Membrane Permeability

100 Years since Ernest Overton

Guest Editors

David W. Deamer

Arnost Kleinzeller

Douglas M. Fambrough

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Tribute

Arnost Kleinzeller, 1914–1997

On February 1, 1997, Arnost Kleinzeller, M.D., Ph.D., D.Sc., and Professor Emeritus of Physiology at the University of Pennsylvania, died at the age of 82 after a long battle with cancer. He was an internationally known physiologist and biochemist whose groundbreaking scientific work demonstrated how kidney cells regulate the contents of water and salt and how they move sugars through tissue membranes.

A leader during the Cold War in building links between the scientific community in Czechoslovakia and the West, Professor Kleinzeller organized a major international scientific congress in Prague in 1960. The work of that congress helped establish the course of research over the next two decades for the emerging field of biological fluid and solute transfer.

The openings he developed with Western scientists brought him increasing political pressure from the orthodox Czech communist regime. In 1966 with the help of American colleagues, Professor Kleinzeller and his family escaped, settling first at the University of Rochester and then in 1967 moving to the University of Pennsylvania, where he was named Professor of Physiology.

At the University of Pennsylvania Professor Kleinzeller taught at the medical school and conducted research in the Department of Physiology. During summers he was a researcher at the Mount Desert Island Marine Biological Laboratory in Maine. He wrote over 150 scientific papers and monographs and was one of the editors of the scientific series *Current Topics in Membranes and Transport.* He continued to campaign for greater openness in international scientific exchange as a member of the Committee on Scientific Freedom and Responsibility of the American Association for the Advancement of Science. He was elected to Germany's prestigious Academia Leopoldina in 1966 and was elected fellow of the College of Physicians of Philadelphia.

Professor Kleinzeller was born December 6, 1914, in Ostrava, Czechoslovakia. In 1938 he graduated from the Masaryk University's Medical School in Brno and published his first scientific report. That same year, following the signing of the Munich Pact, he was forced to flee his native country to England, where continued his scientific studies and worked for the Czech government in exile. He completed his Ph.D. under the Nobel laureate Sir Hans Krebs and continued his postdoctoral work at Cambridge University.

Professor Kleinzeller is survived by his wife of 54 years, Lotte, two daughters, Anna Romancova of Prague and Jana Richman of Forest Hills, New York, and three grandsons.

Friends and Colleagues
Department of Physiology
University of Pennsylvania

Arnost Kleinzeller, to me, was synonymous with *Current Topics in Membranes.* He served as editor for many years and put the force of his intellect and passion for science into it. Working as co-editor with him for 7 years was an experience to be cherished. It was a troubling time because the sciences were in transition from an era of broad, scholarly, archival treatises to something else, involving new speeds of information of exchange and storage. How to guide *Current Topics in Membranes* through this changing world of science was a constant question. Arnost could be fiery in defense of the academic high ground against the inclination of publishers to peek at their bottom line or their occasional lapses of due respect for scholars. Arnost's standards and his sense of the dignity of intellectual endeavor were among his many awesomely admirable traits. What I enjoyed most, however, was the fun of discussing science with him. Whenever we got together to deal with the series, our discussions always turned to science questions: What is most important and exciting in our field? How might we capture this in a volume of the series? Who has the breadth of vision and the skills to be an outstanding guest editor? We always parted with the sentiment that the sheer fun of the science itself more than compensated for our burden of editorial responsibilities. Even during his final suffering, Arnost was intent upon seeing one more volume come into being: a volume celebrating 100 years since Overton's landmark publication in membrane biology. This idea of his, one of many that kept us in animated conversation, has now come to fruition. It is perfectly fitting that this volume be dedicated to the memory of another important figure in the history of membrane biology: Arnost Kleinzeller himself.

Douglas M. Fambrough, Ph.D.
Professor of Biology
The Johns Hopkins University

In his last publication to appear in his lifetime, Arnost Kleinzeller explored the history of the cell membrane, as exemplified by research on the erythrocyte membrane and the early contributions by William Hewson (Kleinzeller, 1996). Kleinzeller describes how the earlier insights into the existence of a cell membrane were disregarded for the greater part of the 19th century, as was the concept of a cell membrane as a distinct and functionally important cellular structure, a concept that was revived by Overton and which, in the 20th century, has played a signal role in cellular physiology. It is a concept to which Arnost Kleinzeller contributed throughout his professional life, as both experimenter and theorist. In a 1994 publication (Kleinzeller *et al.,* 1994), he and colleagues focused on the two choline transport systems in lung cell membrane vesicles; in 1992 he coauthored a study of the polarity of glucose transport in cultured renal epithelia (Miller *et al.,* 1992), glucose transport and kidney cells having been topics of life-long interest. Still in 1992, he published an experimental study on cellular volume regulation (Ziyadeh *et al.,* 1992), again a topic that challenged him throughout his professional career. These publications are witness not only to his undimmed intellectual drive and curiosity, but also to his tenacity, as he suffered from chronic and debilitating illness for several years after his formal retirement in 1985.

I first met Arnost Kleinzeller in the late 1960s, after he and his wife, Lotte, had emigrated to the United States. It was the second time he had had to leave his native Czechoslovakia because of a totalitarian regime. When we met, I was struck by his biochemical approach to physiological events and therefore asked him to join me as co-editor of the series that became *Current Topics in Membranes and Transport.* That the term membranes appeared in the series title may well have been due to Arnost's interests and expertise. As editor, Arnost Kleinzeller applied the same demands of rigor to others that he applied to himself, stimulating critical analysis and encouraging a well-formulated viewpoint, but mindful of contrary evidence.

In the Preface to the first volume of *Current Topics in Membrane and Transport* we wrote ". . . of necessity, recognition [the first step of biological transport of solutes] must occur at the system boundary, most frequently the membrane boundary of cells or organelles." Arnost Kleinzeller devoted his life to understanding how this boundary permitted communication, yet provided individuality. He shall be missed.

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Felix Bronner, Ph.D.
Former editor of Current Topics in Membranes and Transport

It is difficult to know how to begin when writing about Arnost Kleinzeller, for he was a man who had a significant impact on so many different areas of my life.

I first came to know him while working in his laboratory at MDIBL in the mid-1970s, washing dishes and doing basic laboratory routine. I quickly came to find that nothing was routine to Arnost, that the smallest tasks were as important as the largest. He taught me the importance of discipline and perseverence, in life and in science, and these lessons have helped me throughout my career.

One trait that stands out is Arnost's ability to maintain a sense of humor while pushing forward with his ideas. His trademark rubbing of his hands and face in a moment of intense laboratory work was one of his many memorable characteristics. Arnost's unique personality made him a joy to work with and helped take the sting out of the "bad days" where everything that could go wrong did.

Although Arnost had high expectations, he had great compassion and treated me as family as well as student. I will remember Arnost as the person who started me on my way in many aspects of my life. He will surely be missed as a teacher and friend.

Jonathan M. Goldstein
Department of Neurology
Yale University School of Medicine

Arnost Kleinzeller was a wonderful colleague and friend. It was a pleasure to go to him with a particularly difficult scientific problem and reap the benefit of his encyclopedic knowledge and wide experience. He would often think about the problem and come back in a day or two with a suggestion that often turned out to be quite helpful and insightful.

Arnost had little tolerance for sloppy thinking or sloppy work, but that was balanced by his soft, compassionate side. He was the first to offer guidance to those who needed help and was particularly generous to students and junior colleagues working with him. This help often extended to guiding his "offspring" in both their careers and their personal lives.

I remember Arnost as a great friend who knew when to listen and when to offer advice. I miss our conversations, scientific and nonscientific. But most of all I miss the pleasure of sharing his zest for life.

Leon Goldstein
Division of Biology and Medicine
Brown University

In 1965 the Department of Physiology of the School of Medicine of the University of Pennsylvania needed to increase its strength in cell physiology and biophysics and began considering a number of potential faculty. We heard that Arnost Kleinzeller had managed to slip out of Czechoslovakia with his family and was now at the University of Rochester under the wing of Aser Rothstein. Arnost had a superb reputation as a junior colleague of Sir Hans Krebs in England, from whence at the end of World War II he returned loyally to Prague, taking posts at the Technical University and then Charles University and finally becoming Head of the Laboratory for Cell Metabolism of the Czechoslovakian Academy of Sciences. He established a great reputation abroad while there. He was also famous for having his laboratory operate in different languages on different days. He and Lotte visited us in Philadelphia and he eventually agreed to join our department in June of 1967, giving us instant recognition in cell physiology.

Arnost was a precise and demanding teacher and preceptor, no nonsense about science. He organized many activities, large and small, while at Penn. Probably the first was a wonderful course in cell physiology techniques, making available for the first time in our medical school bench-side instruction in many new methods, cell fractionation, membrane transport by radioactivity measurements, and many basic methods now commonplace, but then new. Students, and even some professors, came from all over the school. The faculty was drawn from many parts of the university, even from abroad.

He was an encyclopedia of information on physiology and biochemistry, having grown up with the latter field. His research concentrated on the kidney, which so neatly brought together physiology, biochemistry, and biophysics. He kept abreast of new techniques and was able to review with knowledge a wide area of physiology, which enabled him to edit this wonderful series with Felix Bronner. His great interest in history led him later to do research on Overton, whom he considered ahead of his time and deserving of more credit for developing our concept of the cell membrane than he received.

As a colleague he was tremendously loyal to our department and a great support to me as chairman, even when he thought my judgment was wrong, a not infrequent occurrence. I sought his advice all the time on scientific matters.

Arnost was the very model of a cultured European academic. He knew music and played the violin, though unfortunately I never heard him. He knew Greek and Latin, about which I often consulted him, and was familiar with the great classics of Western civilization.

As he grew older he suffered a considerable series of illnesses, including the ignominy and near devastation of a childhood infectious disease, all of which he bore with fortitude. He visited me frequently all these later years with recent humorous anecdotes and some pungent gossip. Except for a brief factual bulletin concerning the state of his health, he voiced no complaints.

It is impossible to say anything about Arnost's long career here without mentioning Lotte, who supported him in all things but never hesitated to be sure that he did not feel he was too far above the salt. When he was ill, she was an avenging angel.

Arnost Kleinzeller was a tremendous scientist, a wonderful friend, a delight to know, and I miss him.

Robert E. Forster II, M.D.
Isaac Ott Professor Emeritus
University of Pennsylvania

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CHAPTER 1

Charles Ernest Overton's Concept of a Cell Membrane

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I. A BRIEF BIOGRAPHY OF ERNEST OVERTON

A century has elapsed since (Charles) Ernest Overton (1865–1933) presented his views on the osmotic properties of cells in the period 1895–1902. He is justifiably recognized as a pioneer in the development of a comprehensive concept of the cell membrane (Höber, 1926; Collander, 1965; Smith, 1962; Kleinzeller, 1995). As noted in Collander's appraisal of Overton's achievements on the occasion of his 100th birthday, he established several milestones in the field:

1. A "lipid-impregnated boundary layer" is a determinant of the osmotic properties of living cells. Both diffusion- and metabolism-linked active processes function in solute exchange between cells and their immediate environment.
2. The lipids of the cell (membrane) are involved in the phenomenon of narcosis.

[1] Deceased.

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3. An exchange of sodium and potassium across the membranes of muscle and nerve cells is responsible for their excitability.

Most of Overton's papers were published in German. This chapter discusses the experimental basis for these crucial elements of a comprehensive concept of the structure and function of a cell membrane. Such an evaluation of necessity must take into account the framework of knowledge of the field at the time of Overton's creative life.

Ernest Overton (in his publications, Overton used only this first name) was born in 1865 in Stretton, Cheshire, England. On his mother's side he was distantly related to Charles Darwin. Because of his mother's chronic illness, the family moved in 1882 to Switzerland. In 1884, Overton entered the University of Zürich as a student of botany under the preceptorship of Professors A. Dodel and E. Strasburger (in Bonn, Germany), defended his Ph.D. thesis in 1889, and was appointed "Docent" (roughly equivalent to lecturer) in biology in 1890.

During his time in Zürich, Overton was scientifically most productive. In the period of 1888–1893, Overton published seven papers related to his botanical interests (1888, 1889, 1890a,b; 1891, 1893a,b). His observations (1893a,b) on the reduction of chromosomes (meiosis) in certain plant cells gained him the reputation of a skilled and innovative investigator. Overton returned to strictly botanical subjects only in three papers published in 1897, 1899a, and 1899b. He established his scientific status primarily by his papers on the osmotic properties of cells, research that was also begun in Zürich.

In 1901, Overton joined the renowned Department of Physiology at the University of Würzburg (Germany) as assistant to the electrophysiologist Professor M. von Frey. In 1907 he accepted a call to become chairman of the newly established Department of Pharmacology at the University of Lund (Sweden) where he served until his death in 1933. It was in Lund that Overton married Dr. Louise Petrén, a member of a well-known Swedish academic family, and raised his family of four children. His contributions to science from the Lund period were rather limited. Comments of his daughters (Dr. S. Thesleff of Lund, personal communication) would suggest that protracted health problems and difficulties in mastering the Swedish language may have been a contributing factor.

Although he had not received formal medical training, his contribution to the medical field was acknowledged by honorary medical degrees by the universities of Lund and Jena.

II. EXCHANGE OF SOLUTES ACROSS THE CELL BOUNDARY

Overton's interest in this field was prompted by his genetic experiments, which required a substance that would enter plant cells rapidly. The work of Nägeli and Cramer (1855), de Vries (1871, 1884), and Pfeffer (1877) had established the existence of an osmotic barrier between the protoplasm of plant cells and their environment. The evidence was based on observations of protoplasmic permeability: while healthy cell protoplasts were readily permeable to water, they were impermeable to many solutes, including the plant pigment anthocyan (Nägeli and Cramer, 1855) or sucrose from beet roots (de Vries, 1871). This permeability barrier was lost following cell death or major injury, as judged by changes in the appearance of the protoplasm and cessation of protoplasmic streaming.

The phenomenon of plasmolysis was firmly established, that is, a retraction of the protoplasm from the cellulose wall of plant cells exposed to impermeable external solutes, as shown in Fig. 1. Intracellular vacuoles also reduced their volume under plasmolytic conditions, and the phenomenon was reversible (deplasmolysis). The observations were interpreted as osmotically induced flows of water across a thin osmotic barrier, for which

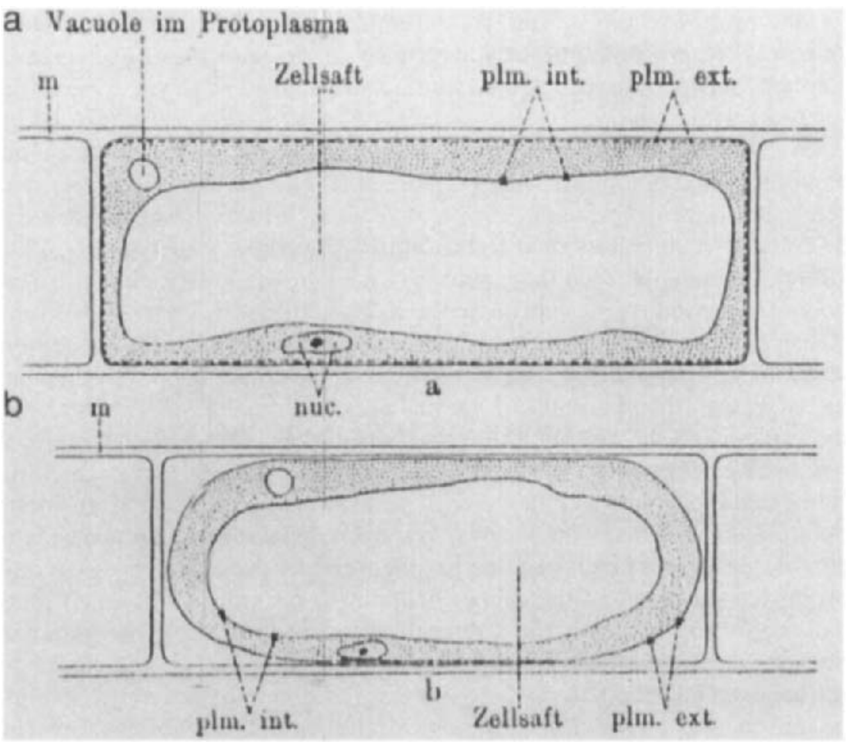

FIGURE 1 Scheme of a plant cell from the osmotic point of view. (a) Normal and (b) plasmolysed state.

Pfeffer used the equivalent terms plasma skin ("Plasmahaut", 1877, 1891), skin layer ("Hautschicht", 1886), or plasmatic membrane (1900). He suggested (1891) that the membranes facing the external and inner (vacuolar) spaces had identical properties and could actually fuse. In his view, the nature of the plasma membrane was proteinaceous, rejecting Quincke's proposal of an oil-like protoplasmic surface (1888). The idea that membranes were composed of proteins had many adherents, particularly when Ramsden (1904) demonstrated the spontaneous formation of solid films on the interface of protein solutions and air.

Investigators in the field employed solutions of sucross or salts as osmotic agents. There were some early observations that not all hypertonic solutes produced plasmolysis. For instance, Klebs (1887) found that glycerol was a poor plasmolytic agent, and this observation was confirmed and extended by de Vries (1888). At the time, no explanation of the phenomenon was offered.

Pfeffer's (1886) careful study of the cellular uptake of dyes allowed him to consider additional properties of the putative osmotic barrier. He proposed that in addition to the "static" properties of the plasma skin (reflected by simple osmotic phenomena), living cells also had the capacity to take up or exclude solutes. At the time, his suggestion that the plasma skin was a "protoplasmic organ capable of regulating the exchange of solutes" between the cell and its immediate environment was a cry in the scientific wilderness.

Overton's systematic exploration of the relationship between the chemical constitution of solutes, mainly organic, and their plasmolytic effects, with reference to the properties of the putative osmotic barrier, was presented in a series of three papers read to the Zürich Naturalist Society (1895, 1896, 1899c). Having observed that solutions of ethanol and other aliphatic alcohols did not produce plasmolysis in the cells of the alga *Spirogyra,* Overton's concept of the phenomenon was broad. Given van't Hoff's (1887) demonstration of basic similarities between the laws governing the physical behavior of gases (the relationship among pressure, volume, and number of molecules) and the osmotic properties of solutions, Overton illustrated his concept by comparing a plant cell to a soccer ball (Fig. 2). Here, the elastic rubber balloon (k) was inflated to 1.5 atmospheres pressure, in turn also inflating the external leather cover (l). Because rubber is impermeable to air whereas leather is permeable to all gases, any pressure increase would produce a shrinkage of the rubber balloon (to k_i). If, however, the rubber ball were permeable to the external gas, an equilibration of pressures outside and inside the rubber balloon would take place, and hence no retraction. Thus, the absence of balloon retraction indicates a permeability of the balloon to the gas. Similarly, a putative cell membrane (or skin) permeable to water would display plasmolysis when the cell was exposed to