SOME REMARKS ON ION TRANSPORT ACROSS EXCITABLE MEMBRANES.
I. THE STATIONARY STATE

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Physical theories describing ion transport across channels in biological membranes are critically reviewed. Special emphasis is put on their restrictions due to the very small channel dimensions.

INTRODUCTION

A biological membrane is characterised by a very low dielectric constant (≈ 2) as compared to that of its surrounding (≈ 80, a typical value for water electrolyte solutions). This is due to both the structure and the composition of the membrane, which may be concerned as a lipid bilayer with integral proteins embedded into it.

The energy needed for a mole of ions to pass the border between a water solution and the membrane phase, called the Born energy ΔG, may be calculated according to the following equation:

$$\Delta G = \frac{z^2 e^2 N}{8\pi r}\left(\frac{1}{\varepsilon_m} - \frac{1}{\varepsilon_w}\right) \qquad (1)$$

where z means the valency of the ion, r stands for its radius, ε_m and ε_w are the dielectric constants of the membrane interior and of the bathing water solution, respectively, e is the elementary charge, and N means the Avogardo number. Putting $\varepsilon_m = 2$ and $\varepsilon_w = 80$ we get from (1), for a small univalent ion, a value of about 300 kJ/mole, which is two orders of magnitude greater than the energy of one-dimensional thermal motion at room temperature. A correction to (1), resulting from the finite thickness of a biological membrane, may be calculated from electrostatic considerations (Neumcke & Läuger 1969; Parsegian 1969). If this effect is taken into account, a lowering of the Born energy is obtained of approximately 10%. Still, membrane permeabilities for physiologically important ions like Na^+, K^+, Ca^{++} and Cl^-, corresponding to such a large value of the Born energy, are several orders of magnitude smaller than those measured on biological membranes. The observed, relatively great ionic permeabilities of biological membranes are due to the fact that during evolution structures have developed in these membranes, which essentially lower the energetic barrier for hydrophilic ions. These structures are integral proteins whose hydrophobic parts interact with the lipid hard-core of the membrane whereas their hydrophilic parts form:

- relatively broad, nonselective aqueous pores like those present in the nuclear membrane,
- specific ionic channels, which open in response to various physical and chemical stimuli, and may be divided into voltage-dependent, temperature-dependent, ligand-dependent and mechanical stress-dependent ones,
- carriers or transporters. They selectively bind extracellular and/or intracellular ions, what causes them to undergo some structural change resulting in transporting the ions across the membrane. Dissociation of the carrier- transported ion complex takes place at the other side of the membrane. This kind of ion transport may be driven by energy supplied by metabolic reactions (in most cases ATP hydrolysis) – then it is called the active transport. The other possibility is that the transport occurs at costs of the free energy of the system - in this case it is called the facilitated diffusion.

The just afore-named transport structures are very important from the viewpoint of cell homeostasis. Namely, they are all involved in controlling the composition and the volume of the cell as well as the membrane potential, defined as electric potential difference between the interior of the cell and the extracellular fluid. They also often play an important role in initialising and controlling many aspects of the cell function, only to mention the

basic role of ionic channels in the phenomenon of excitability.

In this paper we shall focus our attention on ion transport through channels. This phenomenon has been investigated since the early fifties in a continuously growing number of laboratories and a very rich data has been assembled since that time. Simultaneously, some more or less complicated physical theories describing ion transport across channels have been constructed and applied to explain the data. In spite of these efforts, the mechanism of ion movement across biological membranes is still not fully understood, and cannot give any satisfying, detailed description of the phenomenon. In this article a short review of basic physical aspects of the problem will be presented.

ION CHANNELS

The extensive work of Hodgkin and Huxley on the action potential in nerve cells of *Loligo*, carried out in the late forties and the early fifties, suggested that in an excitable membrane there exist some selective, „active paths" for sodium and potassium ions whose conductance depends on the membrane potential (for review see Meves, 1984). These „active paths" are nowadays called „voltage-dependent ionic channels", and their presence has been shown in practically all types of cells. As was mentioned above, there also exist channels which open in response to other stimuli, like a change in temperature, a change in mechanical stress or binding of a ligand (agonist).

The most important difference between broad, nonselective aqueous pores also present in biological membranes and ionic channels is that the latter are highly selective, guaranteeing at the same

time a high turnover number, exceeding the value of 10^6 ions/second.

Our knowledge of ionic channels has been essentially enriched since Neher and Sakmann invented the patch-clamp technique, which made it possible to measure ionic currents flowing across single channels (Sakmann & Neher, 1995 and references therein). Using this method many types of ionic channels have in particular been identified, differing from each other in the transported ionic species (mainly K^+, Na^+, Ca^{++} and Cl^-), the factors opening them and their function (Hille, 1992).

As suggested in the title of this article, we shall below restrict our considerations to voltage-dependent channels. The modern methods of molecular biology allowed to determine the primary structure of proteins forming many of these channels, what in turn made it possible to propose the three-dimensional arrangement of the proteins within the membrane. By cloning the genes, which are responsible for voltage-dependent channel proteins coding, a close similarity between the „transporting" structural parts of the whole channel family has been shown (Terlan & Stühmer, 1998 and literature therein). This similarity occurs in spite of the fact that sodium and calcium channels are formed by a single protein molecule, whereas potassium channels are tetramers. The α-subunits of these channels proteins penetrate the whole membrane and consist of four homological domens called I, II, III and IV. Each of these domens is made up of 6 transmembrane segments (S1-S6), see Fig. 1.

In the S4 segment there are relatively numerous charged amino acid residues which, most probably, play the role of „voltage sensors". The „pore" itself is formed by a protein_chain loop between
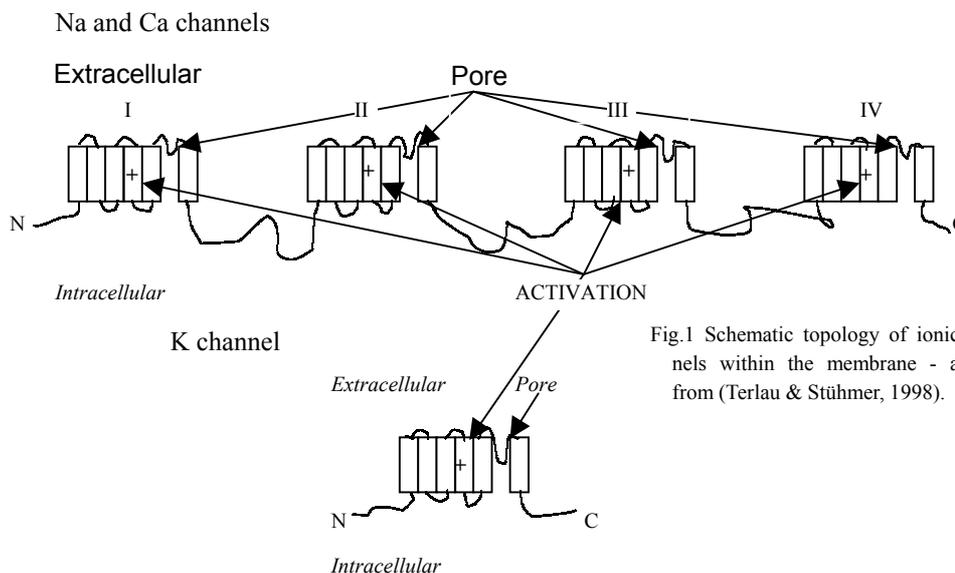
Na and Ca channels

Fig.1 Schematic topology of ionic channels within the membrane - adapted from (Terlau & Stühmer, 1998).

the segments S5 and S6. As for the „selectivity filter", the neurotoxin binding data suggests that it is located at the extracellular mouth of the pore.

This picture of the voltage-dependent channels structure has recently been confirmed by rentgenographic investigations of potassium channels (Doyle, Cabral, Pfuetzner, Kuo, Gulbis, Cohen, Chait & MacKinnon, 1998). The results of these investigations encourage us to imagine the channel pore as drawn in Fig. 2. Such a channel geometry, together with the chemical composition of the channel, explains both the selectivity for K^+ ions and the high transport rate (Roux & MacKinon, 1999). Two K^+ ions, one at the *fore-part* of the selectivity filter and another at its end, have already completely shed their hydration shells. The size of both the filter and the K^+ ion allow a coordination of the ion with four oxygen atoms of the carbonyl residues of the channel protein. The sodium ion, essentially smaller than the potassium ion, does not „fit energetically" the filter which makes the channel selective. The third K^+ ion in Fig. 2 has already entered the main channel pore where approximately 50 water molecules are present. Mutual electrostatic repulsion between all the transported ions assures a high transport rate (Miller, 2000). In this way the basic properties of voltage-dependent potassium channels have been explained. On the other hand, one should of course remember that X-ray investigations deliver a purely static picture of the channel protein.

A similar model has been proposed for calcium channels (Corry, Allen, Kuyucak & Chung, 2000; 2001). There are some minor differences between the two models, concerning mainly the channel dimensions and the polar groups present in both the selectivity filter and the channel vestibule.

MEMBRANE POTENTIAL

Ion transport across a membrane is equivalent to an electric current which charges the „membrane capacitor" to a certain membrane potential. In the steady state conditions the net electric current is equal to zero and the membrane potential does not depend on time in this case — we call it then the resting potential. The value of resting potential is influenced by ion movement across all channels present in the membrane which in turn depends on both the transport properties of the membrane and ion concentrations inside as well as outside the cell. To properly model the resting potential dependence on these physical parameters of the system, we need have, as a starting point, a plausible physical theory of ion transport across channels.

In view of the structure of ion channels, in particular their geometry, one should choose the molecular dynamics as this theory. All the needed calculations, however, would, in the case of a realistic channel model, take several years, even when the most fast present-day workstations would be employed (Levitt, 1999; Eisenberg, 2000)! In order to shorten essentially the duration of the calculations, one is forced to simplify the channel model by taking into account only some chosen elements of the channel protein. There is also another limitation of this approach, namely we still cannot describe exactly all the channel molecules-permeating species interactions.

To simplify the model one assumes that the structure of the channel protein does not depend on time, and that water molecules both inside the channel and in its surrounding form a continuum (Cooper, Jakobsson & Wolynes, 1985; Levitt, 1999; Corry, Allen, Kuyucak & Chung, 2000, 2001). Treating the permeating ions as Brownian particles, one may then choose as the equation of motion of individual ions the following Langevin equation:

$$m_k \frac{d\mathbf{v}_k}{dt} = m_k f_k \mathbf{v}_k + \mathbf{F}_R(t) + q_k \mathbf{E} , \qquad (2)$$

where m_k, $\mathbf{v}_k$, q_k and f_k are the mass, the velocity, the charge and the friction coefficient of k-th ion, respectively, $\mathbf{F}_R$ is the stochastic force resulting

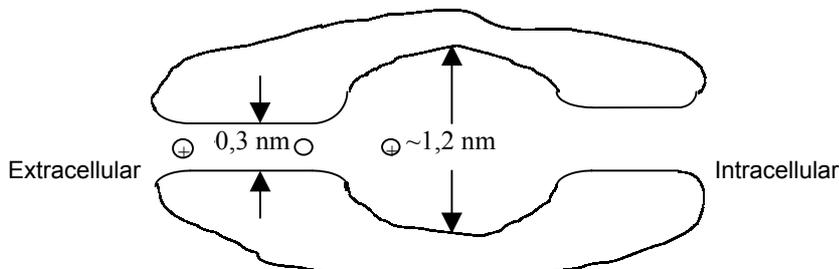Extracellular   0,3 nm   ~1,2 nm   Intracellular

Fig. 2. Schematic representation of ion channel geometry – adapted from (Lipscombe, 2000; Miller, 2000; Corry, Allen, Kuyucak & Chung, 2001).

from ion thermal collisions with both water molecules and the pore wall, and **E** is the electric field strength.

However, random walk of ions inside the channel, especially in the region of the (very narrow) selectivity filter, is of a completely different nature than that in the solutions, bathing the membrane. What more, due to the small dimensions of an ion channel, one is not allowed to neglect the ion-ion interaction within the channel, even if only a few of them permeate the channel at the same time. For the same reason the interactions of permeating ions with the electrically charged groups of the channel protein need be taken into account. One should also mention here a quite general weakness of the Langevin approach, namely the splitting the total force acting on an ion into the three separate contributions, appearing on the right-hand side of Eq. (2), which is not fully justified (Anselm, 1978).

Once the boundary and the initial conditions have been specified, Eq. (2) should be integrated by the discrete time intervals method. Since the dynamics of neither the channel protein nor water molecules inside the channel is allowed for, the time needed for a computer to calculate the stochastic path of an ion permeating the channel shortens significantly when compared to that in the molecular dynamics method. In both approaches - the Langevin dynamics and the molecular dynamics - the critical factor is the specifying of the initial ion distributions inside the channel as well as the energy of their interactions. Not going into details we may say that the Brownian dynamics is effective only for channels of a well-known molecular structure (Levitt, 1999).

For the reasons discussed above it is clear that effective simulations of ion transport through membrane channels by the Brownian dynamics, and still more so by the molecular dynamics, are still the matter of future.

The framework of theoretical transmembrane ion transport investigations is therefore the theory of electrodiffusion (Weiss, 1996 and the literature therein), either in the simple form applied for the first time to the problem more than fifty years ago (Goldman,1943; Hodgkin & Katz, 1949) or in the extended one, developed by Eisenberg and co-workers (Eisenberg, 2000 and references therein). In both cases a mean-field theory is considered with the Nernst-Planck equation written down as the starting point. For wide multiion channels, neglecting ion-ion interactions and assuming the frictional forces in the system considered to be sufficiently great, we may derive this equation from the following Smoluchowski equation:

$$\frac{\partial}{\partial t} p(x,t) = \frac{1}{f}\left(-F\frac{\partial p(x,t)}{\partial x} + kT\frac{\partial^2 p(x,t)}{\partial x^2} \right),$$

where $p(x,t)$ is the probability of finding an ion at time t in the region $(x,x+dx)$, f is the friction coefficient, F is the electric force derivable from a potential and kT the Boltzmann factor. The derivation includes of course the assumption that the term „concentration" retains its physical sense within the channel. This may, however, not be the case in very narrow channels of excitable membranes (Miller, 1999).

A simplified electrodiffusion description of permeation may be derived by combining the following equation delivered by physical chemistry and nonequilibrium thermodynamics:

$$\mathrm{J}_k(x,t) = -u_k c_k(x,t)\frac{\partial}{\partial x}\widetilde{\mu}_k(x,t), \qquad (3)$$

where J_k stands for ionic flux of k-th species and u_k, c_k and $\widetilde{\mu}_k$ are mobility, concentration and electrochemical potential of the same ionic species, respectively, with the Poisson equation:

$$\frac{\partial^2 \varphi(x,t)}{\partial x^2} = -\frac{\rho(x,t)}{\varepsilon} = -F\sum_{k=1}^{n} z_k c_k(x,t) - \rho_z(x,t)$$
$$, \qquad (4)$$

where φ means the electric potential, ρ is the electric charge density, z_k – the valency of k-th ionic species and F the Faraday constant. Two separate contributions to the local charge density $\rho(x,t)$ appear in Eq. (4). The first of them represents the permeating ions charge density, whereas the second one, denoted by $-\rho_z(x,t)$, results from the (local) presence of channel protein electric charges. In the general case equations (3) and (4) have to be considered in the three-dimensional form (e.g. Kurnikova, Coalson & Nitzan, 1999). It should be mentioned that Eq. (3) neglects any coupling between ionic fluxes.

However, in the most of the related papers, especially in the older ones, further significant assumptions are made. The system considered is unidimensional and stationary, and the ideality of the permeating species is assumed. Then Eq. (3) assumes the form:

$$\mathrm{J}_k = -u_k c_k \frac{d}{dx}(RT\ln c_k + z_k F\varphi), \qquad (5)$$

which is equivalent to

$$J_k = -u_k RT \frac{dc_k}{dx} - u_k c_k z_k F \frac{d\varphi}{dx} , \qquad (5a)$$

or to:

$$J_k = -c_k u_k RT \frac{d}{dx}\ln\left[c_k \exp(\frac{z_k F\varphi}{RT})\right] , \qquad (5b)$$

In equations (5) – (5b) R means the gas constant and T is absolute temperature, whereas all the remaining symbols preserve their meaning defined earlier.

An analytic expression for the flux J_k may immediately be obtained from any of the equations (5) - (5b) if electroneutrality is assumed within the channel, that is, when the following condition is fulfilled:

$$\frac{d\varphi}{dx} = \frac{\Delta\varphi}{d} = \frac{V_m}{d}$$

where d is the thickness of the membrane and V_m stands for membrane potential. Though this conditions never strictly holds within a real ion channels because of permeating ions mobility differences, deviations from electroneutrality are, on the other hand, small when there are no membrane bound electric charges present (MacGillivray & Hare, 1969). When the condition is assumed to hold, we get after integrating (5a) across the membrane:

$$J_k = z_k u_k F \frac{V_m}{d} \frac{c_{k,o} - c_{k,i}\exp(-z_k F V_m / RT)}{1 - \exp(z_k F V_m / RT)} \quad (6)$$

where the subscripts „o" and „i" mean „extracellular" and „intracellular", respectively.

Integrating in turn Eq. (5b), this time with no additional assumptions, yields:

$$J_k = z_k F\left(\int_0^d \frac{dx}{c_k u_k}\right)^{-1}(V_m - V_k) , \qquad (7)$$

where $V_k = \frac{RT}{z_k F}\ln\frac{c_{k,o}}{c_{k,i}}$ is the Nernst (equilibrium) potential for k-th ionic species. Multiplying equations (6) and (7) by the molar electric charge of k-th species $z_k F$, we obtain corresponding formulae for ionic current densities I_k. Equation (7) assumes then the form:

$$I_k = G_k(V_m - V_k) \qquad (7a)$$

where $G_k = z_k^2 F^2(\int_0^d \frac{dx}{c_k u_k})^{-1}$ is called the chord conductance of k-th ionic species.

Equation (7a) resembles of course the Ohm's law. It should be stressed, however, that ionic concentrations appearing in the quantity G_k depend – from the formal point of view *via* the Poisson equation – on the electric potential within the channel, that is, the chord conductance G_k may in general be a function of membrane potential V_m. Thus, the $I(V_m)$ relationship does not need to be linear.

Treated formally, relations (3) – (7a) are valid for media, which are homogeneous in the direction perpendicular to that determined by ionic fluxes. On the other hand, ion transport across biological membranes takes place *via* channels, that is, from the viewpoint of its transport properties, a biological membrane should be seen as a mosaic with „non-permeable" and „permeable" regions fitting together. In this case the ion flux per unit area of the membrane may be simply written down as a sum of ion fluxes flowing through all individual channels present in the whole unit area. For instance, relation (7a) may then be written down as follows:

$$I_k = \gamma_k N_k p_k(V_m - V_k) = G_k'(V_m - V_k) \qquad (7b)$$

where the symbols γ_k, p_k and N_k refer to a channel permeable to the k-th species and they mean its conductance, open probability and number of channels per unit area, respectively. Formally, there are no differences between the relations (7a) and (7b). On the other hand, the physical sense of the conductances G_k and G_k' is quite different!

When a cell is in a stationary state, the net electric current across its membrane must be equal to zero. The membrane potential is then time-independent and we call it the resting potential. When analytic formulae for electric currents carried by all the permeable ionic species are specified, an appropriate expression for resting potential is easily obtained by requiring the sum of the currents to vanish. In the case of nervous cells the ionic species taken into account are usually K^+, Na^+ and Cl^- only. Then, putting the total electric current equal to zero, we get, after some simple algebra, from Eq. (6):

$$V_m = \frac{RT}{F}\ln\frac{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^+]_i} \ (8)$$

where V_m is the resting potential V_m and $P_k = \dfrac{RTu_k K_k}{d}$ is the permeability of k-th species, K_k being the respective coefficient of ion distribution between the bulk and the membrane phase. Formula (8), the famous Goldman equation, does, of course, not take into account the active transport of ions which, in the stationary state of a living cell, counterbalances the passive one. However, the contribution to the resting potential of the ion active pumping is usually relatively small (a few mV only). Besides, the presence of active transport may be allowed for in an analytic way (Weiss, 1996). It is important to remember that Eq. (8), either in the original form or in the augmented one, with active transport contribution included, is derived from Eq. (6), that is, it „contains" the constant electric field assumption.

In the literature Eq. (8) is very often written down for K^+ i Na^+ ions only, especially for excitable cells. The most often used explanation of this Cl^- ions omission is the argument that at excitable cell rest chloride ions should be in thermodynamic equilibrium. This argument is supported by the fact that so far no active transport of chloride ions has been detected in nervous cells. On the other hand, the axoplasm and extracellular chloride ions concentrations are 120 mM and 470 mM, respectively. When inserted into the Nernst equation, these concentrations produce a chloride equilibrium potential of 34 mV, whereas the measured values of resting potential lie between 50 and 70 mV. In other words, the axoplasm concentration of chloride ions is 2–4 times greater than that estimated with the use of Nernst equation (Rusell, 1984)! This obvious contradiction might be explained by the experimental finding, that there exists a coupled, ATP-dependent transport of Cl^-, Na^+ K^+ ions in the axon membrane (Russell, 1984; Altamirano, Breitwieser & Russell, 1999).

We should remember that the membrane potential described by equation (8) is in fact merely a diffusion potential. In other words, equation (8) does not take into account any possible electric potential difference between the surface of the membrane and the adjacent electrolyte solution (Kotyk & Janacek, 1977). Finally, an analytic expression for the membrane potential may be derived within the constant field formalism, only if all the permeating ions are monovalent. As a matter of fact, permeating bivalent ions presence may be allowed for (Lewis, 1979), but the resulting equation for the resting potential is of a complicated character and numerical methods must be employed to solve it.

All what has been said above indicates clearly that equation (8) describes the resting potential in an approximate way.

One of course may derive an equation for the resting potential starting with the $I(V_m)$ relationship given by Eq. (7a) or (7b). Requiring the sum of all passive ionic currents to vanish, one then immediately obtains the following result:

$$V_m = \frac{G_K V_K + G_{Na} V_{Na} + G_{Cl} V_{Cl}}{G_K + G_{Na} + G_{Cl}}, \qquad (9)$$

which expresses the resting potential in terms of chord conductances and equilibrium potentials of the permeating ions. The equivalent electric circuit is shown in Fig. 3.

We should remember, however, that equation (7a) (or (7b)) has been obtained by integrating the time-independent form of the Nernst-Planck equation. The corresponding chord conductances are thus related to the stationary state. What more, the conductances cannot be, within the approach, explicitly expressed in terms of the extra- and intracellular ionic concentrations, so that we are forced to treat them as a quite complex system parameters to be determined experimentally.

We have been a little bit critical when discussing above the physical basis of equations (8) and (9), and pointing out the assumptions needed to get them. On the other hand, as already stated above, neither the molecular nor the Brownian dynamics may deliver today a better physical description of the resting potential established across a real biological membrane, the reason being the complexity of the system. As for any extended mean-field electrodiffusion theory, it has been recently shown that such an approach cannot be successful when channel dimensions are smaller than the appropriate Debye length (Moy *et al.*, 2000; Corry *et al.*, 2000). Unfortunately, this condition applies to most, if not to all, ionic channels of biological membranes.

One more remark concerning the origin of resting potential of nervous cells. Though, from the formal point of view, the same transport coefficients G_k' appear in equations (7b) and (9), their
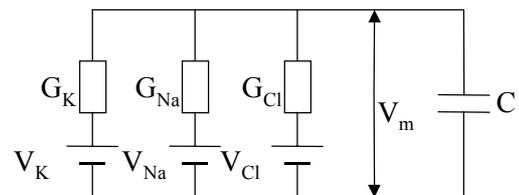

Fig. 3. Equivalent circuit of a membrane unit area.

microscopic interpretation may be quite different in various biological situations. Whereas at sufficiently high membrane potentials the (macroscopic) chord conductance G_k' in Eq. (7b) may practically be interpreted in terms of single channel conductance, open probability, and density of voltage-dependent channels only, this does not need be the case at rest! Recent studies have namely shown that the „resting" potassium current is carried not by voltage-dependent channels but by voltage-independent ones, belonging to the so-called „twin-pore" family and known as TASK-1 (Brown, 2000). The possibility that the axon resting conductance may be due to the action of some voltage-independent „leakage channels", has already been discussed previously (Darnell, Lodish & Baltimore, 1986; DeFelice, 1997). In other words, when applying Eq. (9) to describe membrane potential at nervous cell's rest, we most probably deal with a „new type" of chord conductance G_k', completely *different* from that introduced by Hodgkin and Huxley to describe by Eq. (7b) the $I(V_m)$ relationship in the axon membrane.

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