by itself suffices to explain the Liesegang phenomenon. As a matter of fact, Liesegang (*16*), Hedges (*2*), Bradford (*7*), and Holmes (*17*) observed that between two precipitate bands the solution in the gel is completely or almost completely depleted of one of the forming ions (generally the ion initially present in the lowest concentration is the one that is lacking). Stanfield (*18*) observed that a strong concentration difference between the two reagents predisposes to the formation of macroscopic multiple bands [see also Bradford (*7*) and Holmes (*17*)]. Wilson and Pringle (*19*) and Salvinien and Kaminsky (*20*) noticed the same tendency with immunological precipitates.

Our explanation of the Liesegang phenomenon is most nearly approached by McLaughlin and Fischer's hypothesis (*10*), which states that while the precipitate bands may be somewhat permeable to many substances, they certainly are impermeable to the forming substances. According to these authors, the barriers then lose their impermeability after some time, by a mechanism for which they do not offer a satisfactory explanation. The only thing lacking in their theory, to make it conclusive, is the condition from the Hirsch effect: that these precipitates constitute a membrane that is impermeable to the forming substances only as long as the forming substances are both present on either side of the membrane. Thus, once a band is formed by precipitation of two reagents that meet in a gel (or another porous medium such as filter paper) [see Van Oss, Fontaine, Dhennin, and Fontaine (*9*) and Milone, Cetini, and Ricca (*21*)], that band remains impermeable until one of the reagents is exhausted by precipitation or by absorption on the precipitate, at least in the immediate vicinity of the membrane [see Bradford's adsorption experiments (*7*)]. Only from that moment can the other reagent cross the barrier, until it again encounters farther on a sufficient amount of the first reagent, with which it will form a second band, and so on.

Our theory may also throw some light on the formation of immunological precipitate bands in porous media, where it is of great importance to be able to avoid confusion of Liesegang bands with bands due to a multiple immunological system [Wilson and Pringle (*19*); Salvinien and Kaminsky (*20*); Van Oss, Fontaine, Dhennin, and Fontaine (*9*)]. The mobility of the bands [Oudin (*22*)] does not necessarily exclude the occurrence of a Liesegang phenomenon, particularly not in the cases where the precipitate is soluble in an excess of one of the reagents, like colloidal and immunological precipitates (Bechhold, *23*), and even sometimes precipitates of electrolytes [Pringsheim (*24*), and Cetini and Ricca (*25*)]. The best way to avoid the formation of multiple macroscopical Liesegang bands is to operate with equivalent concentrations of reagents.

Many other phenomena of periodic structure may be explained in a similar way: the very creation of the first structural elements opposes a temporary barrier to the transport of a forming substance. The barrier then loses its function as such for reasons inherent to its growth, thus leaving the way free to the construction of the next structural element. The periodicity is determined by the magnitude of negative feedback arising out of the interaction between the formation and the degeneration of the barrier.

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Prolongation of Response of Node of Ranvier by Metal Ions

Abstract. The response of the node of Ranvier can be prolonged up to 5 to 6 seconds by addition to the medium of minute amounts of certain metal ions. This prolongation involves a change from a triangular to a rectangular configuration. The properties of the prolonged nerve-fiber responses are very similar to those of heart muscle.

Electrical responses of the node of Ranvier whose falling phase is markedly prolonged can be obtained by exposure of the node to such alkaloids as brucine, emetine, sinomenine, and heroine (*1*), to certain derivatives of morphine (*3*), and to strychnine (*4*), as well as by exposure to hypertonic solutions (*2*). Prolonged responses were also obtained in the giant axon of the squid with the intracellular injection of tetraethylammonium chloride (*5, 6*). Following repetitive stimulation, many excitable cells show a type of "memory" in the form of a prolonged response. This phenomenon has been observed in the node of Ranvier of the toad (*7, 8*), giant axon of sepia (*9*), aplysia nerve cell (*9*), the ommatidium of the horseshoe crab (*10*), the supramedullary ganglion cell of the puffer fish (*11*) and in *Noctiluca,* a protozoan (*12*). Somewhat less marked prolongations of nerve fiber responses have been obtained with low temperatures (*13*), high pressures (*14, 15*) and by replacing H_2O in the fluid medium with D_2O (*16*).

The prolonged responses (especially the markedly prolonged responses) show many properties in common with those of the normal response of heart muscle. These similarities involve the configuration, the instability of the duration, the time course of the impedance changes, the refractory period, the resistance to lowered sodium or increased potassium, and the effects upon the duration and configuration of temperature changes, of pressure changes, of polarization, of frequency of stimulation, and of a large number of chemicals (*5, 7, 17*). In the experiments reported here (*18*), a prolongation of the response of the node of Ranvier was obtained by the external application of metal ions. Brief reference to these experiments was made in a previous publication (*6*).

Both the node of Ranvier and the giant axon preparation were used in these experiments. Single myelinated nerve fibers were isolated from the nerve innervating the semitendinosus or sartorius muscle of the toad (*Bufo marinus*). This technique has been described previously (*1*). Action currents of the node of Ranvier were recorded by the "bridge-insulator" technique (*1*). Action potentials

of the node of Ranvier were recorded by the method of Tasaki and Frank (*19*). Intracellular action potentials from the squid (*Loligo pealii*) were recorded by a modification (*5, 15*) of Hodgkin and Huxley's (*20*) and Curtis and Cole's (*21*) methods. Intracellular injections into the giant axon were carried out with a modification (*7*) of the methods of Arvanitaki and Chalazonitis (*22*), Hodgkin and Keynes (*23*) and Grundfest *et al.* (*24*). The control frog Ringer's solution contained 0.22 percent $NaHCO_3$, 0.014 percent KCl, 0.65 percent NaCl, and 0.012 percent $CaCl_2 \cdot 2H_2O$. In some experiments 0.001 percent NaH_2PO_4, 0.2 percent glucose, or both, was added. Experiments were carried out at room temperature (21° to 25°C).

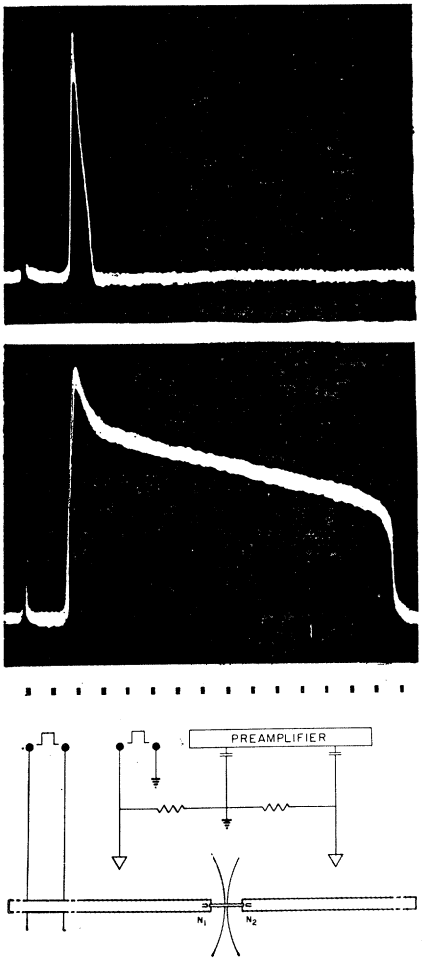

Fig. 1. Prolongation of response of node of Ranvier by metal ions. (Top) Before application of $NiCl_2$. (Middle) After application of $5 \times 10^{-4}M$ $NiCl_2$. Temperature, 23°C. Bar at right subtends 1×10^{-9} amp. Time marker, 1 msec. (Bottom) "Bridge-insulator" method used in recording action currents.

The following metal ions were employed: Ni^{++}, Co^{++}, Be^{++}, and Cu^{++}. The upper two frames of Fig. 1 illustrate the effect of Ni^{++} upon the duration of the action current. The "bridge-insulator" method for recording the action current is illustrated at the bottom of Fig. 1. The response of the node in normal Ringer's solution is shown in the top frame of Fig. 1. In the middle frame, the response was recorded after replacement of the fluid surrounding the node with Ringer's solution containing $10^{-4}M$ $NiCl_2$. The response is prolonged by development of a plateau in the falling phase. The extent of prolongation obtained with Ni^{++} was variable. Increases in duration of more than 10,000 times normal have been obtained.

Results obtained with Be^{++} and Co^{++} were generally comparable. With Cu^{++} the effects were not always clearly demonstrable. The minimal concentration of Ni^{++} required to produce the prolongation was found to be variable. In some experiments, an effect could be demonstrated with $5 \times 10^{-6}M$ $NiCl_2$. In most instances, however, higher ($10^{-4}M$) concentrations were required. The threshold concentration for the other metal ions was not determined. These ions were effective in concentrations of $10^{-4}M$ or higher. When much higher concentrations of any of these ions were used, the prolongation was less marked, and the amplitude was lower, or the node became completely inexcitable. When the node was freed of the adhering tissues, the effect of Ni^{++} was instantaneous. Within a certain range an increase in the concentration of Ni^{++} resulted in an increase in the prolongation. Often Ni^{++} resulted in a slight increase in the amplitude of the response.

When the treated node was washed with normal Ringer's solution, the effect of the ions was sometimes reversible. Very often, however, the effect was not reversed. Sometimes, but not always, under these conditions, where the prolonged response cannot be shortened by washing with normal Ringer's solution, washing with Ringer's containing a small amount of Versene resulted in a reversal of the effect.

The effect of Ni^{++} upon the action potential of the node was similar to that upon the action current. Extracellular application of Ni^{++} to the giant axon of the squid did not result in a comparable prolongation of the response. The possibility that the lack of effect on this axon was due to an inability of Ni^{+} to penetrate the axonal surface was considered. In this connection, Ni^{++} was injected into the giant axon in such a volume and concentration as to give a final concentration of 10^{-3} to $10^{-4}M$. This procedure did not result in a marked prolongation of the response. The example of an agent that prolongs the response of the node but does not prolong the response of the giant axon is not unique. Repetitive stimulation and hypertonic saline (two conditions known to prolong the response of the node) did not prolong the response of the giant axon of *Loligo pealii*. On the other hand, such agents as low temperature, high pressure, and D_2O affect the duration of both types of fibers in a similar manner.

We have no clear idea about the exact mechanism whereby these metal ions exert their effect. In accordance with the two-stable potential states concept of excitation, the metal ion effect can be described as an increased stabilization of the upper potential level.

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