

PROCEEDINGS OF THE
FOURTH INTERNATIONAL CONGRESS
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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

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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 Huxley², 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 Ling⁶), 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:

$$\ln (M_{in}/M_{out}) = \ln \frac{c_0}{c_1} + \frac{xFE}{RT} + \frac{\Delta w}{D} \int_0^x \frac{1}{A} dx$$

Where Δw 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 stoichiometrically related to the metabolism. Returning now to the problem of active sodium transport, let us first consider the giant cells of the alga *Halcystis 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 10μ thick) layer of cytoplasm. Externally there is a thin cellulose wall of considerable tensile strength.

As shown by Blinks⁴, 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 $\text{e.m.f.} = (RT/F) \ln (K_{\text{sap}}/K_{\text{sea}})$. 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 Zerahn⁶). 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.

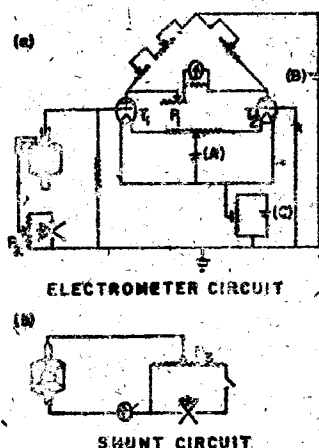


Fig. 1. A schematic diagram of the equipment used in potential difference measurements and effecting a short-circuit between vacuole and environment.

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 Dainty³ 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} ($\mu\text{Mcm}^{-2}\text{hr}^{-1}$)	M_{out}	E (mV)	M_{in}/M_{out} (found)	M_{in}/M_{out} (calculated)
Contr. DNP	0.34	0.093	62	3.66	0.011
	0.25	1.57	-11	0.16	0.15
Contr. DNP	0.445	0.145	82	3.07	0.025
	0.049	0.56	-6	0.087	0.126
Contr. DNP	0.228	0.008	72	28.5	0.017
	0.032	0.216	-7	0.148	0.132

positive relative to outside) is shown in column 3. It is seen that the influx of sodium, as measured with ^{22}Na , is 2-3 times larger than the outflux of sodium, measured simultaneously with ^{24}Na . In other words there is a strong net transport in the inward direction. The table also shows the enormous discrepancy between the flux ratio found and that predicted for an ion with a single positive charge passing the membrane influenced only by the physical forces of electric field and diffusion. Furthermore the table shows that the active transport of sodium is totally discontinued under the influence of dinitrophenol in a dose sufficient to increase slightly the oxygen consumption. After the addition of DNP to the inside bathing solution the sodium ion behaves very nearly as should a strictly passive behaving ion. It is worth mentioning that chloride (and the other halides) behave passively in the frog skin even in the absence of DNP.

The sensitivity of the active sodium transport to DNP and similar uncoupling agents is particularly interesting because it points to energy-rich phosphates as the energy source for the transport. In passing it should be mentioned, however, that the active sodium transport is also inhibited by all agents which interfere with oxidative metabolism, with the citric acid cycle and with the glycolytic cycle. The experiments so far mentioned indicate that sodium can be transported against its electrochemical potential gradient, whereas chloride is not. Thus it was natural to propose that the active sodium transport is the source of the e.m.f. The direct proof of this hypothesis was provided by the short-circuiting technique.⁵ When fully short-circuited (and with identical solutions on both sides) the skin produces a current which is exactly equal to the amount of sodium ions actively transported from the outside to the inside bathing solution (see Table III). The identity of active sodium transport and current output holds true even when the active transport is stimulated by hormones

TABLE III

Short-circuit current and sodium flux values for a number of short-circuited frog skins (Rana temporaria) (Ringer's solution on both sides)

	$\mu\text{A}/\text{cm}^2$			
	Na (in)	Na (out)	Na	Current
I	20.1	2.4	17.7	17.8
II	11.1	1.5	9.6	9.9
III	40.1	0.89	39.2	38.6
IV	62.5	2.2	60.3	56.8
V	47.9	2.5	45.4	44.3

and drugs and when it is inhibited, for instance by low pH. Only in one case does the equality of sodium transport and current production break down: when adrenaline is added to the inside solution there is an extra contribution to the electric current, provided by active transport of chloride in the outward direction. Apparently this active chloride transport resides in the skin glands and not in the ordinary epithelial cells.

TABLE IV

Effect of partial replacement of Na^+ by K^+

(K/Na) x 100 in solutions	Influx ($\mu\text{eq. Na/hr}$)	Outflux ($\mu\text{eq. Na/hr}$)	Na ($\mu\text{eq. Na/hr}$)	Na current (μA)	Total current (μA)
I 35.0	13.2	0.53	12.6	339	289
35.0	9.4	0.81	8.6	230	236
II 35.0	5.80	0.38	5.42	145	157
35.0	6.13	0.35	5.78	155	154
35.0	5.50	0.41	5.09	136	143
III 35.0	4.05	1.04	3.01	81	85
35.0	5.30	1.06	4.24	114	116
35.0	5.25	0.94	4.41	118	121

Even when as much as one-third of the Na^+ in the bathing solution is replaced by K^+ , the total current generated by the short-circuited frog skin is carried by the Na ions. Area of skin: 7.1 cm^2 .

The active sodium transport of the frog skin is highly specific. Even if a large proportion of the cation offered to the skin is potassium, calcium or magnesium, there is no evidence of any net transport in the inward direction. This property of the frog skin is exemplified in Table IV, which shows that if 35% of the cation is K and the rest Na, the total short-circuit current consists of sodium ions. The only cation which can to some extent substitute for sodium is Li. But although this ion is actively transported through the skin it also piles up in the epithelium cells, as if the transport mechanism cannot handle as much Li as is taken up, and this ultimately leads to total inhibition of the ion pump.

The isolated frog skin presents an almost ideal object for the study of the relationship between active transport and oxygen consumption. The active sodium transport can be measured routinely as the short-circuit current, and tracer experiments or chemical analyses are only necessary whenever a new agent is tested for its effect to ascertain that it does not upset the one-to-one relationship of sodium transport to current output. The oxygen consumption associated with the active sodium transport of the frog skin has been measured by Zerahn⁷ and Leaf and Renshaw⁸. The

following account is based mainly upon Zerahn's work, but in almost every respect his work was verified by Leaf and Renshaw. Two main points have emerged from this work. Firstly, there must exist a stoichiometric relationship between the metabolic rate and the rate of active sodium transport. The relationship is expressed schematically in Fig. 2, which shows the oxygen consumption as a function of the rate of active sodium

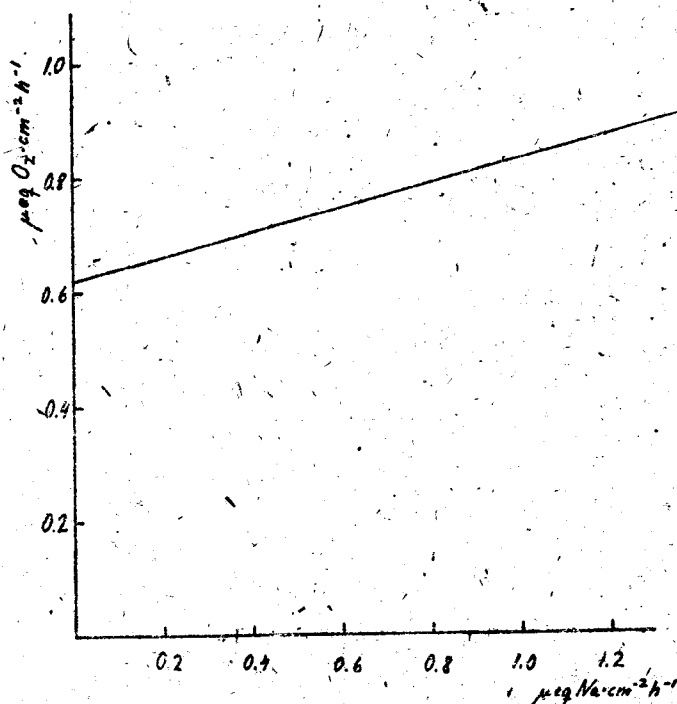


Fig. 2. The relation between net sodium transport and oxygen consumption of the isolated short-circuited frog skin.

transport. Clearly, even in the absence of any net sodium transport, there must be a certain oxygen consumption. This is partly an expression of the fact that the transporting cells have other functions than to transport sodium, but also one must realize that the skin, besides the actively transporting epithelium, contains connective tissue cells, smooth muscle fibres and gland cells, all of which must consume a certain amount of oxygen. The state of no active sodium transport can be brought about most simply by leaving out Na from the outside bathing solution. Distilled water, MgCl_2 , CaCl_2 , and KCl all give essentially the same result. Also the active transport can be stopped simply by applying a large potential which opposes the sodium pump. Some 180–200 mV inside positive suffice to