the ion-exchange resin hypothesis can always claim that the binding properties are only characteristic of the intact cell and its specific internal structure.

If we are to choose between the apparently equally unreasonable hypotheses of active ion transport in the membrane and selective binding in the cytoplasm, it may be well to remember that no matter what we choose, we have to accept the existence of active sodium transport in many cell membranes anyhow, the evidence being, as far as I can see, quite compelling. In the present context we shall understand by active transport a transfer which is not due to the physical forces of diffusion, electric field and solvent drag (that is, the friction exerted upon the species in question by mass flow of the solution through pores in the membrane). When these forces are excluded, there is every reason to believe that the transport is due to chemical reactions in the membrane phase in which the ion in question is taking part. From these considerations it appears that in order to single out the transport processes which must be considered active, one must be able to characterize the behaviour of an ion which is not subject to anything but physical forces and which moves uncombined with other moving particles. If the existence of pores through which there is a net flow of solvent can be ruled out or disregarded, simple passive diffusion through any membrane, homogeneous or composite, is characterized by the flux equation:

$$M_{in}/M_{out} = (c_o/c_i) \exp (zFE/RT)$$

where $M_{in}$ is the inward flux, $M_{out}$ the outward flux, $c_o$ is the concentration of the ion in the outside solution, $c_i$ its concentration in the inside solution, $E$ is the potential difference between the outside and inside solutions, and $z$, $F$, $R$ and $T$ have their usual meaning.

If there are pores in the membrane through which there is a net flow of water (either due to osmosis or to electro-osmosis) there is an additional term, the magnitude of which is determined by the linear rate of flow in the pores, the length of the pores and the diffusion coefficient of the ion in question. The magnitude of the drag term can be estimated by suitable test substances. This term can be very important in membranes where there is a large net transfer of water.

Under such conditions the flux equation becomes:

$$\ln (M_{in}/M_{out}) = \ln \frac{c_o}{c_i} + \frac{zFE}{RT} + \frac{\Delta w}{D} \int_0^{x_0} \frac{1}{A} \, dx$$

Where $\Delta w$ is the volume rate of water flow through unit area of membrane, $D$ is the free diffusion coefficient of the ion in question, $A$ is the fraction of the area which is available to flow, $x$ is the distance from the outside boundary of the membrane area, and $x_0$ the total thickness of the membrane. The value of the integral can be estimated from the behaviour of a suitable test substance. In the cases discussed in the following, the drag term is of secondary importance and can usually be disregarded.

The above two equations describe the behaviour of an ideal passive ion. Deviations from this behaviour are always found in cases of active transport. But deviations are also seen when the ions associate with moving membrane constituents (exchange diffusion) or when they are interdependent during the passage through the membrane (single file diffusion). Thus active transport is most unambiguously identified when the transport proceeds "uphill", that is, when it overcomes the sum of the forces of diffusion, electric field and solvent drag. As will appear from the following, still another criterion of active transport may turn out to be important in the future, namely that the transport is stoicheiometrically related to the metabolism. Returning now to the problem of active sodium transport, let us first consider the giant cells of the alga *Halicystis ovalis*. This organism lives in the sea along the shores of the west coast of America. The cells are spherical, of the size of a gooseberry, and have a large central vacuole surrounded by a thin (about 10$\mu$ thick) layer of cytoplasm. Externally there is a thin cellulose wall of considerable tensile strength.

As shown by Blinks[4], the cell can easily be impaled with a narrow glass tube and the cytoplasm will heal around it. Thus it is possible to obtain reliable measurements of the composition of the sap as well as of the electric potential difference between the sap and the surrounding sea water. The sap is a dilute, watery solution of inorganic ions, slightly hypertonic with respect to the sea water. The composition is shown in Table I. It is seen that the dominating ions are K, Na and Cl. The chloride concentration is not very different from that of the surroundings, Na is definitely lower, and

TABLE I

*Ionic composition of the cell sap of* Halicystis ovalis (mM)

| Na | Ka | Cl | Potential outside zero |
|---|---|---|---|
| 257 | 337 | 543 | −80 |

K much higher than in the sea. The table also shows the potential difference between sap and sea. It is seen that the sap is some 80 mV negative relative to the surroundings. When this is taken into account, it turns out that the potassium ions of sap and sea water are in electrochemical equilibrium. Thus, by the Nernst equation we find that e.m.f. $= (RT/F)$ ln $(K_{sap}/K_{sea})$. For the sodium ion, however, there is a gross discrepancy between the concentration required by electrochemical equilibrium and that actually found. The same is the case with Cl, only that chloride is present far in excess of the equilibrium concentration whereas sodium is present in a concentration which is several times too low. In the case of this cell there can be no question of specific sites being responsible for the high potassium concentration or for the low sodium. Also the potential measurements can be made with greater assurance than can most intracellular potential determinations, because the effect on the activity coefficients of proteins and other polyelectrolytes is absent. In order to explain the ionic composition of the sap of this cell, it is thus necessary to postulate an active transport of sodium ions out of, and chloride ions into the sap.

The existence of the two active transport processes has been demonstrated most beautifully by Dr. Blount from U.S.A., who for the time being is working in our laboratory. In order to measure the active transports quantitatively, Dr. Blount used the "short-circuiting" technique (compare Ussing and Zerahn[5]). The principle is the following: If identical solutions are placed in contact with the two sides of a living membrane, and if, furthermore, the membrane potential is totally short-circuited, ions which are not subject to active transport should pass through the membrane at the same rate in both directions and cannot give rise to any net transport of electricity, once a condition of steady state is obtained. A net transfer of any ionic species is, on the other hand, indication of active transport, and by comparing the ionic currents with the total current passing through the short-circuit it is possible to estimate the contributions of the individual active transport processes to the total output of electricity. Diagrammatically the circuit used for shorting the cell is shown in Fig. 1. In order to short-circuit the *Halicystis* cell, it is impaled by a double-barrelled micropipette. Through the inner pipette sea water is

infused, whereas excess fluid is drained off through the outer tube. After rinsing, the cell can be constantly perfused with recirculated sea water. The inner pipette also serves as bridge for the potential measurement, whereas the outer pipette serves as current bridge for the short-circuit. The ionic fluxes in the inward and outward directions can be determined with isotopes and the net ionic currents are found as the differences between inward and outward fluxes. To make a long story short, it has now turned out that electric current which can be drawn from the *Halicystis* cell can be totally accounted for as the sum of the active sodium transport in the outward direction and the active chloride transport in the inward direction.

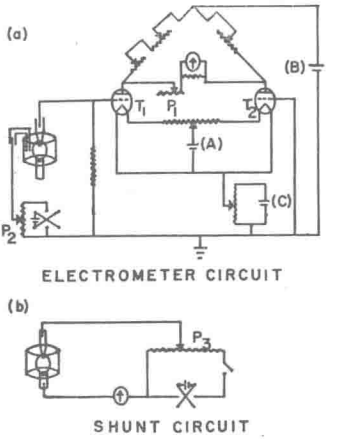


Fig. 1. A schematic diagram of the equipment used in potential difference measurements and effecting a short-circuit between vacuole and environment.

The two active transports seem to be at least partly independent, since the active sodium transport can be inhibited with DNP added to the outside bathing solution without any immediate effect on the part of the current which is due to active chloride transport.

It is interesting that MacRobbie and Dainty[6] have found convincing evidence for the same two active transports in the giant cells of the brackish water alga *Nitellopsis*. Even here there is an active chloride transport mechanism located in the tonoplast (the cell membrane facing the vacuole) and an active sodium transport in the outward direction (the cell membrane facing the surroundings). The potassium of the sap is in electrochemical equilibrium with that of the surroundings. Thus it appears that an intracellular phase (the sap) owes its ionic composition to active ion transports and to the potential created by these transports, whereas specific binding plays no role whatsoever. Actually MacRobbie and Dainty found the ratio Na/K to be the same in the cytoplasm and in the sap, indicating that not even in the thin layer of cytoplasm surrounding the sap is there

any measurable discrimination between K and Na due to specific binding sites. The whole discrimination then must depend upon substances and processes located in the outward-facing membrane.

In the giant plant cells discussed above we could account for the electric asymmetry on the basis of two active transport processes, one for Cl and one for Na. There is, however, a whole group of membranes where the active transport of sodium seems the only factor responsible for the production of electric energy. Thus this is the case for many epithelia: The skin of frogs, toads and other amphibia, the urinary bladder of the toad, the mucosa of the large intestine of toad, bullfrog and rat, the coecum of the guinea pig, and the epithelium of the rumen of ruminants. Furthermore there is strong evidence that active sodium transport is one of the major active processes in kidney, and probably most other glands. Of these organs the frog skin has been studied most exhaustively and it may be appropriate to recapitulate some of the main characteristics of the ion transport in this organ.

The isolated frog skin will transport Na from the outside, not only when both sides are bathed with Ringer solution, but also when the outside solution is quite dilute with respect to sodium. Since the inside solution is positive relative to the outside solution as long as traces of sodium are present in the outside solution, this means that sodium can be transported inwardly against the combined effects of electric potential and concentration gradients.

This behaviour is exemplified in Table II (control periods). In the experiments in question the outside solution was 1/10 Ringer and the inside solution Ringer. The potential difference across the skin (inside

TABLE II

*The influence of DNP. An in- and outflux of* Na *through the frog skin. Solution bathing the outside—*1/10 *Ringer. Solution bathing the inside—Ringer*

| | $M_{in}$ ($\mu$Mcm$^{-2}$hr$^{-1}$) | $M_{out}$ ($\mu$Mcm$^{-2}$hr$^{-1}$) | $E$ (mV) | $M_{in}/M_{out}$ (found) | $M_{in}/M_{out}$ (calculated) |
|---|---|---|---|---|---|
| Contr. | 0.34 | 0.093 | 62 | 3.66 | 0.011 |
| DNP | 0.25 | 1.57 | –11 | 0.16 | 0.15 |
| Contr. | 0.445 | 0.145 | 82 | 3.07 | 0.025 |
| DNP | 0.049 | 0.56 | –6 | 0.087 | 0.126 |
| Contr. | 0.228 | 0.008 | 72 | 28.5 | 0.017 |
| DNP | 0.032 | 0.216 | –7 | 0.148 | 0.132 |