JGP 100th Anniversary

The founding of *Journal of General Physiology*: Membrane permeation and ion selectivity

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This essay begins with a description of the founding years of *Journal of General Physiology* (JGP) and a historical overview of the content of the journal. It then turns to key conceptual articles published in JGP that advanced the field of membrane permeation and ion selectivity. Much of this information comes from reading the online archives of JGP and searches in PubMed.

Foundations

General physiology was traditionally a special introductory section of physiology books that dealt with general mechanistic and physical principles needed to understand life (e.g., Ludwig, 1852, 1856; Bayliss, 1918). That section would typically include physical chemistry and properties of protein solutions (colloids); viscosity; enzyme reactions; osmosis; properties of gases; electricity; laws of diffusion; temperature effects; oxidation–reduction; growth; properties of cells; effects of salts, acids, and bases; muscle mechanics, and more. General physiology [*allgemeine Physiologie*] was exemplified by disciples of Johannes P. Müller (1801–1858) and their circle, particularly Hermann von Helmholtz, Emil Du Bois-Reymond, Ernst Wilhelm von Brücke, Carl Ludwig, and Ludwig's teacher, Adolf E. Fick. Paul Cranefield (editor of JGP 1966–1995) cites Ludwig as saying in 1847 that "We four imagined that we should constitute physiology on a chemico-physical foundation, and give it equal rank with physics" (Cranefield, 1957). Ludwig was referring to his contemporaries Helmholtz, du Bois-Reymond, von Brücke, and himself. When they were 26–30 years old, they were declaring the demise of vitalism and the ascent of physical explanations in biology. von Brücke (1843) proposed pore theories of biological membranes; Fick (1855) proposed the laws of diffusion, even specifically for pores in membranes; and Helmholtz measured the conduction velocity of the nerve action potential. The great physiologist Claude Bernard (1813–1878) also could be said to promote general physiology with his emphasis on the scientific method and experiments to establish ultimate causes of physiological phenomena and his insistence that the laws of life are the same as those for inanimate objects.

Founding JGP. While in Jena in 1894, Max Verworn (1863–1921) first published his textbook *Allgemeine Physiologie* (Verworn, 1895), which emphasized the cellular underpinnings of physiology, and in 1902 he founded the journal *Zeitschrift für allgemeine Physiologie*. This German journal was published only until 1923, two years after Verworn died. In 1912, Jacques Loeb (biologist, Rockefeller Institute) published his first book, *The Mechanistic Conception of Life* (Loeb, 1912), and in 1918 he founded JGP in New York with himself and Winthrop J.V. Osterhout (botanist, Harvard) as coeditors. Both frequented the Marine Biological Laboratory (MBL) at Woods Hole in the summers—a powerhouse of general physiologists working on marine animals, plants, and model systems. Before 1918, Loeb had been publishing many times a year in *Science* magazine and in *Proceedings of the National Academy of Sciences of the USA*. After he founded JGP, he switched to publishing in this new journal, and amassed 61 articles within six years until he died at age 64. Osterhout, too, had published frequently in *Science*, but with the founding of JGP, he switched and eventually published 124 papers in its pages. One of them was an elegant, analytical, informative, and admiring obituary of his senior colleague, Jacques Loeb (Osterhout, 1928). In 1926, Osterhout moved to the Rockefeller Institute to replace Loeb, continuing as an editor until 1964. John Northrop (biochemist, Rockefeller Institute) and William J. Crozier (visual physiologist, Rutgers University, later moving to Harvard University) were subsequently added as coeditors. In 1946, Wallace O. Fenn (physiologist, University of Rochester) was added as well. Northrop published 121 papers in JGP, Crozier, 119, and Fenn, 23.

These early events set a pattern. JGP started as a Rockefeller journal, a vehicle noted for the papers of its early editors that was edited in house. As well, there were papers from many other Rockefeller faculty and

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from others elsewhere. The journal also had an immediate association with the MBL in Woods Hole, and when much later the Society of General Physiologists was founded (1946), the Society and JGP maintained a loose association with each other and with the MBL. The number of JGP papers that included work from the MBL was considerable. Table 1 provides more information on selected scientists who published in JGP, emphasizing the early days. Inter alia, it shows their years of publication in the journal, the number of publications, and whether they were associated with the journal editorial board or with the Rockefeller Institute. A thoughtful, scholarly, and more complete discussion of the founding of JGP, the philosophy of the founding editors, the editorial process, and the early content is found in an editorial by Olaf Andersen (editor of JGP 1995–2008; Andersen, 2005).

From the beginning, papers in JGP ranged broadly in biology and biological physical chemistry with a quantitative or "biophysical" style. In the first eight years, subjects included cow, chicken, and human growth curves; infection of bread; tropisms in plants (helio-, geo-, photo-, and galvanotropisms); osmosis, temperature effects on many biological processes; and nervous responses of comb jellies, anemones, sea worms, *Limulus*, *Planaria*, and *Drosophila*. The cascade of papers from the editors was membrane- and permeation-biased, with titles like "A comparative study of permeability in plants" (Osterhout, 1919), "Influence of the concentration of electrolytes on the electrification and the rate of diffusion of water through collodion membranes" (Loeb, 1919), "Influence of the concentration of electrolytes on some physical properties of colloids and of crystalloids" (Loeb, 1920), "Donnan equilibrium and the physical properties of proteins: I. Membrane potentials" (Loeb, 1921), and "Conductivity and permeability" (Osterhout, 1921). They began the focus on penetration of electrolytes and water into cells or across model membranes. By way of reminder, collodion (from nitrocellulose) can form an inert, porous, semipermeable membrane used for the classical dialysis sac, and the Donnan (Gibbs-Donnan) equilibrium (Donnan, 1911) describes the unequal ion, potential, and osmotic distributions at equilibrium across a semipermeable membrane when a dissolved component on one side is charged and impermeant. Loeb used what he called gelatin chloride as the impermeant molecule, and found for example that trivalent ions and pH changes modified the permeability of the collodion membrane. Amusingly, after 14 articles and a book on the Donnan equilibrium from Loeb, Archibald V. Hill (1923a,b) wrote a brief complaining note in the *Proceedings of the Royal Society* and then in JGP proclaiming his impatience with too much ado about Donnan. He said the results were self-evident from the second law of thermodynamics and they were not logical proofs of the Donnan mechanism. Loeb's junior colleague David I. Hitchcock (physical chemist, later at Yale) wrote a rebuttal in JGP (Hitchcock, 1923). Loeb's extended focus on membrane potentials in Donnan equilibria at that time may have contributed to a residual invocation even today in some textbooks of Donnan potentials for discussions of the plasma membrane potential of excitable cells. Donnan potentials can arise between blood and tissue fluids across the vasculature, but the Goldman-Hodgkin-Katz (GHK) style of explanation is usually a better choice for excitable membranes, which are never at equilibrium.

E. Newton Harvey published a long series of papers on bioluminescence (1919–1941). Northrop and Moses Kunitz published many papers on proteins, their first crystallization, their ionic properties in solutions, their kinetics, and their digestion by acid. For example, Northrop concluded from collodion membrane di-

Table 1. **Founders and key authors on permeation**

Name	Publications in JGP			Ed?	Born	Deceased	RI?
	No. of papers	First year	Last year				
Jacques Loeb	61	1918	1924	Ed	1859	1924	Y
W.J.V. Osterhout	124	1918	1956	Ed	1871	1964	Y
Leonor Michaelis	14	1925	1941	Ed	1875	1949	Y
Wallace O. Fenn	23	1919	1968	Ed	1883	1971	N
Moses Kunitz	55	1923	1962		1887	1978	Y
John H. Northrop	121	1919	1968	Ed	1891	1987	Y
William J. Crozier	119	1919	1950	Ed	1892	1955	N
Kenneth S. Cole	25	1928	1975		1900	1984	N
Karl Söllner	16	1940	1960		1903	1986	N
H. Keffer Hartline	12	1923	1983		1903	1983	Y
Harry Grundfest	46	1932	1974		1904	1983	Y
Torsten Teorell	7	1936	1959		1905	1992	N
David E. Goldman	7	1943	1968		1910	1998	N
Ichiji Tasaki	9	1951	1976		1910	2009	N
Arthur K. Solomon	50	1952	1983	EB	1912	2002	N
Ahron Katchalsky	2	1961	1963		1914	1972	N
Lorin J. Mullins	31	1942	1986	EB	1917	1993	N
Gilbert N. Ling	4	1960	1967		1919		N
John W. Moore	20	1960	1979		1920		N
Susumu Hagiwara	20	1957	1980	EB	1922	1989	N
Daniel C. Tosteson	29	1955	1991	EB	1925	2009	N
George Eisenman	9	1953	2011		1929	2013	N
Paul Horowicz	8	1965	1984	EB	1931	1995	N
Clay M. Armstrong	29	1964	2014	EB	1934		N
Knox Chandler	27	1965	2006	EB	1933	2017	N
Alan Finkelstein	41	1958	2015		1935		Y
Bertil Hille	39	1967	2016	EB	1940		Y
Robert S. Eisenberg	22	1967	2009	EB	1942		N
Francisco Bezanilla	54	1969	2013	EB	1944		N
David C. Gadsby	23	1977	2015	EB	1947		Y
Olaf S. Andersen	36	1976	2015	Ed	1945		Y
Henry A. Lester	27	1975	2014	EB	1945		N
Christopher Miller	39	1979	2014	EB	1946		N
Ted Begenisich	24	1974	2012	EB	1947		N
Michael D. Cahalan	21	1976	2014	EB	1948		N

The table includes founding contributors and representative authors focused on permeation with publications in JGP that began before 1980. Ed, editor; EB, editorial board; N, no; RI, some direct association with the Rockefeller Institute or its successor, The Rockefeller University; Y, yes.

alysis experiments that trypsin acts like a monovalent cation between pH 2.0 and 10 (Northrop, 1924) and pepsin acts like a monovalent anion between pH 1.0 and 7 (Northrop, 1925). Northrop was later awarded a Nobel Prize for his work on crystallization of proteins and viruses.

Subsequent years. After Loeb died, collodion membrane work in JGP was continued by Leonore Michaelis (Berlin, Johns Hopkins, then Rockefeller Institute), starting in 1925. He and Maud M. Menten had earlier developed the famous Michaelis-Menten equation for enzyme kinetics (Michaelis and Menten, 1913). Michaelis was mechanistic. He considered the molecular dimensions of pores. The titles sound familiar, e.g., "Studies on permeability of membranes: VII. Conductivity of electrolytes within the membrane" (Green et al., 1929). In the period 1924–1941, Hugo Fricke and then Kenneth S. Cole were publishing many papers in JGP on impedance and capacitance measurements of red blood cells, marine eggs, giant algae, and eventually of the squid giant axon, including the landmark "Electric impedance of the squid giant axon during activity" (Cole and Curtis, 1939). Such impedance measurements set the stage for a new style of membrane biophysical studies in JGP a few decades later. The 1930s also began continuing studies on vision from H. Keffer Hartline, Selig Hecht, Maurice Henri Pirenne, George Wald, and W. J. Crozier. By the late 1950s, JGP was publishing flux work with radioisotopes and explicit studies of ion pumps and active transport. The 1960s brought the glass micropipette electrode, calcium actions, voltage clamp of excitable membranes and planar lipid bilayers, and studies of ion channels of excitable cells. The 1970s added vertebrate and invertebrate muscle contraction.

Search queries on PubMed show that JGP has remained solidly in the general physiology camp over its 100 years. PubMed shows ~8,250 JGP articles altogether (June 2017). Using terms for organisms suggests that many articles did not concern a specific organism: frog (17% of articles); rat or mouse (15%); plant terms (6%); bacteria, squid, and fish (3 to 4% each); virus or bacteriophage (2%); yeast (2%); and fly (0.4%). At the tissue level, we find cell (63%), nerve and neuron terms (29%), muscle (20%), and epithelia (5%). On the subject level, we find electrical terms, membrane, ion, and protein (39–45% each); channel (30%); transporter (30%); permeability (15%); enzyme (12%); water (10%); gene and nucleic acid terms (6%); and mitochondria, antibody, nucleus, and cytochrome (~1% each). Thus historically, cell, membrane, electricity, ion, protein, channel, and transporter predominate. The gene revolution starting with Watson and Crick (1953) was not reflected quickly in JGP, nor was the contemporaneous membrane biophysics revolution of Hodgkin and Huxley (1952). Indeed, the Hodgkin-Huxley work was sufficiently complex, different, and complete that for more than a decade, there was very little follow-up, and there were relatively few citations in any journal.

Permeation

We turn now to ideas important for understanding membrane permeability and ion selectivity, concepts that were dear to the early editors. Many seminal papers and series of investigations appeared in JGP. Although the term "permeability" yielded only 15% of the published JGP articles, the background ideas come from many more. My choice of articles to highlight is narrow, subjective, and not comprehensive. It emphasizes the first 60 years. JGP has played a central role in these concepts, although much important work has appeared in parallel in many other journals.

Julius Bernstein (German physiologist, trained with du Bois-Reymond and von Helmholtz) postulated that cells are surrounded by a membrane that is selectively permeable to K^+ ions at rest (Bernstein, 1902, 1912). This would account for the negative resting potential of excitable cells. He called his hypothesis "the membrane theory." The absence of intracellular microelectrodes, radioisotopes, electron microscopy, or experimental distinction among various possible permeation mechanisms meant that for many decades, the membrane theory remained one hypothesis among several. During the first half of the twentieth century, experimentalists gradually recognized ways to measure fluxes and membrane potentials, to distinguish plasma membranes of individual cells from tissue membranes formed by sheets of cells, to distinguish flux mechanisms like passive diffusion from active transport, and to measure one mechanism while preventing others. At the same time, new model lipid membranes were developed, and permeation and transport theories were elaborated.

Michaelis and Osterhout: Pores and carriers. The pore hypothesis was developed by von Brücke (1843); he called them *kanäle*) for osmosis in pig bladders, and extended to glomerular filtration by Ludwig (1856). The hypothesis was repeated by many in the following 100 years but not accepted for cell membranes until the 1960s. A history of the pore concept is given in Hille (2001). Michaelis was a clear proponent in many papers in JGP measuring diffusion across "dried collodion" membranes. His was an appropriate mechanistic view, as seen in the following excerpt from Weech and Michaelis (1928): "In offering an explanation for the large differences in the diffusion rates of different substances through the same membrane there are two theories requiring consideration. On the one hand we may conceive of the membrane as a phase working as a solvent for the diffusing substance [the "solubility theory"]. In this case the partition coefficient would determine the

gradient of concentration of the substance in the membrane and thus control the rate of diffusion. On the other hand it is possible that the specific mobility of the substance expressed as a diffusion coefficient has a different value within the membrane from that in water. In this case it is not necessary to use a concept of solubility although the two theories do not exclude each other…. In previous papers we have treated the dried collodion membrane as a sieve membrane with pores almost of molecular size. When the membrane channels are as minute as this a distinction between a molecule in solution in the membrane and one within a channel becomes doubtful…. In attempting then to form a mental picture of the mechanism of diffusion in our experiments which would account for the observations made two possibilities were kept in mind. We thought it probable that the great difference in the diffusion rates between molecules of different sizes was due to the fact that the membranes contained *pore channels* [emphasis mine] of varying sizes, that only the largest of these channels would serve for the passage of a large molecule such as glucose, and that many more channels could be used by the smaller molecules such as acetone and urea…. However it is possible to formulate another hypothesis in which the great difference with which molecules of larger and smaller size diffuse may be attributed to increased frictional resistance of the larger molecules against the pore walls rather than to variations of pore size."

These passages reiterate thinking that dates back as far as von Brücke and Ludwig (Ludwig, 1856) and their successors. They were written as conditional or subjunctive hypotheses, but they are a nice anticipation of what was to come much later for permeation in real biological cell membranes. Recall that here they are about dialysis sacs. In other papers, Michaelis postulates selective adhesion and charges on the pore wall as accounting for differences in the "transfer number" of anions and cations, and he anticipates effects of pore saturation with higher solute concentrations and related clogging of pores by the permeating substances.

At the same time, Osterhout was publishing his many studies of penetration of substances into giant algae in JGP. Experiments with NH_3 entering *Valonia* showing saturation of influx at higher concentrations led him to propose a carrier-like theory in a *Proceedings of the National Academy of Sciences of the USA* paper entitled "How do electrolytes enter the cell?" (Osterhout, 1935): "A very simple explanation (suggested by the study of models) is that electrolytes enter by combining reversibly with a constituent HX of the protoplasm. The simplest assumption is that NH_3 enters the nonaqueous layer [we would now say *membrane*] and reacts with HX…assuming that under the conditions of these experiments the rate of entrance is proportional to NH_4X at the outer surface of the protoplasm (which we may call NH_4X_0). Adsorption at the surface might give a curve somewhat like [the experimentally observed saturation curve] if adsorption occurred on a micelle [protein?] at the outer surface and if the micelle moved across the outer nonaqueous protoplasmic surface layer [outer face of the membrane] and reacted in the aqueous layer of the protoplasm to form a salt which then passed through this layer, and repeated the process at the inner nonaqueous layer [inner face of the membrane]…. It is not impossible that strong electrolytes also enter by combining with one or more constituents of the protoplasm."

Thus by the 1930s, membrane solubility, pores, and saturable carriers were being advanced in JGP as hypotheses for substances to cross into cells. None of them had been established, and some authors were still cautious about the use of the word *membrane* and about implicating specific classes of biochemicals or macromolecules.

Fricke and Cole: Capacitance and cell membrane. Fricke published five papers in JGP on capacitance measurements in suspensions of red blood cells or ghosts (1924–1935) and gave a molecular interpretation. He extracted a fair value for the specific membrane capacitance from which he estimated that, if the membrane was "lipoid," its thickness would be "from 20 to 30 carbon atoms, if we assume that the distance between two neighboring carbon atoms of an organic molecule is 1 to $1.5 \cdot 10^{-8}$ cm [1 to 1.5 Å]," citing Langmuir's estimates with fatty acids (Fricke, 1925). Cole continued with a series of impedance measurements in JGP, mostly on whole cells or suspensions of cells (1927–1941). He was a physicist who interpreted his measurements as linear electrical elements in an electrical equivalent circuit. He was not a physiologist, and he did not think about molecular explanations so much as formal electrical ones. Cole's most cited papers were in physics, concerning the electrical properties and phase–angle behavior of physical capacitors. Nevertheless, his exquisite biological measurements eventually showed that marine eggs, giant algal cells, and the squid giant axon were surrounded by membranes of very high resistance and capacitance (0.6–1.1 $\mu F/cm^2$) and contained "sap" that was several-fold less conductive than seawater. The measurements from Fricke and Cole significantly increased the probability that the postulated invisible membrane actually existed and that it was a major but exceedingly thin permeability barrier at the cell surface. Consistent with his formal electrical and nonmolecular style, Cole's papers from this period may have mentioned only once (Cole, 1928) that Fricke had used membrane capacitances (values similar to Cole's later numbers) to calculate a membrane thickness. All of these papers used only extracellular electrodes. They preceded intracellular recording.

Two of Cole's papers in JGP had very special significance. Curtis and Cole (1938) initiated study of the squid giant axon at the MBL. Noting the difficulty of studying impedance of nerve bundles, they said, "It is therefore necessary to further simplify matters by making transverse measurements on a single axon, but this was not considered possible until we were introduced to the squid giant axon by Dr. John Z. Young (1936). We are also very much indebted to him for his assistance in preliminary experiments which were made during the summer of 1936 at the Biological Laboratory, Cold Spring Harbor, Long Island."

This was followed by the milestone discovery (Cole and Curtis, 1939) of large impedance changes during the propagated action potential of the squid giant axon as well as in *Nitella* (Cole and Curtis, 1938). It was through spending a month working with Cole and Curtis at the MBL in 1938 and observing these impedance experiments that Alan L. Hodgkin first learned about the squid axon preparation and, as he says in his autobiography (Hodgkin, 1992), that working as a team beats doing complex experiments alone. The key discovery of these squid papers was that the conductance of the membrane rose 40-fold during the action potential, and yet the capacitance did not change, leading Cole and Curtis (1939) to say, "We may reason, as we did for Nitella, that the conductance is a measure of the ion permeable aspect of the membrane and we see that the maximum conductance is far from a complete permeability [i.e., the membrane does not break down fully]. And indeed the capacity, which represents the ion impermeable portion of the membrane, has not been encroached upon by more than 2%. Thus if the change on excitation is uniform throughout the structure of the membrane it must be so delicate as to leave the capacity and phase angle nearly unchanged and conversely if there are drastic changes they must be confined to a small fraction of the membrane area."

The last 17 words here are as close as Cole got to thinking about ion channels, a concept that even in the 1970s he, Gilbert N. Ling, and Ichiji Tasaki (American cell physiologists born in China and Japan, respectively) were unwilling to accept (personal communication from K.S. Cole and I. Tasaki). For Cole and Tasaki, it was the entire membrane that became permeable to ions rather than specific structures within the membrane. For Ling (1960), there was no membrane.

Theories for passive fluxes, membrane potentials, and ion selectivity. Understanding formal physical rules of diffusion was a requirement for analyzing permeation in membranes. Once the concept of free ions was established by Svante Arrhenius, Nernst (1889) could write the formula for equilibrium potentials, and Planck (1890) could formulate the equations of free diffusion of dissolved ions in concentration gradients based on individual ionic mobilities and concentrations solved in conjunction with the Poisson-Boltzmann equation. The Planck formulation was conceptually simple but led to difficult mathematical expressions in practice. Several practical simplifications were introduced by electrochemists. Henderson (1907) had given a formula for the steady-state potential (electromotive force) developed at the junction of two electrolytes in terms of free-solution mobilities and concentrations, based on the assumption that the concentrations varied continuously as a linear gradient across the liquid junction. Expressions like his are the basis for our modern liquid-junction potential calculations and were used until the 1950s as the basis for concluding that K^+ is by far more "mobile" than Na^+ or Cl^- in resting membranes of many cells (e.g., in JGP, Osterhout, 1930, 1939; Damon, 1932).

Over the years, many variations on the Nernst-Planck theory have been published in JGP. In the period 1939–1959, Torsten Teorell advanced the theory of thick membranes containing a dense fixed charge (e.g., Teorell, 1953), with further tests by Karl Sollner and by Alexander Mauro and Alan Finkelstein (1958). Ora Kedem and Aharon [Katzir-] Katchalsky (Israeli physical chemists) described how to bring the phenomenological coefficients (mobilities) into accordance with the Onsager relations of irreversible thermodynamics (Kedem and Katchalsky, 1961). However, the most influential theoretical paper was one that assumed a constant electric field in the membrane (Goldman, 1943).

In his PhD thesis and in JGP, Cole's student David E. Goldman used the Nernst-Planck theory to give a widely adopted formulation for the diffusion potential across a membrane with different ion mobilities (Goldman, 1943). He assumed that the electric field varied linearly across the membrane diffusion regime (constant field) and followed an integration method used by Neville F. Mott (British solid-state physicist) who had assumed a constant electric field in a theory for copper–copper-oxide rectifiers. Goldman obtained two equations: the ion fluxes for individual ions and the membrane zero-current voltage, equations that we usually call the GHK equations. For a single salt with concentrations n_1 and n_2 on the two sides, producing cations (+) and anions (−) with mobilities u_+ and u_-, he wrote the membrane voltage as

$$V = (RT/F)\ln[(u_+ n_2 + u_- n_1)/(u_+ n_1 + u_- n_2)].$$

Hodgkin and Katz (1949) reexpressed this in terms of membrane permeabilities and as sums for several ions on the inside (i) and the outside (o) of the membrane, illustrated here for Na^+, K^+, and Cl^-:

$$V = (RT/F)\ln\left[\frac{(P_{Na}[Na]_O + P_K[K]_O + P_{Cl}[Cl]_i)}{(P_{Na}[Na]_O + P_K[K]_O + P_{Cl}[Cl]_O)}\right].$$

This equation subsequently became the workhorse for interpreting reversal potential measurements in terms of relative ion permeability. These relative permeabilities often were taken as an experimental definition of ion selectivity. For a deeper discussion of such constant-field equations and their considerable impact on work in JGP, see the JGP Milestone article by Alvarez and Latorre (2017).

Solomon and Mullins: Pore radius and selectivity. The period 1935–1955 saw theoretical discussions of effects of pore size on ultrafiltration accompanied by measurements in collodion membranes. Pores were represented as cylinders, and solutes as spheres. In JGP, John D. Ferry (1936) introduced the notion of the pore as a target of molecular collisions that failed if the solute hit the rim of the pore. As in a basketball hoop theory, only solutes that missed the pore rim could enter. Conceptually, for capture, the center of the solute had to strike within a reduced circle whose radius was equal to the pore radius minus the solute radius. As the area of this capture circle decreased, so the predicted permeability decreased. Again, in JGP, Eugene M. Renkin supplemented this with theory from physical chemists and engineers to account for friction of sliding along long pore walls (Renkin, 1954). The friction was expressed as a polynomial expansion of the ratio of solute radius to pore radius. Thus, from Renkin we have functions derived from a theory of entry and flow of spheres of different sizes through a viscous medium in a long, narrow cylinder. The theory was a continuum theory because, although the test solute molecules were represented as spheres of approximately the right size, the medium in the cylinder was treated as a viscous continuum rather than as a realistic collection of water molecules and a wall made of molecules. Again, a solute that almost spanned the pore diameter was less permeant that one that was smaller.

By the late 1950s, serious efforts were made to define the molecular dimensions of pores in plasma membranes. In a series of JGP papers, Arthur K. Solomon (biophysicist, Harvard University) determined what he called the equivalent pore radius of hypothetical water pores in the red blood cell membrane. Studying osmosis, he measured initial swelling or shrinking rates of the cells as they were placed in media supplemented with small nonelectrolytes as osmoticants. Water would flow out of cells if the nonelectrolyte was large and impermeant and therefore osmotically active, but not if the solute was small and readily permeant. Some molecules like glycerol, urea, and several of their relatives fell in between these extremes, and their dimensions could be used to define a smoothly decaying function ending in absolute cutoff. The results were fitted to Renkin's continuum theory. By these criteria, the equivalent water pore radius in human red blood cells was 4.2 Å (Goldstein and Solomon, 1960). Several decades later, we understand that the water is flowing through aquaporin water channels and that some subtypes of aquaporin pores are permeable to glycerol and urea.

For discussion of ion selectivity in the period between 1940 and 1970, there was a gradual shift in the physiological literature from considering the hydrated radius of ions to the crystal radius. The hydrated radius is a fictitious number that derives from the aqueous mobility of ions. To account for the lower mobility of Na^+ compared with K^+ in electrophoresis and in diffusion, Na^+ is assigned a larger effective hydrated radius even though it has a smaller crystal (ionic) radius. This was convenient to try to explain the greater permeability of K^+ in the resting cell membrane using a pore (sieve) theory. However, from the perspective of molecular structure, one cannot construct a particle with this fictitious hydrated radius, which in some examples would be a number like, e.g., 1.2 times the ionic radius. Instead, one must start with the crystal ionic radius and add individual discrete water molecules as required. This kind of realistic approach was developed to describe ion exchange at binding sites. Thus, Gilbert N. Ling developed his association-induction model for binding of small cations to cytoplasmic anions (Ling, 1960), and George Eisenman developed his model for ion-selective glass electrodes on the basis of naked small cations trading waters of hydration for an anionic binding site (Eisenman, 1962). From the energetics, they realized that a series of different binding selectivity patterns among cations arise as the radius of the anionic site is made smaller ("stronger field strength"). New crystal structures of highly ion-selective ionophore complexes supported the idea of a bare cation binding to polar atoms of the carrier molecule. Thus, the valinomycin-K^+ complex showed K^+ coordinated by six carbonyl groups pointing at it and forming a tight cage (Pinkerton et al., 1969).

Lorin Mullins considered realistic atomistic particles and pore sizes to develop the first comprehensive model for an excitable membrane (Mullins, 1959a,b, 1960). His was a model where a close fit was best for permeation.

If an ion is to permeate through a membrane composed of small pores, it must replace the water molecules that are serving as hydration with other molecules (the pore walls), which will serve this function (Mullins, 1959a).

This is similar to the way that selective pores are described today. He postulated that pores in the resting membrane of a frog muscle or squid giant axon would have a narrow, bell-shaped distribution of radii. At rest, the distribution was centered on a radius that closely fit a K^+ ion surrounded by exactly one layer of water molecules (Fig. 1 A). The fraction of pores fitting the one-layer hydrated Na^+ ion was only 4%. However, when the

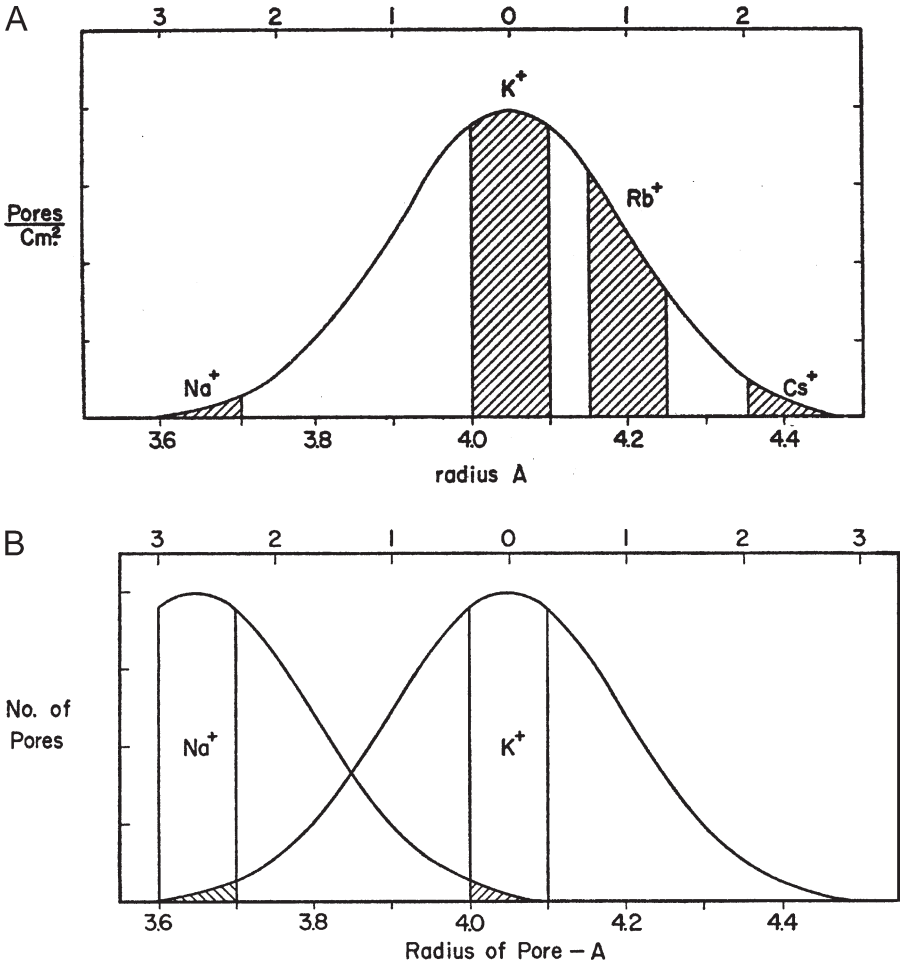

Figure 1. **The Mullins model for pore selectivity based on a distribution of pore sizes.** The curves show an assumed Gaussian distribution of pore radii, and the vertical bars represent the fraction of pores matching the indicated ion. **(A)** Distribution of radii in a resting polarized cell with many pores passing K^+ ions (from Mullins, 1959a). **(B)** Original curve and, to the left, the new distribution of radii during membrane depolarization. Pores have "compressed" to smaller sizes suited for passing Na^+ and no longer permeable to K^+ (from Mullins, 1959b).

membrane was depolarized, all the pores were "compressed" so that they acquired a size optimal for Na^+ ions and too small for K^+ ions (Fig. 1 B). The compression would account for the rise of Na^+ permeability (activation of Na^+ channels) with depolarization. Mullins said that similar models would also work if cations with no waters or with two layers of waters were the permeant species. In this ingenious and parsimonious model, K^+ channels and Na^+ channels were the same molecules in different states of expansion, a view that Mullins espoused for at least another decade (Mullins, 1968).

Armstrong, Bezanilla, and Hille: Selectivity filter geometry. During the 1960s, the membrane came to be regarded more clearly as having one set of permeability properties because of the lipid bilayer and another set because of intrinsic proteins that were boldly called ion channels, pumps, and transporters. The functions of these intrinsic transport proteins could be dissected by use of specific blockers and inhibitors. The arrangement of membrane proteins in the membrane was described as proteins swimming in a sea of lipids in the fluid mosaic hypothesis of Singer and Nicolson (1972). The concept of single channels came into sharper focus in JGP as electrical measurements on model membranes revealed stepwise increases of ionic current in the picoampere range as pore-forming materials were introduced into the membrane (Bean et al., 1969; Fig. 2).

Between 1965 and 1980, the ionic selectivity of several ion channels was probed in detail by voltage clamp (Chandler and Meves, 1965; Hille, 1971, 1972, 1973; Bezanilla and Armstrong, 1972; Adams et al., 1980; Dwyer et al., 1980). The measured reversal potential changes and relative permeabilities from the GHK voltage equation gave the selectivity for a wide range of small cations. Except for the Chandler and Meves study, all were published in JGP. For example, Chandler and Meves (1965) and Hille (1971, 1972) found that the voltage-gated Na^+ channel of axons is permeable to 11 small organic and alkali metal ions and impermeable to 18 only marginally larger ones. Similarly, Bezanilla and Armstrong (1972) and Hille (1973) found that K^+ channels of axons are permeable to four small cations and impermeable to seven others. Such measurements were interpreted in terms of a close fit of the largest permeant ion to a relatively short rigid pore lined with oxygen dipoles and, in the case of the Na^+ channel, with an acid group ($-COO^-$) as well. These biophysical studies led to very

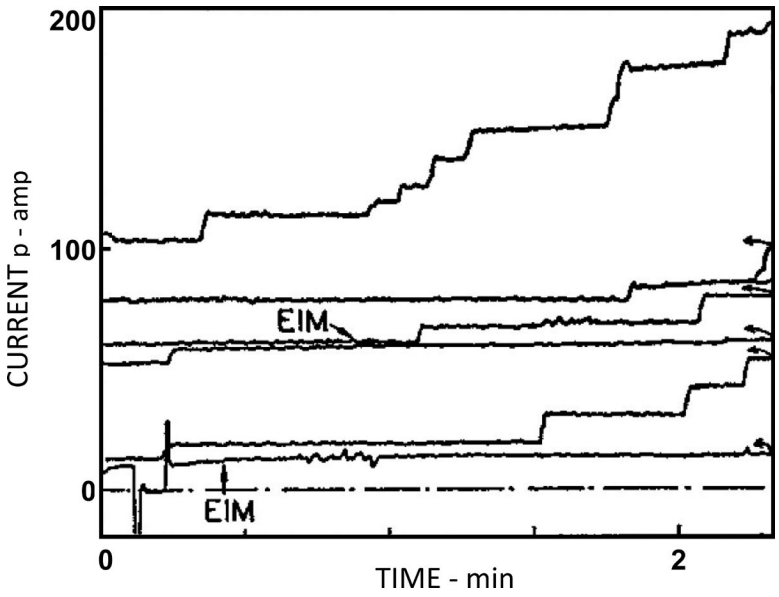

Figure 2. **Stepwise unitary currents induced by bacterial "excitability inducing material (EIM)."** A planar lipid bilayer was treated twice with a small amount of EIM at arrows. The membrane potential is held at −15 mV starting just before the first EIM addition. The record shows a total of 14 min, reset to the left edge each time it goes off the right edge (Bean et al., 1969). This paper in JGP may be the first publication to show single-channel currents.

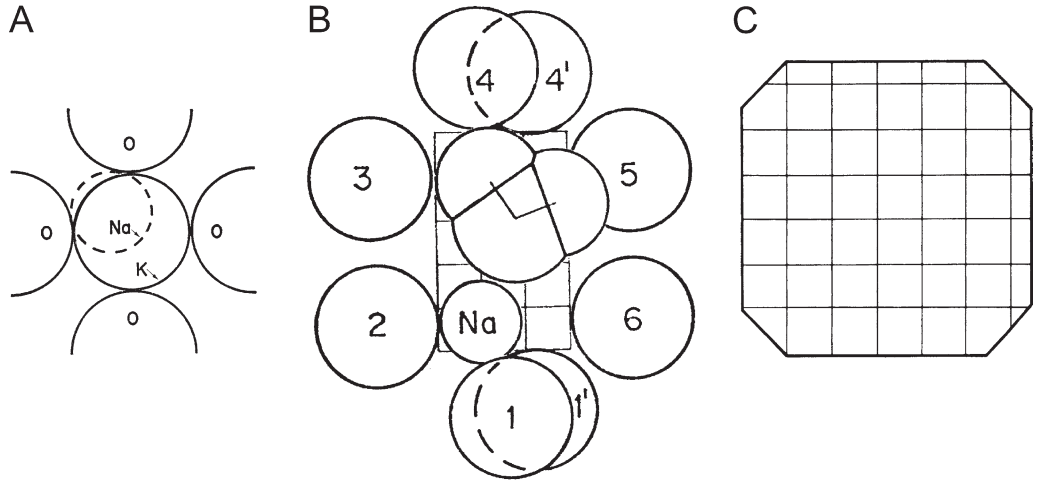

Figure 3. **Drawings of proposed pore dimensions from the JGP archive.** The three pores are drawn to the same scale and crystal radii have been used for ions. **(A)** Voltage-gated K^+ channel of squid axon showing four oxygen dipoles with close contact to a K^+ ion and less perfect contact to a Na^+ ion (Bezanilla and Armstrong, 1972). **(B)** Voltage-gated Na^+ channel of frog node of Ranvier showing Na^+ and a water molecule fitting into the pore formed by eight oxygen dipoles. The bottom two (1, 1′) were proposed to be from an ionized carboxylic acid. The grid in the background is 1-Å squares (Hille, 1971). **(C)** The large nicotinic acetylcholine receptor pore of frog muscle represented only as a 1-Å grid would fit an alkali metal ion together with several water molecules (Dwyer et al., 1980).

specific speculative predictions of the pore dimensions (Fig. 3, A and B). Organic ions capable of forming hydrogen bonds with the oxygen dipoles were allowed the closer approach permitted by H-bonds. Similarly, Dwyer et al. (1980) and Adams et al. (1980) found that the nicotinic acetylcholine receptor of frog motor endplates is permeable to 55 small organic ions and 15 alkali and alkaline earth metal ions and not measurably permeable to 7 marginally larger ones. This clearly larger and poorly selective pore could be described by the minimum rigid-pore approach (Fig. 3 C) and alternatively by the Ferry-Renkin continuum approach, which gave a pore radius of 3.7 Å (Dwyer et al., 1980), similar to Fig. 3 C. Only after several more decades have these several geometric hypotheses begun to be tested by direct x-ray and cryo-electron microscope structures of the channels (Doyle et al., 1998; Payandeh et al., 2011; Morales-Perez et al., 2016). The early biophysical predictions have been gratifyingly validated, at least at the 1.5–4 Å resolution level available so far. Still remaining to resolve is how much the channel pore flexes and accommodates during the passage of each ion.

Flux ratio, selectivity, saturation, and block. The initial thinking about fluxes in ion channels had assumed (1) free diffusion as embodied in the early description of Fick (1855) or at least, a century later, (2) ions moving independently as in the independence principle of

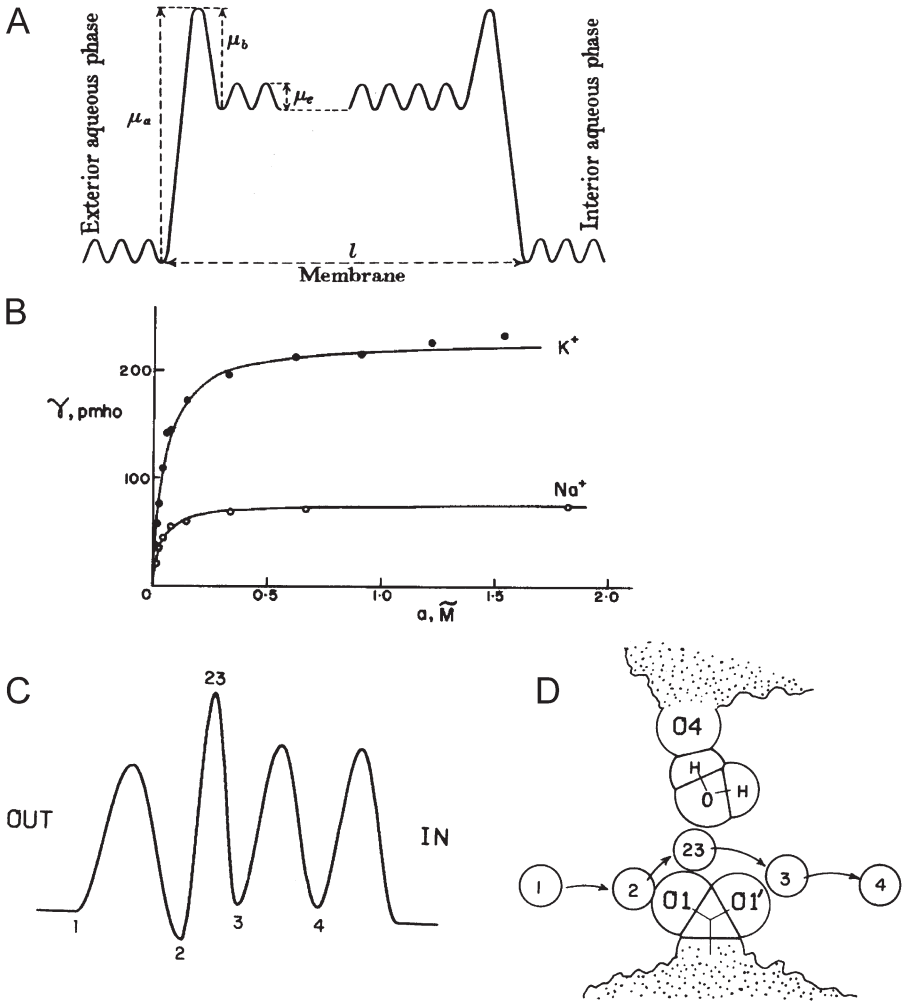

Figure 4. **Energy profile representations for selectivity and saturation. (A)** Early conceptual model of activation energy barriers (μ) for a permeating molecule entering and passing through the membrane. The higher the barriers, the slower the permeation (Danielli, 1939). **(B)** The conductance (in pS) of a cation channel in the sarcoplasmic reticulum reaches a saturating value as the concentration (activity) of the permeant ion is increased (Coronado et al., 1980). **(C)** Proposed free-energy barriers and **(D)** their molecular interpretation for the passage of Na^+ ions through voltage-gated Na^+ channels (Hille, 1975). The sketch in D is a view orthogonal to the selectivity filter drawing of Fig. 3 B with a water molecule above and a carboxylic acid below the permeating Na^+ ion. This corresponds to the transition state labeled "23" in C.

Hodgkin and Katz (1949). These assumptions were implicit when Danielli (1939) described solutes crossing the membrane as traversing a series of energy barriers (Fig. 4 A) and when Eyring et al. (1949) used rate theory to add a voltage drop and electrical forces on the ions for such energy diagrams. The assumption of independence had to be revised when Hodgkin and Keynes (1955) found that the ratio of inward and outward isotopic fluxes of K^+ ions in K^+ channels of squid giant axons could be described better by a model with several ions moving coordinately in single file in a long pore and not by free diffusion.

In JGP, evidence accumulated that fluxes in voltage-gated Na^+ channels and in a sarcoplasmic reticulum cation channel became saturated with increasing ion concentration (Hille, 1971; Coronado et al., 1980; Fig. 4 B). These would be deviations from independence. Just as Michaelis and Menten (1913) had done before for enzymes, and Osterhout (1935) for transporters, it became necessary to introduce occupancy and saturable binding sites in channels. The first examples in JGP were the model by Woodhull (1973) of proton permeation and the model by Hille (1975) (Fig. 4, C and D) of Na^+ permeation in voltage-gated Na^+ channels, both using concepts of rate theory. The former explained voltage-dependent proton block at the channel selectivity filter, and the latter, saturation by binding of permeant Na^+ in the channel. Ion selectivity was explained by supposing that at the transition state (the highest energy state), the ion lost water and gained interaction with an acid group of the selectivity filter, and the field strength of this interaction favored Na^+ over K^+ in the same way as in the glass electrode theory by Eisenman (1962). Following leads by Hodgkin and Keynes (1955) and Heckmann (1972), these models were solved by writing down a list of the occupancy states of the channel and the voltage-dependent kinetic equations for transitions among them in terms of rate theory. An explicit long-pore model of the K^+ channel was solved in the same way with occupancy states that included several ions in the pore (Hille and Schwarz, 1978). That model gave the right flux ratio deviations and predicted a steep voltage-dependent block by poorly permeant particles as in inward rectification and anomalous mole-fraction effects for mixtures of ions. As rate-theory energy diagrams with wells and barriers

proliferated in JGP, the editor Olaf Andersen had to remind authors that the relation between absolute rates and barriers required an unknown "frequency factor," so these diagrams had only qualitative significance and should not be presented as absolute (Andersen, 1999).

Finkelstein: Solubility theory. Much of the cell membrane surface area forms what Cole and Curtis (1939) had called "the ion impermeable portion of the membrane." Because a significant part of it is lipid bilayer, it is permeable to hydrophobic species. We already quoted Weech and Michaelis (1928): "We may conceive of the membrane as a phase working as a solvent for the diffusing substance. In this case the partition coefficient would determine the gradient of concentration of the substance in the membrane and thus control the rate of diffusion." In JGP, Alan Finkelstein made careful tests of that theory, showing that permeability in planar lipid bilayers and the oil-water partition coefficient are proportional (Finkelstein, 1976; Orbach and Finkelstein, 1980). The proportionality constant did vary with the lipid bilayer composition, e.g., the amount of cholesterol. Thus, both the solubility theory and the sieve theory that Michaelis described 90 years ago for dialysis sacs apply to biological membranes.

Conclusion

JGP was founded and has adhered to the principles of general physiology as a mechanistic science. JGP has played a key and formative role in fostering the elucidation of ion channels as molecular pores that mediate selective ion permeation across excitable membranes. Selectivity and permeation remain as exciting today as before, and we can expect many more important contributions to appear in these pages.

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