Phase Transitions in Cell Biology

Gerald H. Pollack · Wei-Chun Chin
Editors

Phase Transitions in Cell Biology

Springer

Editors

Gerald H. Pollack
Department of Bioengineering
Box 355061
University of Washington
Seattle WA 98195
USA

Wei-Chun Chin
PO Box 2039
Merced CA 95344
USA

ISBN: 978-1-4020-8650-2 e-ISBN: 978-1-4020-8651-9

Library of Congress Control Number: 2008928026

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

Preface

Why phase transitions?

Phase transitions occur throughout nature. The most familiar example is the one that occurs in water: the abrupt, discontinuous transition from a liquid to a gas or a solid, induced by a subtle environmental change. Practically magical, the ever-so-slight shift of temperature or pressure can induce an astonishing transition from one entity to another that bears little resemblance to the first.

In a sense, this transition is a kind of amplification: a subtle change inducing a radical change. So "convenient" a feature is seen throughout the domains of physics and chemistry, and one is therefore led to wonder whether it might also be common to biology. Capitalizing on this kind of amplification would seem to be a sensible approach for Mother Nature to have taken as she built the domain of life. Small change yields big response. What could be more sensible?

The idea that such a phenomenon might be pervasive throughout biology was first proposed in a book written by one of us (GP*). Cells, Gels and the Engines of Life* proposed that the phase transition is indeed a central protagonist in the drama of life. Many of the most fundamental cellular processes are arguably attributable to radical structural shifts triggered by subtle changes that pass above a critical threshold. These processes include transport, motion, signaling, division, and other fundamental aspects of cellular function.

Largely on the basis of that book, a symposium was organized in Poitiers, France, to bring together people who have additional evidence for the role of phase transitions in biology, and this book is largely, albeit not completely, a compendium of some of the more far-reaching presentations. Contributions from several scientists who were unable to attend are included as well.

The book should be suitable for anyone interested in the nature of biological function, particularly those who tire of lumbering along well trodden pathways of pursuit, and are eager to hear something fresh. The book is replete with fresh interpretations of familiar phenomena, and should serve as an excellent gateway to deeper understanding.

It is our hope to awaken to the reader the idea that phase transitions play a role as important in biology as they play in physics and chemistry. Biology, after all, is little more than applied physics and chemistry. Is it not?

Seattle, WA, USA — Gerald H. Pollack
Merced, CA, USA — Wei-Chun Chin

Contents

Contributors

Adriano M. Alencar
Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

Mark M. Banaszak Holl
Department of Chemistry and Program in Macromolecular Science and Engineering, Michigan Nanotechnology Institute for Medicine and Biological Science, 930 N. University Avenue, University of Michigan, Ann Arbor, MI 48109-1055

H. Berry
INRIA, Team Alchemy, Parc Club Orsay Université, 3, rue J. Rostand, 91893 Orsay Cedex France, hugues.berry@inria.fr

James P. Butler
Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

I.L. Cameron
University of Texas Health Science Center, San Antonio, USA, CAMERON@uthscsa.edu

E. Chen
School of Engineering, University of California, Merced, CA, 95344, USA

W.-C. Chin
School of Engineering, University of California, UC Merced, P. O. Box 2039, Merced, CA, 95344, USA, wchin2@ucmerced.edu

James Clegg
Section of Molecular and Cellular Biology, and Bodega Marine Laboratory, University of California, Davis, 2099 Westside Road, Bodega Bay 94923, USA

Y.X. Ding
Department of Bioengineering, University of Utah, Salt Lake City, UT 84112, USA

Ben Fabry
Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

Jeffrey J. Fredberg
Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

G.D. Fullerton
University of Texas Health Science Center, San Antonio, USA

F. Gallyas
Department of Neurosurgery, University of Pécs, H-7623 Pécs, Rét utca 2, Hungary

S. Giraudier
Errmece, University of Cergy Pontoise, BP 222, 95302 Pontoise, France

D.P. Kharakoz
Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Institutskaya street 3, 142290 Pushchino, Russia

V. Larreta-Garde
Errmece, University of Cergy Pontoise, BP 222, 95302 Pontoise, France

Guillaume Lenormand
Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA, glenorma@hsph.harvard.edu

R. Lux
Department of Dentistry, UCLA, Los Angeles, CA, USA

J. Pál
Department of Neurosurgery, University of Pécs, H-7623 Pécs, Rét utca 2, Hungary

J. Picard
Errmece, University of Cergy Pontoise, BP 222, 95302 Pontoise, France

Gerald H. Pollack
Department of Bioengineering, Box 355061, University of Washington, Seattle WA, 98195, USA, ghp@u.washington.edu

Ichiji Tasaki
Bldg. 13, Rm. 3E-25, LIMB, NICHD and LCMR, NIMH, National Institutes of Health, Bethesda, MD, 20892, USA, itasaki@erols.com

Xavier Trepat
Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

Pavel Voráek
Lund Observatory, Box 43, SE-221 00 Lund, Sweden, postmaster@astro.lu.se

Bohumil Vybíral
Department of Physics, Pedagogical Faculty, University of Hradec Králové, Rokitanského 62, CZ-500 03 Hradec Králové, Czech Republic, Bohumil.Vybiral@uhk.cz

K. Yang
Department of Physiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada

L. Yeghiazarian
Department of Bioengineering, currently at the Department of Biostatistics, School of Public Health, UCLA, Los Angeles, CA, USA

En-hua Zhou
Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

On the Reversible Abrupt Structural Changes in Nerve Fibers Underlying Their Excitation and Conduction Processes

Ichiji Tasaki

Abstract The cortical gel layer of nerve fibers has the properties of a cation-exchanger. Hence, this layer can, and actually does, undergo a reversible abrupt structural change when monovalent cations (e.g. Na^+) are substituted for the divalent counter-ions (e.g. Ca^{2+}). This structural change brings about a sudden rise in the water content of the layer which in turn produces a large enhancement of cation mobilities accompanied by a shift of ion-selectivity in favor of hydrophilic cations. Based on these grounds, it is argued that the electrophysiological processes known as "nerve excitation and conduction" are, basically, manifestations of abrupt structural changes in the cortical gel layer. In recent studies, we have shown that several aspects of the excitation phenomena can actually be reproduced by using synthetic polyanionic hydrogels in place of living nervous tissues. It is noted that these studies of synthetic model systems lead us to a better understanding of the process of divalent-monovalent cation-exchange in natural and artificial polyanionic gels.

Keywords Nerve excitation and conduction · structural phase transition in nerve fiber · divalent-monovalent cation-exchange

1 Introduction

The polypeptide chains in solutions can be reversibly converted, as is well known, from the random coil to the helical form. Hydrogen bonds formed between different groups in one long polypeptide chain lead the whole chain into the helical form. This structural transformation is very sharp; that is, a change of a few degrees in temperature or a few percent of solvent composition is sufficient to complete the transformation. Hence, the term "phase transition" has been employed to describe this reversible structural change (see Doty and Yang, 1956; Zimm and Bragg, 1959; Ptitsyn et al., 1968).

I. Tasaki
National Institutes of Health, Bethesda, MD, 20892, USA
e-mail: itasaki@erols.com

G.H. Pollack, W.-C. Chin (eds.), *Phase Transitions in Cell Biology*,

Negatively charged polyelectrolyte gels in salt solutions can be converted from the swollen state to the compact state when the monovalent counter-ions are replaced with divalent cations. This structural transformation is also sharp; that is, it can be initiated and completed by a small change in the salt composition of the surrounding solution (see Katchalsky and Zwick, 1955; Kuhn, 1962; Tanaka, 1981; Tasaki and Byrne, 1992). There seems little doubt that such reversible, abrupt changes in the gel structure are a process of very common occurrence in various biological systems.

The objective of the present article is to demonstrate that the reversible abrupt structural changes occurring in the cortical gel layer of nerve fibers are at the base of the process of excitation and conduction. There is abundant evidence to show that these structural changes are associated with divalent-monovalent cation-exchanges taking place in the negatively charged sites in the cortical gel layer.

2 Abrupt Structural Changes in Synthetic Polyanionic gels

In this section, attempts are made at elucidating the role of divalent-monovalent cation-exchange in the production of abrupt structural changes in polyanionic gels. To achieve this end, we describe, in some detail, the results of several observations which we have made on synthetic polyanionic gels during recent years. In the following section, we treat the results obtained from living nerve fibers on the basis of our knowledge about the abrupt structural changes in synthetic gels.

2.1 Discontinuous Volume Transition

We now know that the volume of a small piece of cross-linked Na-polyacrylate or Na-polymethacrylate gel can change *discontinuously* when Na-ions in the gel are replaced, by gradual steps, with divalent cations (Tasaki and Byrne, 1992, 1994; Tasaki, 1999). In most of our studies, we have examined the effects of application of Na- and Ca-salts to the gel. However, we have observed on several occasions that similar results can be obtained by using other divalent cations, such as, Mg^{2+}, Sr^{2+}or Ba^{2+} in combination with other monovalent cations, such as, Li^{+}, K^{+}, Rb^{+}, Cs^{+} or tetraalkylammonium ions.

An example of our results is shown in Fig. 1. Here, small spherical beads of cross-linked polyacrylate gel of approximately the same diameter were placed in a series of petri dishes containing 40 mM NaCl solution (kept at pH 7.4). Small aliquot quantities of a concentrated $CaCl_2$ solution was added to the dishes, and the diameters of the gel beads were determined when equilibrium was reached between the gel beads and the surrounding salt solution.

It is seen in the figure that the Ca-salt added to the solution produced a gradual decrease in the gel diameter initially. However, at the point where the Ca^{2+} concentration in the dish rose to about 1.2 mM, there was a *discontinuous decrease* in the

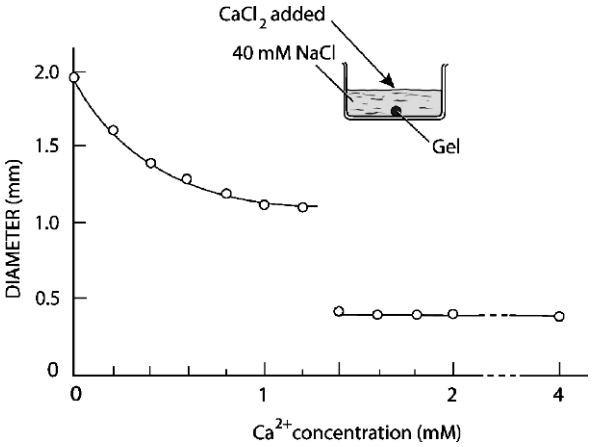

Fig. 1 The diameter of a small spherical gel bead immersed in a 40 mM NaCl solution, plotted against the concentration of Ca^{2+} added to the solution (Tasaki and Byrne, 1992)

gel diameter. The volume of the gel bead fell at this point by a factor of roughly 1/10. We now wish to know how this discontinuous fall in the gel volume is brought about.

As the first step toward achieving our goal, we made measurements of the quantities of $Na^+$and Ca^{2+} *inside* individual gel beads, as a function of the concentration of the Ca-salt in the dish. After some initial difficulties, we were able to obtain reasonably reproducible results, indicating that the quantity of Na^+ *inside the bead* falls and that of Ca^{2+} rises smoothly (but rather precipitously) as the Ca^{2+} concentration *outside the beads* is raised gradually from zero. As expected from the existence of a high density of fixed negative charges in the gel beads, the sum of the quantities of $Na^+$and Ca^{2+}(expressed in unit of equivalents) was found to remain constant within the experimental uncertainty. We *could not* detect any sign of discontinuity in the quantity of Na^+ or Ca^{2+} *inside the gel beads* in the entire range of Ca^{2+} concentration outside.

It is known that the COO^--groups in macromolecules overwhelmingly prefer Ca^{2+} to Na^+ (Williams, 1970; Levine and Williams, 1982). In fact, we have seen that, at the point of discontinuity of the gel volume, roughly 80% of the entire negative charge *inside the gel bead* was neutralized by $Ca^{2+}$and only about 20% by Na^+. We have noted already that, at this point, the external concentration of Ca^{2+} was only about 1/33 (=1.2/40) of that of Na^+. Such a remarkably high selectivity for Ca^{2+} had never been considered in previous studies of living excitable tissues.

We expect that the external Ca^{2+}-concentration required for inducing a discontinuous volume transition varies according to the NaCl concentration in the dishes. In fact, when the NaCl concentration in the dish was raised from 25 to 150 mM, there was a nearly proportionate rise in the required external Ca^{2+} concentration. Consequently, the concentration ratio $[Ca^{2+}]/[Na^+]$ at the point of volume discontinuity was found to be insensitive to the variation in the NaCl concentration in the dish.

Interestingly, this ratio $[Ca^{2+}]/[Na^{+}]$ at the point of volume discontinuity appears to vary inversely with the density (and probably with the regularity of distribution) of the fixed negative changes in the gel. We have seen that lowering the pH of the surrounding salt solution down to 5 or less brings about a noticeable increase of this ratio. Furthermore, copolymerization of acrylic acid with acrylamide in gel synthesis was found to bring about a marked rise of the ratio $[Ca^{2+}]/[Na^{+}]$, even when the uncharged component (acrylamide) was only 10% of that of the negatively charged component (acrylic acid). No macroscopic discontinuity was observed in the gel volume when the acrylamide content was 33% or more.

Finally, we note in Fig. 1 that, in the range of Ca^{2+} concentration *higher than* about 1.3 mM, the size of the gel bead was practically independent of the external Ca^{2+} concentration. It is known that Ca-ion is capable of forming a complex, bridging $C00^{-}$-group of one chain with another COO^{-}-group in a neighboring chain (see Williams, 1970). The size of such a complex is probably determined roughly by the exclusion volumes of the hydrocarbon chains, the Ca-ions, the ligands and water molecules involved. Thus, we visualize the compact, Ca^{2+}-rich structure of the gel bead as stabilized by Ca-bridges between the COO^{-}-groups in the gel bead (see Tasaki, 2005a).

2.2 Propagation of the Boundary Between Swollen and Compact Regions of a Gel Strand

We now describe a phenomenon that is directly related to the *instability* of the structure which constitutes the boundary between the compact and swollen regions of a gel strand. When one end of a strand of cross-linked polyacrylate gel in its compact (Ca^{2+}-rich) state is immersed in a NaCl solution, swelling of the gel starts at this end, thus creating a gel strand which consists of two structurally distinct regions, compact and swollen. The transitional region, or the boundary between the two regions, of the gel strand was found to be remarkably short and sharp. By using such a gel strand, it was found possible to induce continuous displacement of the boundary with the aid of an electric current applied to the strand.

The diagram at the top of Fig. 2 shows the arrangement employed. Here, a gel strand in its compact state was placed across a 10 mm wide platform separating the solution of NaCl from the $CaCl_2$ solution in a plastic chamber. After covering the surface of the gel strand with a thin layer of liquid paraffin, an electric current was delivered to the strand. The current was directed from the portion of the gel strand immersed in the NaCl solution toward the other end immersed in the $CaCl_2$ solution. As expected, the portion of the gel in the NaCl solution began to swell. And then, the boundary between the compact and swollen portions started to move towards the compact side.

With this experimental setup, there was a layer of salt solution on the surface of the gel strand and also on the platform. Consequently, the current employed was considerably stronger than the intensity expected from a simple $2Na^{+} \rightarrow Ca^{2+}$

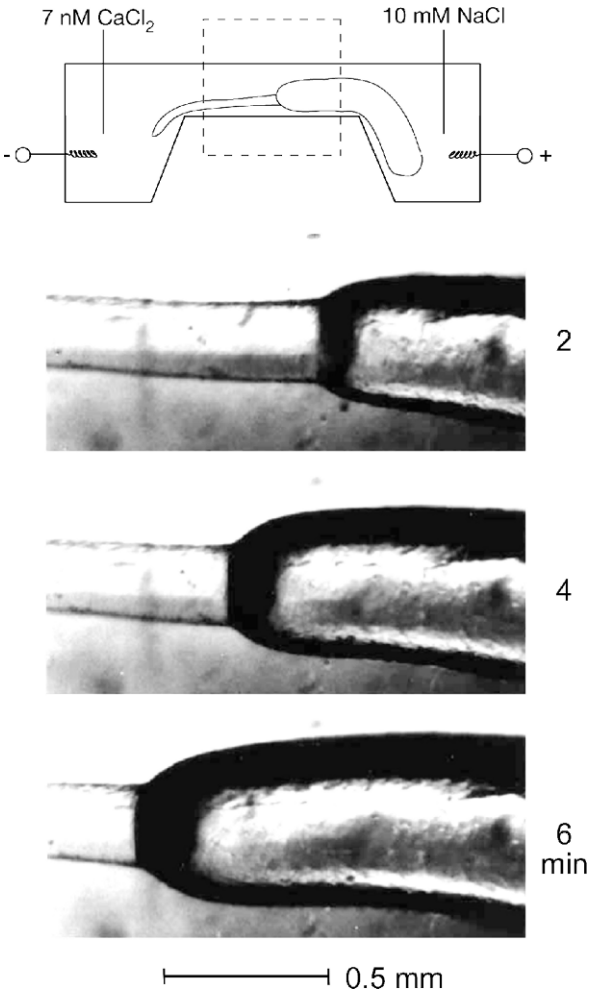

Fig. 2 Photomicrographs showing displacements of the boundary between the compact and swollen regions of a gel strand induced by electric current of 1 mA (Tasaki, 2002)

exchange at the sharp boundary. When the boundary was located on the platform, propagation of the boundary from the compact side of the strand to the swollen side could be induced by application of a current flowing in the reverse direction.

2.3 Formation of Bundles of Polyelectrolyte Chains

We now describe an interesting pattern of binding of divalent cations to the negatively charged chains in polyelectrolyte gels. We first demonstrate that exposure of

a swollen polyelectrolyte gel to a solution of divalent cation salt can produce highly refractile bundles of polymer chains in the gel (Tasaki, 2005b).

Strands of cross-linked Na-polyacrylate gel in their swollen state are wholly transparent and there is no structure recognizable in the strand under dark-field illumination. Individual polymer chains in the gel are highly hydrated and a considerable portion of the Na-ions in the gel are loosely associated with the chains (see Kern, 1939; Huizenga et al., 1950; Ikegami, 1964). When a swollen gel strand is exposed to a $CaCl_2$ solution, the surface of the gel immediately becomes visible, and soon highly refractile bundles of polymer chains begin to appear on the surface. These bundles originate usually from the cut end or some irregular spots of the gel surface and spread gradually into the interior of the gel stand.

The photomicrograph in Fig. 3, left, shows highly visible bundles of polymer chains stretching from the compact region to the swollen region of the gel strand. Because of a large change in the diameter of the strand at the boundary, the polymer chains near the surface are under tension which tends to align the chains in the longitudinal direction. This parallel alignment of the polymer chains is considered as the condition favorable for creating Ca^{2+}-bridges and forming bundles of polyelectrolyte chains.

When Ca-ions are delivered directly into the interior of a swollen gel by the aid of a glass pipette, a quite different pattern of bundle formation is observed (see Fig. 3, right). Here, a pipette filled with a $CaCl_2$ solution was pushed into the gel and an outwardly directed current was delivered to the gel by using an Ag-AgCl wire inserted in the pipette. The photomicrograph of the bundle formation in the figure was taken shortly after delivering a 0.1 mA current for about 15 s. It is noted in the figure that the pattern of bundles reflects the distribution of the stretched polymer chains created by insertion of the pipette. It is noted also that the process of bundle formation initiated by the Ca-ions in the vicinity of the orifice of the pipette spreads *cooperatively* along these chains.

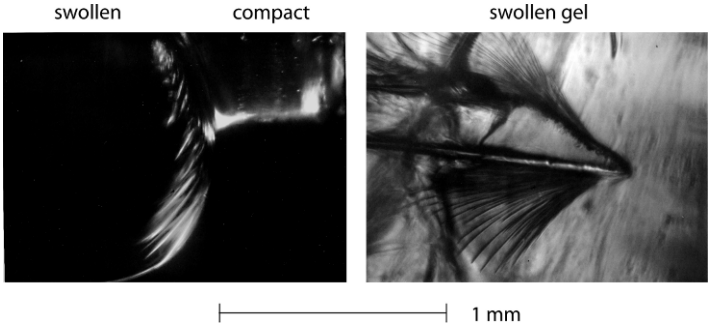

Fig. 3 *Left*: Highly refractile bundles of polymer chains formed in the transitional zone between the compact and swollen regions of a cross-linked polyacrylate gel strand. *Right*: Bundles of polyelectrolyte chains formed inside a swollen gel by application of an electric current by use of a glass pipette containing a $CaCl_2$ solution (Tasaki, 2005b)

Since these bundles of polymer chains formed by the delivery of a current pulse are surrounded by many Na^+-acrylate units of the chains in the gel, they tend to fade away little by little within a few minutes. When, however, a pulse of an inwardly directed current is delivered to the gel, the portions of the bundles located in the vicinity of the orifice of the pipette promptly disappear as a consequence of the rapidity of the Ca^{2+}-Na^+ exchange process.

The formation of bundles of biopolyelectrolyte chains with polyvalent cations appears to be a quite general phenomenon in biology (see Tang et al., 1996).

2.4 Abrupt Changes in Electric Impedance of the Gel Associated with Ca^{2+}-Na^+ Exchange

When a compact gel layer undergoes a transition to a swollen state in association with a divalent-monovalent cation-exchange, there is a sudden change in the electric properties of the layer. The ion mobilities in the gel layer are greatly enhanced by the rise in the water content of the layer. It is therefore easy to demonstrate that the a.c impedance abruptly falls when a Ca^{2+}-Na^+ exchange induces a structural change in the superficial layers of a compact gel layer (see Fig. 4).

The diagram at the top of the figure schematically illustrates the setup employed. A 1 kHz a.c. was applied to a cylindrical gel rod by use of a pair of platinized platinum electrodes, placed one inside and the other outside the gel. The surface of the internal platinum electrode was completely insulated except for a short portion located inside the gel rod. The intensity of the a.c. was adjusted to give rise to an alternating voltage of about 20 mV (rms) across the gel layer. This voltage was amplified, half-wave rectified and was passed through a resistor-capacitor circuit. The resulting non-alternating (d.c.) voltage output was taken as a measure of the impedance of the layer.

It is seen in the record shown in the figure that, following application of a 100 mM NaF solution to the surface of a compact gel rod, there were repetitive abrupt falls of the impedance. Note that *fluoride* (or *phosphate*) salts, instead of *chloride* salts, of monovalent cations had to be employed in these experiments. It is known that, in the *lyotropic series* of common anions, F^- and HPO_4^{2-} are the anions most effective in precipitating various proteins (see Tasaki et al., 1965). We visualize the abrupt structural change observed in polyanionic gels as a transition from a *compact* state to a *swollen* (i.e. *hydrated*) state. Reflecting its position in the lyotropic series, chloride salts tend to raise the water content of the gel; consequently, it is unfavorable to evoke abrupt changes in the a.c. impedance of the gel repetitively.

Abrupt changes in the a.c. impedance can be demonstrated in a compact gel sheet (about 1 mm in thickness) compressed between two thin plastic plates, each of which has a small (about 1 mm in diameter) hole at a matching position. When one of the small surfaces of the layer is exposed to a 66 mM $CaCl_2$ solution and the other small surface to a 100 mM NaF solution, repetitive abrupt falls in the impedance followed by fairly *rapid recovery* are observed. Under these conditions,

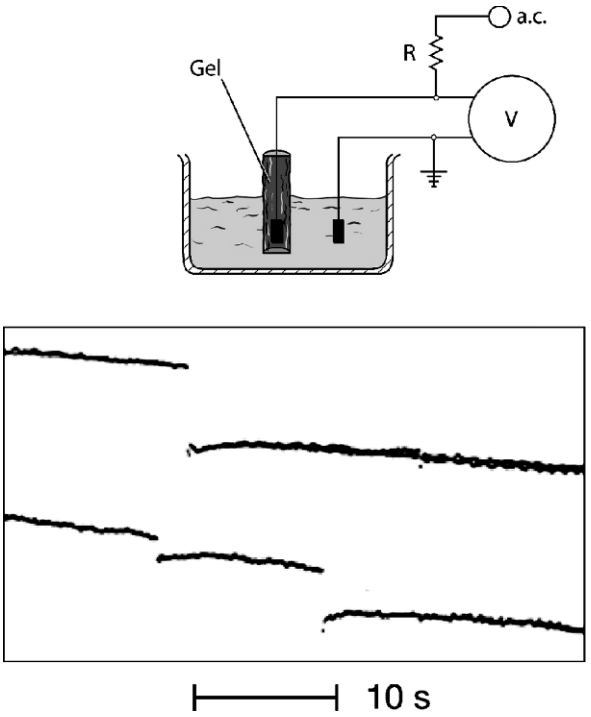

Fig. 4 Abrupt fall of the electric impedance across the superficial layers of a compact gel rod immersed in a 100 mM NaF solution. The impedance at 1 kHz was 1.1 kΩ, and the change observed in the upper trace was about 1% initially. (Tasaki, 2005b)

the gel surfaces exposed to the salt solutions are subjected to a considerable mechanical stress. This stress constrains the polymer chains near the gel surface and this is considered as the predominant factor that brings about a rapid recovery of the impedance loss.

2.5 Electric Potential Changes Associated with Abrupt Structural Transitions

The variation of the potential difference across a compact gel layer can be induced by application of a monovalent cation salt under a variety of experimental conditions (Tasaki, 2005b). An example of those observations is presented in Fig. 5. Here a compact, Ca-rich gel rod was compressed by means of two plastic plates separating a NaF solution from a $CaCl_2$ solution. The plastic plate facing the NaF solution had a small (0.5 mm diameter) hole, and the other plate had of a larger hole at the matching position.

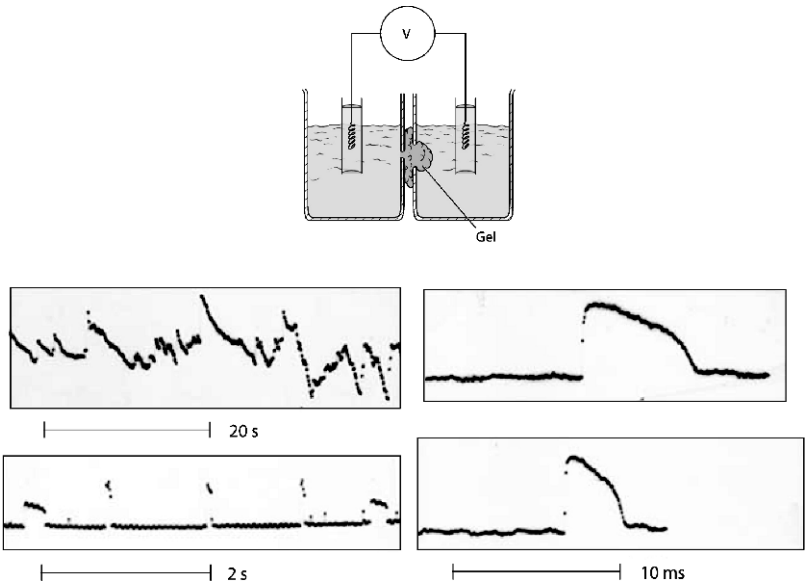

Fig. 5 Variation of the potential difference across a compressed gel layer that were exposed to a 100 mM NaF solution on one side and to a 66 mM $CaCl_2$ solution on the other side. The amplitudes of the abrupt potential changes were between 1 and 24 mV (Tasaki, 2005b)

Many records of the potential variation taken under these experimental conditions were found to have time-courses that resemble those taken from living nerve fibers (see the figure). A sharp rising phase, followed by a slower falling phase, is characteristic of these "responses" of the compact gels. The variability of the amplitude of these recorded "responses" is considered to arise from the non-uniformity of the size of the activated patches on the gel surface.

It is possible to evoke similar "responses" by application of an electric current to a thin layer of polyacrylate gel separating salt solutions containing monovalent and divalent cations. To record such "responses", however, A.C.-coupling of the output signals from the gel layer to the recording preamplifier input is required, because the applied current generates a large, slowly varying potential drop across the layer.

3 Excitation Processes in Nerve Fibers

This section is devoted to the description of several observations demonstrating that the electrophysiological phenomena known as "nerve excitation and conduction" are, basically, manifestations of reversible abrupt structural changes occurring in the cortical gel layer of the nerve fiber. The process of divalent-monovalent cation-exchange in the layer assumes the principal part in the present discussion.