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PREFACE

A primary aim of the *Annual Review of Physiology* series is to track recent advances that have occurred in our field. Our task is daunting, not only because the scope is broad and constantly in flux, but also because we need to limit our overall coverage to provide useful reviews. As our readers are aware, we have approached this task by sectionalizing our subject areas, utilizing an adopted "theme" within each section, with these themes being cycled through different topics in subsequent years, depending on the changes that have taken place in each field. This format, together with the inclusion of special topics, has held us in good stead over the years as judged by the favorable reaction of many readers and by more objective measures such as the Impact Factor. But having said this, we are also on the alert and concerned with how we could improve our publication not just by altering the format but in choosing our various subjects for review. I believe the Editorial Committee would welcome suggestions that would enable us to better serve our constituencies. One reason for raising this question is the almost constant preoccupation of how our fellow physiologists view the current and changing faces of our field. We have seen academic departments of physiology (particularly in the United States) modify their names to include, in various forms, cellular and molecular, biophysics, or cell biology and, more recently, integrative. It is interesting that comparative physiology has long bridged all of these areas. Now come new concerns or at least new catch phrases such as functional genomics, translational physiology, and ecogenomics (see References 1–5). Although the intent and experimental enticements underlying this new lexicon have been in the physiologists arsenal for many years, they do emphasize the need for a broadening of focus, using the tool boxes from the reductionist approach, to study whole animal physiology as well as pathophysiology. It is clear that new horizons are appearing that beckon the discovery of new physiological treasures by drinking from the genomic spring. This subject has been commented on in this space in previous volumes (see *Annual Review of Physiology*, Volumes 54, 55, 58, and 63).

This year's volume contains, in addition to the traditional sections, our prefatory chapter written by Ian Glynn, in which he reflects on his work with the sodium pump, and two special topics. The special topics are aimed at informing our readers of important developments in related fields that lie outside our normal coverage. One special topic, edited by James B. Hurley, is on G protein effector mechanisms. The other special topic, edited by Gerhard Giebisch, concerns transportopathies. As said before, we are open to suggestions and encourage comments about our

publication. We can be reached at www.annualreviews.org. It should also be noted that this year's articles, as well as those from previous years, are available on our web site.

Joseph F. Hoffman
Editor

1. Chapman RW. 2001. EcoGenomics—a consilience for comparative immunology? *Dev. Comp. Immunol.* 25:549–51
2. Hochachka PW, Mommsen TP, Walsh PJ, eds. 2001. Knowledge explosion in comparative physiology and biochemistry: its causes and its future. *Comp. Biochem. Physiol. B* 130: 125–26
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4. Hall JE. 2001. The promise of translational physiology. *Am. J. Physiol. Cell Physiol.* 281:C1411–C14
5. Murer H. 2001. From the Editor-in-Chief. *Pflügers Arch.* 443:2

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Regulation of G Protein-Initiated Signal Transduction in Yeast: Paradigms and Principles, Henrik G. Dohlman and Jeremy W. Thorner

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Ian Glynn



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ERRATA

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A HUNDRED YEARS OF SODIUM PUMPING

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Key Words sodium, potassium, pump, ATPase, Na,K-ATPase

■ **Abstract** This article gives a history of the evidence (a) that animal cell membranes contain pumps that expel sodium ions in exchange for potassium ions; (b) that the pump derives energy from the hydrolysis of ATP; (c) that it is thermodynamically reversible—artificially steep transmembrane ion gradients make it run backward synthesizing ATP from ADP and orthophosphate; (d) that its mechanism is a ping-pong one, in which phosphorylation of the pump by ATP is associated with an efflux of three sodium ions, and hydrolysis of the phosphoenzyme is associated with an influx of two potassium ions; (e) that each half of the working cycle involves both the transfer of a phosphate group and a conformational change—the phosphate transfer being associated with the occlusion of ions bound at one surface and the conformational change releasing the occluded ions at the opposite surface.

INTRODUCTION

It has been the convention in recent years for the author of the first chapter in each *Annual Review of Physiology* to take a more historical and more personal view of the topic being discussed than would be appropriate in the rest of the volume. Since it is now seven years since I forsook the sodium pump for the less tractable problems of the origin and machinery of the mind, I am glad to be able to make a virtue of necessity and follow that convention.

THE FIRST FIFTY YEARS

In 1902, Ernest Overton (1) reported a discovery that, he tells us, left him so totally staggered (*vollständig verblüfft*) that he took several hours in the open air to think about it. Overton was an Englishman, and grandson of the man who as a boy had introduced Charles Darwin to beetles. Working in Würzburg, he had been studying the osmotic behavior of frog muscles, and what left him so *verblüfft* was the observation that muscles suspended for some time in an isotonic solution of sucrose were inexcitable. Adding sodium chloride to the solution restored excitability, and although the nature of the anion was not critical, only lithium could substitute for sodium. He concluded that sodium ions must have a specific function, and

he suggested that, during a very brief period following stimulation, the muscle membrane becomes permeable to both sodium and potassium ions, leading to an exchange of intracellular potassium for extracellular sodium. There was, he pointed out, a corollary to this hypothesis:

Consider that, in the course of 70 years, heart muscle cells contract about 24×10^8 times and respiratory muscles about 6×10^8 times. If some sodium ions enter and some potassium ions leave during each contraction, then the differences between internal and external cation concentrations would gradually be levelled out unless there is some mechanism at work which opposes this equilibration. In actual fact, our muscles contain, so far as I am aware, just as much potassium and as little sodium in old age as they do in early youth. (Translation by Bernard Katz.)

What makes this argument for the existence of a sodium pump so remarkable is not the argument itself, which is straightforward, but the premises on which it is based. For Overton was writing before the publication of the famous paper by Bernstein (2), which suggested that the resting potential was roughly a potassium equilibrium potential and that the action potential was the result of a transient loss by the membrane of its selective permeability to potassium ions. How Overton arrived at his hypothesis he doesn't tell us, but I suspect that, like Bernstein's, it owed a great deal to work by Ostwald (3) on so-called precipitation membranes—artificial membranes formed by the precipitation of insoluble material at the interface of two solutions. Ostwald studied the permeability and electrical properties of membranes of copper ferrocyanide, which he formed by putting solutions of copper sulfate and potassium ferrocyanide on opposite sides of thin pieces of parchment. He found that such membranes were permeable to potassium and chloride ions but not to the larger barium or sulfate ions. What is more, when the fluids bathing the membrane contained only a single species of penetrating ion, present in different concentrations on the two sides, he detected transmembrane potentials whose magnitudes fitted the Nernst equation, published the previous year. And Ostwald predicted that analogous behavior in natural membranes would explain not only the electric currents in muscles and nerves but also the mysterious workings of the electric organs of electric fish.

Whether this notion of Overton's debt to Ostwald is right or not, Overton seems to have been equally prescient in his hypothesis and in its corollary. Prescience, though, is recognized only with hindsight, and for Overton's work that hindsight came in the mid-1940s, a decade after his death. Bernstein's paper was published just a month after Overton's and, of course, contains no reference to it; his 1912 book, *Elektrobiologie* (4), has only a brief account of Overton's experiments and no mention of their possible implications. Bayliss's influential *Principles of General Physiology* (5), published in 1915, is similarly inadequate, and Fenn's exhaustive (and exhausting) 1936 review on electrolytes in muscle (6) does not mention the relevant experiments. Andrew Huxley (7) has said that he and Alan Hodgkin were not aware of these experiments when they discovered the overshoot of the action

potential in 1939, and he believes that they would have thought of the correct explanation of the overshoot much sooner if they had been.

By 1940, the widely held notion that the high concentration of potassium and the low concentration of sodium within muscle fibers were maintained simply by the impermeability of the membrane to sodium ions was no longer tenable. In experiments on anesthetized rats, Fenn & Cobb (8) had shown that stimulation of the sciatic nerves for 30 min caused the gastrocnemius muscles to lose part of their potassium and take up a roughly equivalent amount of sodium; both changes were largely reversed during a few hours of rest. Using the then newly available radioactive ^{24}Na , Heppel (9) had shown that it took less than an hour for sodium in the muscles of potassium-deprived rats to equilibrate with sodium in the bathing solution. And Steinbach (10) had shown that frog muscles soaked in potassium-free Ringer's solution gradually lost potassium and gained sodium and that the exchange was reversible. Since, in the experiments of both Fenn & Cobb and of Steinbach, the movements of both potassium and sodium during the recovery phase were against the concentration gradients, and these movements were in opposite directions, it seemed to follow either that at least one species of ion can be pumped through the membrane or that much of the potassium inside the fibers is bound to molecules that are unable to penetrate the membrane and prefer potassium to sodium.

Curiously, none of the experimentalists who produced these striking results showed any enthusiasm for the notion of ion pumps, and their papers contain no mention of the relevant work of Overton. Fenn & Cobb suggested that, following excitation, perhaps "only the surface of the fiber breaks down and exchanges its potassium for sodium, the lower layers being still impermeable to sodium"—a suggestion later dismissed by Fenn under the umbrella of "some rather artificial hypotheses." Steinbach has only a brief discussion of the causes of ion movements during the recovery phase, and favors selective binding of potassium to indiffusible organic molecules within the cell. In a slightly later paper (11), he justifies this rejection of the notion of ions being pumped across the membrane, explaining that to postulate the existence of such a pump "removes much of the charm of the old selective permeability idea, since once an auxiliary mechanism must be assumed, the simplicity and clarity of the scheme is destroyed."

Why selective binding is less destructive of the simplicity and clarity of the scheme than ion pumping is not clear, and in the following year, Dean (12), basing his conclusions on much the same experiments, argued strongly for the existence of an outwardly directed sodium pump.

Work on the storage of blood during the Second World War, by Maizels & Patterson in London, and by both Danowski and Harris in the United States, provided independent evidence for the pumping of both sodium and potassium (13–15). During cold-storage, red cells gradually lose potassium and gain sodium, but it was found that these changes could be reversed by replacing the cells in the circulation or by incubating them with glucose at 37°C . Reversal seemed to depend on energy from glycolysis because it was prevented by fluoride or iodoacetate, but not by cyanide or dinitrophenol.