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).

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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

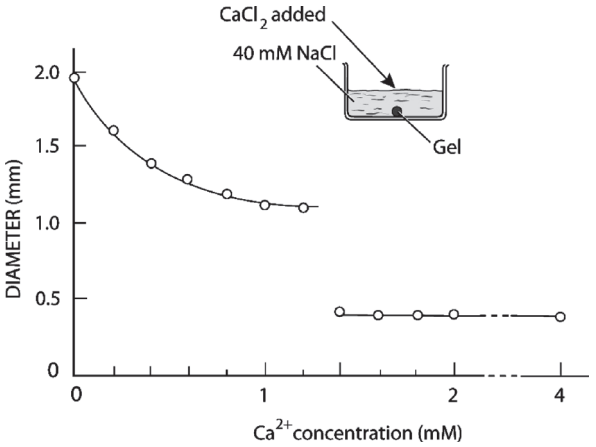

Fig. 1 The diameter of a small spherical gel bead immersed in a 40 mM NaCl solution, plotted against the concentration of Ca^{2+} added to the solution (Tasaki and Byrne, 1992)

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 $2Na^{+} \rightarrow Ca^{2+}$

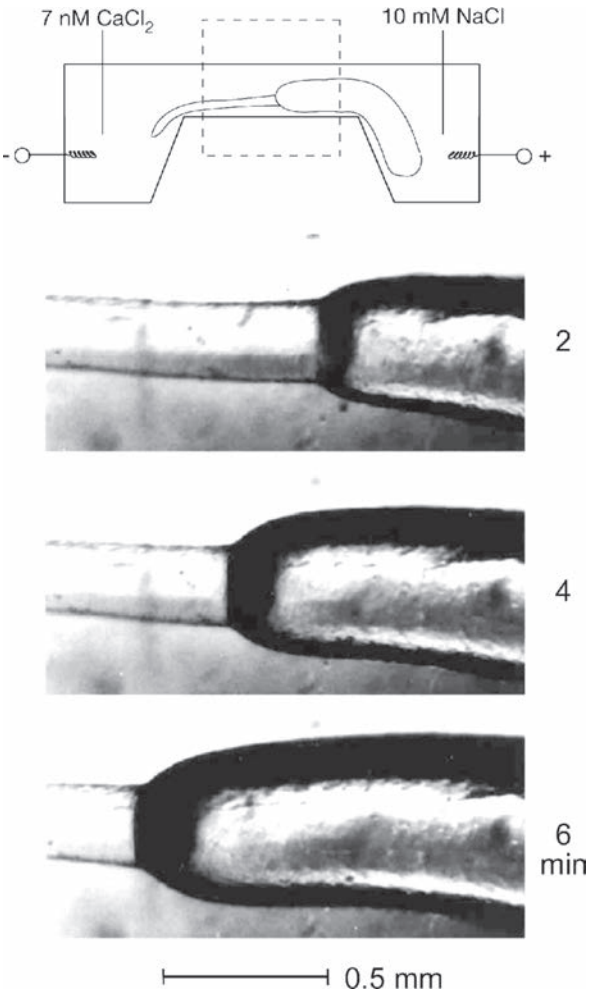

Fig. 2 Photomicrographs showing displacements of the boundary between the compact and swollen regions of a gel strand induced by electric current of 1 mA (Tasaki, 2002)

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.

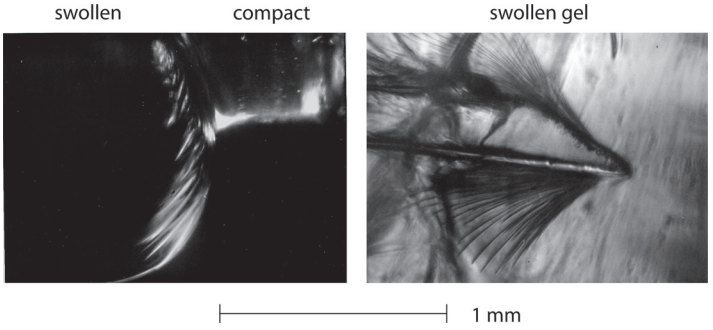

Fig. 3 *Left*: Highly refractile bundles of polymer chains formed in the transitional zone between the compact and swollen regions of a cross-linked polyacrylate gel strand. *Right*: Bundles of polyelectrolyte chains formed inside a swollen gel by application of an electric current by use of a glass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

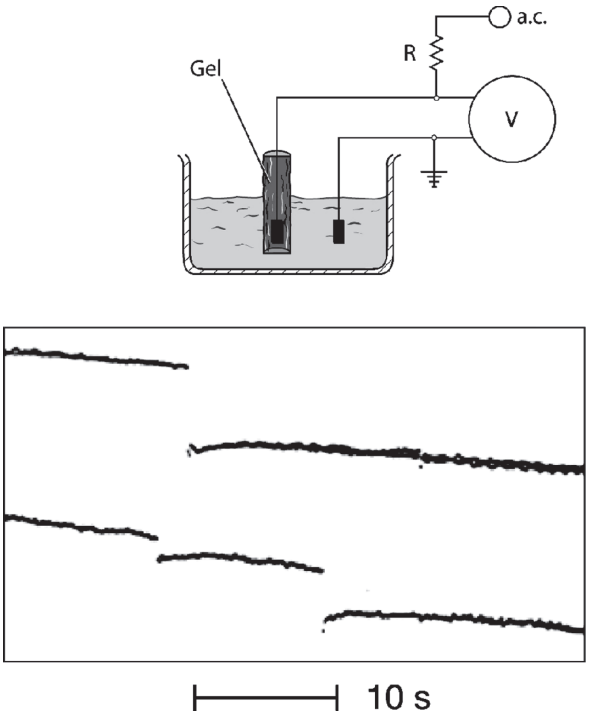

Fig. 4 Abrupt fall of the electric impedance across the superficial layers of a compact gel rod immersed in a 100 mM NaF solution. The impedance at 1 kHz was 1.1 kΩ, and the change observed in the upper trace was about 1% initially. (Tasaki, 2005b)

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.

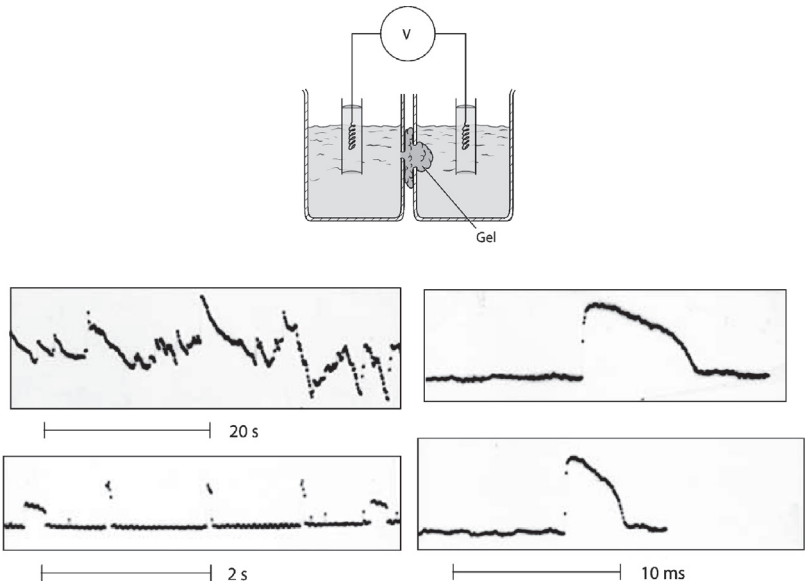

Fig. 5 Variation of the potential difference across a compressed gel layer that were exposed to a 100 mM NaF solution on one side and to a 66 mM $CaCl_2$ solution on the other side. The amplitudes of the abrupt potential changes were between 1 and 24 mV (Tasaki, 2005b)

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.

3.1 The Role of Ca^{2+} in Nerve Excitation: Jacques Loeb's Theory

In 1883, Sidney Ringer published his recipe of the saline solution which is favorable for maintaining the excitability of excised heart muscles and pointed out the importance of Ca-salt in the solution. In the year 1900, Jacques Loeb published an article subtitled "The poisonous character of a pure NaCl solution" and showed that the "poisonous" effect of a pure NaCl solution on excitable tissues can be counteracted, or "antagonized", by addition of the salt of Ca-ions.

Loeb formulated at that time a quite comprehensible theory of nerve excitation. It is highly instructive to see how Loeb visualized the process of nerve excitation a century ago. The following sentences are taken from his paper and monograph: "The salts, or electrolytes in general, do not exist in living tissues as such exclusively, but are partly in combination with proteins or fatty acid. The salts or electrolytes do not enter into this combination as a whole, but through ions. The great importance of these ion-proteid compounds (or soaps) lies in the fact that, by the substitution of one ion for another, the physical properties of the proteid compound change (p. 327 in Loeb, 1900)". "The normal irritability of animal tissues depends upon the presence in these tissues of Na, K, Mg and Ca ions in the right proportion; – any sudden change in the relative proportions – gives rise to an activity or an inhibition of activity (p. 95 in Loeb, 1906)". "The quotient of the concentration of Na ion over the Ca ions, C_{Na}/C_{Ca}, becomes therefore of importance for phenomena of irritability (p. 79 in Loeb, 1906)".

From these sentences, we can see that Loeb had a fairly good grasp of the process of exchange of Ca^{2+} for Na^+ or K^+ in living excitable tissues. It may appear very strange that his theory did not gain much popularity among classical neurophysiologists. The reason for this absence of wide acceptance of his theory might be, in part at least, that the experimental evidence cited in support of his idea was very indirect in nature and probably was not quite compelling. However, the main reason appeared to be that most physiologists at that time were passionately preoccupied with W. Nernst's mathematical theory of nerve excitation dealing with an abstract semipermeable membrane (see Nernst, 1908). Consequently, Loeb's theory of nerve excitation remained dormant for a long time to come.

3.2 The Process of Ca^{2+}-Na^+ Exchange at the Base of Action Potential Production

We have seen already that cross-linked polyanionic hydrogels can generate abrupt potential changes in response to a Ca^{2+}-Na^+ exchange. We now demonstrate that squid giant axons are capable of producing action potentials in association with a simple Ca^{2+}-Na^+ exchange occurring in the cortical gel layer of the axons (see Tasaki, 1982; 1999).

Here, we choose our observations made on squid giant axons under intracellular perfusion. The diagram at the top of Fig. 6 schematically illustrates the experimental

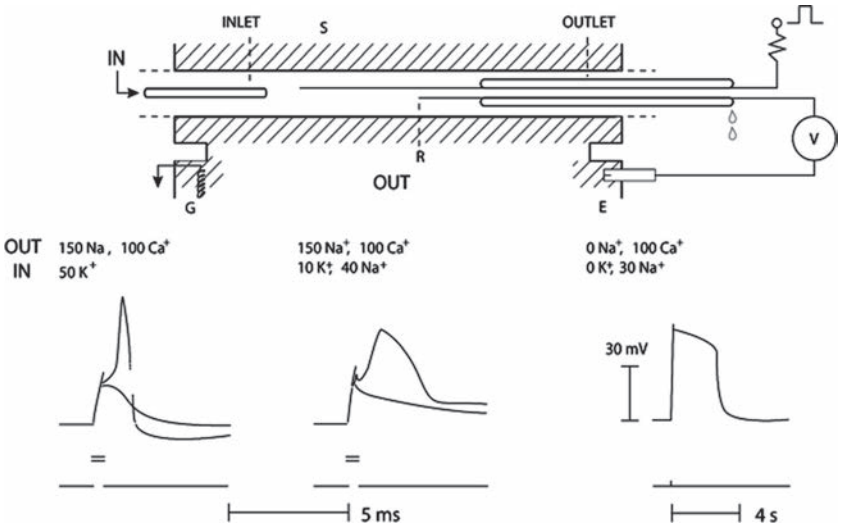

Fig. 6 *Top*: Schematic diagram illustrating the setup for intracellular perfusion of a squid giant axon. *Bottom*: Action potentials recorded from an axon. The concentrations of the cations in the extracellular (OUT) and intracellular (IN) salt solutions are indicated (Tasaki, 1982)

arrangement employed. A giant axon (about 0.5 mm in diameter) is mounted on a plastic platform (30 mm wide). The major portion of the protoplasm inside the axon between the tips of the two cannulae ("inlet" and "outlet" in the diagram) has been removed beforehand (by suction). The inlet cannula is connected to a reservoir of the solution for perfusion (containing KF, NaF, glycerol, etc). The flow of the solution inside the axon (roughly 25 μl/min) is maintained by adjusting the height of the reservoir. The external surface of the axon is exposed to a Ca^{2+}-containg salt solution. Pulses of outwardly directed current are delivered across the cortical layer of the axon by use of an internal metal wire electrode (S in the diagram) and a ground electrode (G). The responses of the layer to the current pulses are recorded with non-polarizable electrodes (R and E).

The left-hand record in the figure was taken from an axon which was intracellularly perfused with a K^+-salt solution and immersed in a chloride salt solution of Ca^{2+} and Na^+. We see that a brief pulse of outward current evoked an action potential which is very similar to those observed in intact axons (without intracellular perfusion). Under these conditions, we can maintain the ability of the axon to generate full-sized action potentials for more than 10 h.

Shortly after the invention of the technique of intracellular perfusion, we found that the excitability can be maintained in axons intracellularly perfused with solutions of the fluoride or phosphate salts of Cs^+, Na^+, choline, tetraalkylammonium ions, etc, as long as the pH and the osmolarity of the intracellular solution are kept in a proper range. Furthermore, it was found possible to evoke action potentials in axons immersed in the solution of the salt of Sr^{2+} or Ba^{2+} substituting Ca^{2+}. [Note, however, that the salt of divalent cations introduced into the axon interior

irreversibly damages the axon.] *No action potential can be elicited from axons when the Ca-ions (or their substitutes) in the external solution are completely replaced with monovalent cations.*

The record furnished in Fig. 6, right, is an example the action potentials taken from axons intracellularly perfused with a dilute NaF solution and immersed in a $CaCl_2$ solution. Here, the cortical layer of the axon is sandwiched between a Na^+-salt solution inside and a Ca^{2+}-salt solutions outside. *There is no Na^+ outside the axon.* The concentration of Ca^{2+} in the external medium is high enough to maintain the cortical layer in the Ca^{2+}-rich, compact state. Hence, delivery of a pulse of outward current to the layer is expected to induce a $2Na^+ \rightarrow Ca^{2+}$ exchange in the cortical layer.

From the finding that all-or-none action potentials can be observed under these simplified experimental conditions, we conclude that the process of Na^+-Ca^{2+} exchange occurring in its cortical layer of the axon is responsible for the generation of the observed action potentials.

3.3 A Wave of Reversible Abrupt Structural Change Running Along the Cortical Gel layer

In 1970, in his Tilden Lecture entitled "The biochemistry of sodium, potassium, magnesium, and calcium", R. J. P. Williams predicted the existence of a mechanical change in the nerve fiber in association with a propagating nerve impulse. He said: "This (propagating nerve impulse) is often pictured as a physical event – electrostatic field changes altering the membrane so that it changes from a potassium to a sodium permeable condition. Could it not rather be that inward diffusion and binding of calcium causing a running wave of structural change along the membrane?" (p. 362 in Williams, 1970). It was known at that time that, during repetitive stimulation of a squid giant axon, radioactively labeled Ca-ions in the surrounding medium are rapidly incorporated into the axon (Hodgkin and Keynes, 1957). Later on, using the method of intracellular perfusion, it was shown that, at the peak of excitation, the influx of Ca^{2+}across the cortical gel layer rises to an intensity 200–300 times as high as its influx at rest (see p. 224 in Tasaki, 1982).

Ten years after William's prediction, we actually found an unmistakable sign of a running wave of structural change—*shrinkage* of the squid giant axon following the production of an action potential (Iwasa and Tasaki, 1980). At the same time, we were gratified to find, in addition, a definite sign of *swelling* of the axon which *preceded* the shrinkage. This discovery of *swelling* had great impact on our discussion of the process of nerve excitation.

The schematic diagram at the top of Fig. 7 illustrates the design of the piezoelectric sensor device employed for detection of small and rapid mechanical changes in squid giant axons. As is well-known, these axons are capable of responding to repetitive brief shocks with production of full-sized action potentials for a long period of time. The record shown below was taken after averaging a large number

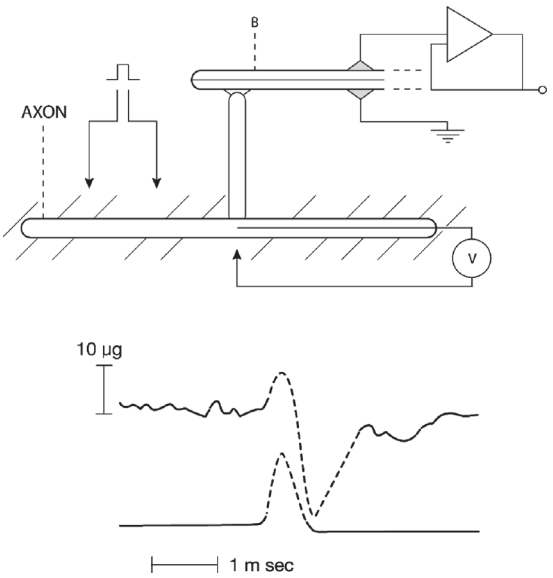

Fig. 7 *Top*: Schematic diagram illustrating the piezoelectric device for detecting small and rapid pressure changes associated with excitation of a squid giant axon. *Bottom*: Mechanical response and action potential recorded simultaneously (Tasaki 1982)

of responses. It is seen that the *swelling* of the axon starts almost simultaneously with the onset of the action potential. Furthermore, the peak of the swelling roughly coincides with the peak of the action potential.

This finding, obtained by use of a piezoelectric pressure sensor, was extended by employing a Fotonic sensor for detection of a small displacement of the axon surface (Tasaki and Iwasa, 1982). It was shown that, during the rising phase of the action potential, the axon surface moves outwards and this surface displacement is generally in the range between 0.5 and 2 nm.

From these observations, we deduce the following conclusion: *The propagating nerve impulse is a running wave of reversible structural change, representing a continuous displacement of the boundary between the site of swelling and the site of shrinkage of the cortical gel layer.*

The abrupt and drastic fall of the a.c impedance of the cortical layer associated with action potential production (Cole and Curtis, 1939) can now be safely attributed to the swelling (i.e. rise of water content) of the cortical layer running along the axon.

3.4 Spectral Analysis of Changes in Extrinsic Fluorescence Associated with Nerve Excitation

In 1968 we made the discovery that nerve fibers stained with appropriate fluorescent molecules can generate “optical responses” representing transient changes in

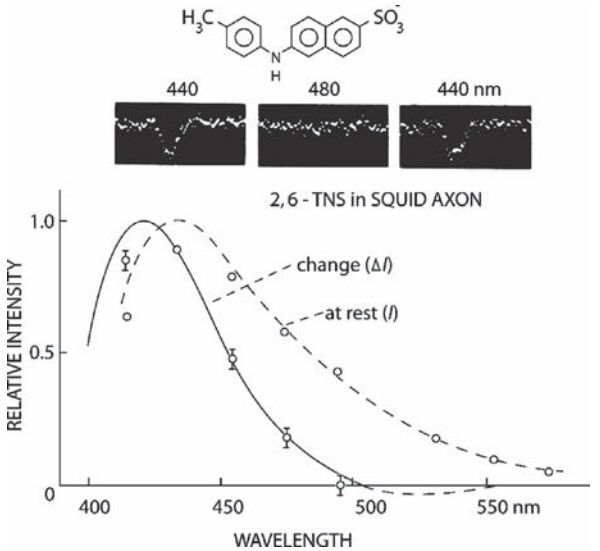

Fig. 8 *Top*: Samples of records of 2,6-TNS fluorescence changes associated with action potential production. *Bottom*: the wavelength dependence of fluorescence of 2,6-TNS injected into a squid giant axon (I) and that of fluorescence changes associated with action potential production (ΔI) (Tasaki et al., 1973)

intensity of the fluorescent light associated with action potential production (Tasaki et al., 1968). Our analyses of such optical responses have yielded much information about the structural changes which take place in the cortical layer of the nerve fiber during excitation (see Tasaki, 1982). In this subsection, we describe one of those analyses (see Fig. 8).

Anilinonaphthalene sulfonate (ANS) and toluidinylnaphthalene sulfonate (TNS) are known to emit intense fluorescent light when they are dissolved in organic solvent (with low solvent polarity), such as ethanol, but they do not fluoresce when dissolved in water. When water is added to an ethanol solution of these compounds, a fall in the fluorescence intensity is accompanied by a red-shift of the emission spectrum. Taking advantage of this property, ANS and TNS have been used for optically probing the *hydrophobicity* of the micro-environment of these molecules inside proteins (see Weber and Laurence; 1954; McClure and Edelman, 1966).

Figure 8 shows the results obtained by using 2,6-TNS injected into a squid giant axon. The broken line in the figure, marked (I), represents the spectrum of the fluorescent light emitted by the 2,6-TNS probe under constant illumination with polarized quasi-monochromatic light of 365 nm. The continuous line, marked (ΔI), shows the transient fall in the fluorescent light intensity brought about by electric stimulation of the axon, plotted against the wavelength of the emitted light. Note that the spectrum of ΔI is shifted to the shorter wavelength side of the spectrum I.

The significance of this finding can easily be understood on the basis of the Ca^{2+}-Na^{+} exchange process taking place in the cortical layer of the axon. In

the resting (Ca^{2+}-rich) state of the axon, the micro-environment of the 2,6-TNS molecules in the layer must be *effectively hydrophobic,* because the fluorescence emission is intense. The transition of the cortical layer to its swollen state drastically enhances the *hydrophilicity* of the micro-environment of these probe molecules and brings about an abrupt fall in light intensity accompanied by a red-shift of the emission spectrum. We found that the observed spectrum of ΔI can be reproduced by subtracting the emission spectrum of this probe molecule dissolved in ethanol-water mixture from that observed in pure ethanol.

In this connection, it is to be remembered that the *hydrophobic* sites in the resting axon are most likely to take up K-ions far more easily than Na-ions (see pp. 343, 350 and 357 in Williams, 1970). In the squid giant axon at rest, addition of K-salt to the external medium readily causes a loss of excitability and a depression of the resting potential of the axon. We may now attribute this distinguishing property of K-ions to the *hydrophobicity* of the cortical layer of an axon at rest revealed by the observation described above.

In the swollen, *hydrophilic* state of the cortical layer, the cation mobilities are high and the selectivity for hydrophilic cations is enhanced. The behavior of Na-ions – and other monovalent cations such as hydrazinium (see p. 211 in Tasaki, 1982) – in the excited state of the axon may now be safely attributed to the running wave of swelling of the cortical gel layer.

3.5 Production of All-or-none Action Potentials by Electric Stimulation of Myelinated Nerve Fibers

In myelinated nerve fibers, the axis-cylinder of the fiber is covered, as is well known, by a layer of myelin sheath except at the nodes of Ranvier. The occurrence of the transient physiochemical events underlying the process of excitation and conduction is limited to the naked portion of the axis-cylinder at individual nodes of Ranvier (see Tasaki, 1982). The naked surface of the axis-cylinder at the node of a bullfrog motor nerve fiber is 0.5–1 μm wide and roughly 10 μm in diameter. Here, we analyze how action potentials are generated by electric stimulation of the node.

The records of action potentials developed by a *single node of Ranvier* furnished in Fig. 9 were taken by use of the following arrangements: The node under study (N_1 in the diagram) was kept in physiological saline solution (containing Na^+ and Ca^{2+}) and was separated from the proximal portion of the fiber (including N_0) by a narrow air-gap. The Ranvier nodes in this portion were rendered inexcitable by use of a dilute anesthetizing solution. The distal portion of the fiber (between N_1 and N_2) was used as a part of the "pipette electrode" for recording action potentials. Brief voltage pulses were applied between node N_1 and the proximal portion of the fiber, whereby generating pulses of outwardly directed current through the node under study, N_1.

It is seen in the figure that action potentials evoked were about 110 mV in amplitude. It is noteworthy that the amplitude was practically independent of the duration

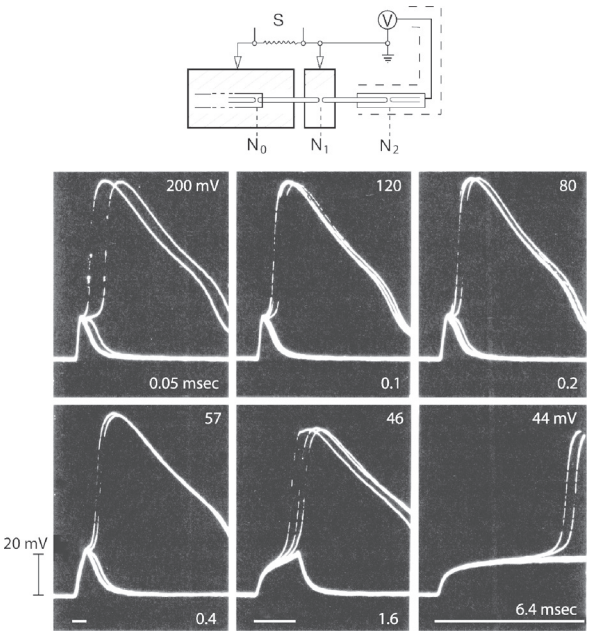

Fig. 9 Action potentials of a single node of Ranvier evokeded by application of brief voltage pulses. The duration and voltage of the stimulating pulse employed are indicated in each of the frames of record. Several records of the responses to the same stimulus strength are superimposed in each frame (Tasaki, 1982)

of the stimulating pulses, as long as the evoked action potential started after the end of the pulse. That is, *the response of a node of Ranvier to a brief stimulating pulse is all-or-none.* The threshold potential was about 22 mV above the resting potential and was also independent of the duration of the brief stimulating pulse employed.

It is known that the production of an action potential at the node is accompanied by a drastic fall in the electric resistance of the cortical layer at the node (see p. 69 in Tasaki, 1982). Hence, it is reasonable to assume that the cortical layer at the node is *swollen* and becomes *hydrophilic* in its excited state. In the resting state, the node is sensitive to "depolarization" by K-salt, implying that the layer is *compac*t and effectively *hydrophobic*. Thus, the mechanism of excitation of a node of Ranvier is considered to be basically the same as that of other excitable tissues.

The potential fall from the peak of the action potential is a reflection of the "relaxation process" which is considered to arise primarily from accumulation of the extracellular cations in the intracellular space (see p. 68 ibid.). The *shoulder* of the action potential is regarded as representing the onset of a *transition* of the cortical layer from its swollen state (modified by the relaxation process) to the compact state (see p. 274 ibid.).

We now analyze the potential changes brought about *directly* by a brief stimulating pulse. It is noted that, at the end of the stimulating pulse of the threshold

intensity, the observed potential change is close to the well-known value *RT/F* (≈ 25 mV), which is not large enough to appreciably alter the cation distribution in a cation exchanger membrane (see p. 264 ibid.). By such a stimulating pulse, only a small fraction of the entire cortical layer at the node is expected to be thrown into the excited (i.e. swollen) state. The state of the node containing swollen spots or patches is unstable. As a consequence of the electric interaction of the swollen spots and patches with the remaining surface area of the node, the cortical layer tends to reach, eventually, either a uniformly swollen state or a uniformly compact state. Note the *bifurcation* of the potential trace in the records following the termination of the applied brief stimulating pulse.

We represent the fraction of the cortical layer at the node excited directly by the stimulating pulse by α. Then, the fraction of the remaining surface at the node area is $(1 - \alpha)$. The intensity of the inwardly directed current through the swollen portion of the surface is given by $\alpha(E_e - V)/r_e$, where E_e represents the e.m.f. of the layer in its excited state, r_e the resistance of the (entire) layer of the node in the excited state and V is the intracellular potential in the "mixed" state. Analogously, the outward current flowing through the remaining (resting) area is given by $(1-\alpha)(E_r - V)/r_r$, where E_r and r_r are the e.m.f. and the resistance of the layer in the compact (i.e. resting) state.

Immediately after the ends of the stimulating pulse of the threshold intensity, namely, during the period in which the *bifurcation* of the potential trace is observed, the net current through the node should vanish. It follows from this that

$$\alpha \frac{(E_e - V)}{r_e} + (1 - \alpha) \frac{(E_r - V)}{r_r} = 0 \tag{1}$$

By solving this equation for α, we obtain

$$\alpha = \frac{1}{\left(\dfrac{E_e - E_r}{V - E_r} - 1 \right) \dfrac{r_r}{r_e} + 1} \tag{2}$$

In the present case, the action potential amplitude, $(E_e - E_r)$, is 110 mV, the potential rise evoked by threshold stimulation, $(V - E_r)$, is about 22 mV, and the ratio of the electric resistance of the layer at rest to that at the peak of excitation, r_r/r_e, is about 10. From these values, we find that the fraction α is about 1/40. The conclusion reached from this analysis is that *activation of a very small portion of the nodal area by an applied stimulus does* "trigger" *transition of the entire nodal surface to its excited state in an all-or-none manner.*

We know that a decrease in the Ca^{2+}-concentration in the external medium or some other chemical treatment of nerve fibers can cause lowering of the threshold stimulus strength. Hence, the fraction α can become even smaller than the value estimated above. We now have good understanding of the origin of the all-or-none behavior in the process of action potential production.

3.6 Local Current, Cable Property and Conduction Velocity of Nerve Fibers

In the Handbook of Physiology published in the year 1879, Ludimar Hermann advanced his *local current* (Strömchen) theory. He proposed in this book that nerve conduction is brought about by successive excitation of the neighboring resting area of the nerve by the local electric current generated by the excited area.

In the following years, the importance of this concept of re-stimulation by virtue of the local current has been fully recognized by every investigator studying the process of nerve conduction. However, the identity of the structural elements in the nerve (trunk) involved in the process of re-stimulation, "core conductor" and "sheath", remained completely obscure for a long time.

Shortly before and after World War II, the cable properties of both myelinated and non-myelinated nerve fibers were thoroughly clarified (see below), and the details of the process of re-stimulation by the local current became abundantly clear. In this subsection, we briefly describe how the velocity of nerve conduction is determined by the cable properties of the nerve fiber involved in the process of re-stimulation.

The electric resistance and capacitance of the myelin sheath were determined by recording the action current (and the quantity of electricity) passing through a short portion of an internodal (myelinated) segment under the condition that *two nerve impulses,* evoked near the two ends of the same fiber, *collide and vanish* at the site of the internode under study (see p. 72, Tasaki, 1982). The resistance of the portion of the myelin sheath was evaluated from the quantity of electricity which traverses the sheath during one complete cycle of rise and fall of the potential associated with the action potentials simultaneously generated at the two nearest nodes. (Note that the capacitive contribution to the measured quantity vanishes in this case.) The capacitance of the portion was determined from the potential and the quantity of electricity observed at the peak of the action potential, after subtraction of the resistive contribution at this moment. By applying the same method to a short portion of a nerve fiber including one node (rendered inexcitable by light anesthetization), the resistance and capacitance of the node at rest were estimated.

These measurements have shown that the resistance and capacitance of the myelin sheath of the amphibian motor nerve fiber are, respectively, $2.9 \times 10^7\ \Omega\,\mathrm{cm}$ and 1.6×10^{-11} F/cm, the resistance-capacitance product being about 0.46 ms. This finding indicated that the myelin sheath does not behave like a good insulator when the potential difference across the sheath is varying rapidly with time. Because of the capacitive flow of electric current through the myelin sheath, the local current generated by abrupt excitation of one node does not reach the neighboring node instantly. Evidently, the major portion of the internodal conduction time (about 0.1 ms across a $2 \sim 2.5$ mm long internode) represents the time required for raising the potential inside the neighboring node to the level high enough to trigger a transition to its excited state (see p. 95 in Tasaki, 1982; Tasaki and Matsumoto, 2002).

We now turn to the cable property of squid giant axons. Owing to their great size (about 0.5 mm in diameter), the electric parameters of these axons can be determined more-or-less directly. The capacitance of the cortical layer (C) is shown to be about 1.0 μF/cm^2, the ohmic resistance of the layer at the peak of excitation (R^*) is 25 $\sim$ 40 Ω cm^2, and the resistivity of the axon interior (ρ) is 30 $\sim$ 70 Ω cm (see Cole and Hodgkin, 1939; Hodgkin and Huxley, 1952).

We have seen that, when a brief stimulating shock is delivered to a squid giant axon, a traveling wave of rapid swelling is induced in the cortical layer of the axon. In the vicinity of the sharp boundary between the swollen (excited) and compact (resting) regions of the axon, the local currents are generated, spreading exponentially to the two sides of the boundary. The conduction velocity is determined by the spread of the local current in the vicinity of the boundary.

Some time after the inception of the technique of intracellular perfusion, Matsumoto and Tasaki (1977) examined the distribution of the local current in the squid giant axon in great detail and found that there is a simple quantitative relationship between the conduction velocity and the electric parameters. The equation describing the velocity, v, of an axon of diameter d is as follows:

$$v = \frac{1}{C}\sqrt{\frac{d}{8\rho R^*}}. \tag{3}$$

By introducing the above-stated values of the electric parameters, the velocity, calculated for axons of 0.05 cm in diameter, is found to be between 15 and 28 m/s. with an average of 22 m/s. The agreement between the calculated and observed values is good. It is noted that, in non-myelinated nerve fibers, the conduction velocity is directly related to the symmetric spread of the local current to the compact and swollen sides of the boundary between the two structurally distinct regions (see Tasaki, 2006).

4 Conclusion

(1) COO^- groups of polyelectrolyte chains in salt solutions bind Ca^{2+} preferentially and reversibly. The compact structure of a Ca^{2+}-rich polyelectrolyte gel is stabilized by calcium-bridges formed between polyelectrolyte chains.
(2) The cortical gel layer of nerve fibers or cells can be converted from the compact state to the swollen state by substituting monovalent cations (e.g. Na^+) for the divalent counter-cations (Ca^{2+}). The cortical layer is effectively hydrophobic in its resting state, and hydrophilic in its excited state.
(3) The process of action potential production is nothing but an electrical manipulation of reversible abrupt structural changes occurring in the cortical gel layer of nerve fibers and cells.

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