ture cells are less responsive to hydrocortisone suppression of hyaluronate synthesis than normal ones are; they also pointed out that this trait is propagated by the cells for more than 20 generations in monolayer culture. The possibility that the persistent differences in the rheumatoid cells are due to a genetic change or the serial passage of an infectious agent deserves consideration. Further studies that compare the properties of normal and rheumatoid synovial cells may reveal additional differences that could explain the pathogenesis of rheumatoid arthritis.

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3 November 1967

Giant Axon of Myxicola: Some Membrane Properties as Observed under Voltage Clamp

Abstract. *A new space-voltage clamped giant (500 to 900 microns) axon preparation is described* (Myxicola infundibulum). *Normal value for resting and action potentials are 71 and 89 millivolts, respectively. This preparation under voltage clamp exhibits relations between current and voltage like those described for the squid axon. The early inward current component is reduced in a solution with low sodium concentration. This preparation, then, acts in all its essential features like the squid giant axon.* Myxicola, *however, can be made available the year around and should prove to be an extremely useful preparation for the study of excitable membranes.*

Much of the present understanding of excitable membranes is based on data and analyses obtained from the squid giant axon (*1*, *2*), which has many characteristics making it a favorable preparation, particularly for observation using the space-voltage clamp technique (*3*, *4*). However, squid are available only seasonally, and they do not survive well in the laboratory. It would be desirable, then, to have an axon preparation also suitable for observation under space-voltage clamp, which is available year around. We now describe voltage clamp studies on the giant axon of the marine polychaete *Myxicola infundibulum*, which is available the year around.

Animals (obtained from Maritime Biological Laboratories, St. Stephen, New Brunswick) were maintained in the laboratory for periods of several weeks to several months in a cold, aerated commercial seawater mixture (Instant Ocean). There was no difficulty in obtaining a good supply of fairly large animals (5 to 6 cm long, when contracted). The prepared axons from such animals have been up to 900 μ in diameter, but they were usually from 500 to 700 μ. To prepare the fibers we first anesthetized animals by immersing them in 5 percent ethanol in Instant Ocean for about 30 minutes. A median dorsal incision, just through the body wall, was made for the entire length of the animal, care being taken not to rupture the gut. The animal could then be pinned ventral side down in a paraffined dish; we exposed the nerve cord by gently moving the gut aside. Most of the nerve cord is the giant axon which dominates the dorsal aspect of the cord (*5*). In this preparation a problem is presented in the intersegmental constrictions in the cord and giant axon (*5*). These constrictions may reduce the diameter of the fiber to as little as one-fourth that of the segmental swellings and so make insertion of the long internal electrode impossible. However, we could relieve these constrictions by carefully stripping off the closely adhering dorsal blood vessel, taking care at each intersegmental constriction to include in the stripping the lateral branches of the blood vessel and the connective tissue bands adhering to the lateral branches. The fiber was then a more uniform cylinder. The fiber and a narrow strip of the body wall to which it was attached, the ends ligated with thread, were removed from the worm. Then, if the body wall was carefully torn away from the fiber, the fiber boundaries could be readily seen when lighted from beneath. Three centimeters of axon were usually prepared in this way.

The dissection probably cuts fine axon branches, as it does in the preparation of the squid giant axon. As judged from the values obtained for resting membrane potentials, action potentials, and the current records obtained under voltage clamp, these cut branches do not seem to have produced any particular problems. Stretch (*6*) was not controlled for, but it was never more than 1.5 times the length of the nerve *in situ*. Better results were obtained if the preparation was kept in cold (10° to 12°C) Instant Ocean during the dissection. The entire dissection usually required from 2 to 3 hours.

The internal coaxial electrode has been described elsewhere (*7*). The chamber and clamp were essentially the same as those used for the squid axon (*7*, *8*). Normal saline used in the recording chamber (ASW) had, in final concentration, the following composition: 430 m*M* Na^+, 10 m*M* K^+, 10 m*M* Ca^{++}, 50 m*M* Mg^{++}, 560 m*M* Cl^-;

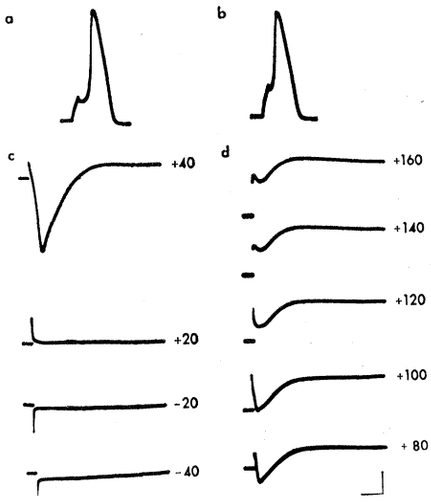

Fig. 1. Membrane current and potential records from the giant axon of *Myxicola*. (a and b) Internally recorded action potentials from the same axon before and after a voltage clamp series. (c and d) Membrane current records recorded under voltage clamp. The figures at the sides of the traces indicate the amplitude of the pulse in millivolts relative to the holding potential. Action potential threshold in this axon was about +22 mv, relative to the resting potential. Scale: (a and b) 20 mv, 2 msec; (c) 0.5 ma/cm², 2 msec; (d) 1.0 ma/cm², 2 msec.

*p*H near 7.4. Solutions with low sodium concentrations were prepared by replacing nine-tenths of the NaCl with choline chloride.

Resting membrane potentials were recorded with a micropipette, as the direct-current shift on withdrawal, in three preparations. These animals were anesthetized as described above, but were much less extensively dissected than those prepared for study with the long internal electrode. Dissection time was only 20 to 30 minutes. The mean resting membrane potential was 70 mv, with the inside negative. This value agrees extremely well with values obtained from the median giant fiber of *Lumbricus*, the only annelid axon from which there are data for comparison (*9*). The mean initial resting membrane potential from four fibers prepared for voltage clamping and recorded with an internal coaxial electrode was 71 mv (inside negative), which agrees very closely with the values that were obtained with a micropipette.

Normal action potentials recorded from five fibers with a coaxial internal electrode ranged from 80 to 104 mv, with a mean of 88.9 mv. These values agree very closely with those obtained from the median giant fiber of *Lumbricus*, which was prepared without anesthetic (*9*). Neither the anesthetic, then, nor the insertion of the long internal electrode seems to have any particular deleterious effects on this preparation.

Figure 1a is a record of the action potential of the giant axon of *Myxicola* recorded just before a voltage-clamp sequence. Figure 1b is a record of the action potential of the same axon just after such a study. The two action potentials are very similar, indicating that this preparation may be voltage-clamped without any accompanying deleterious effect.

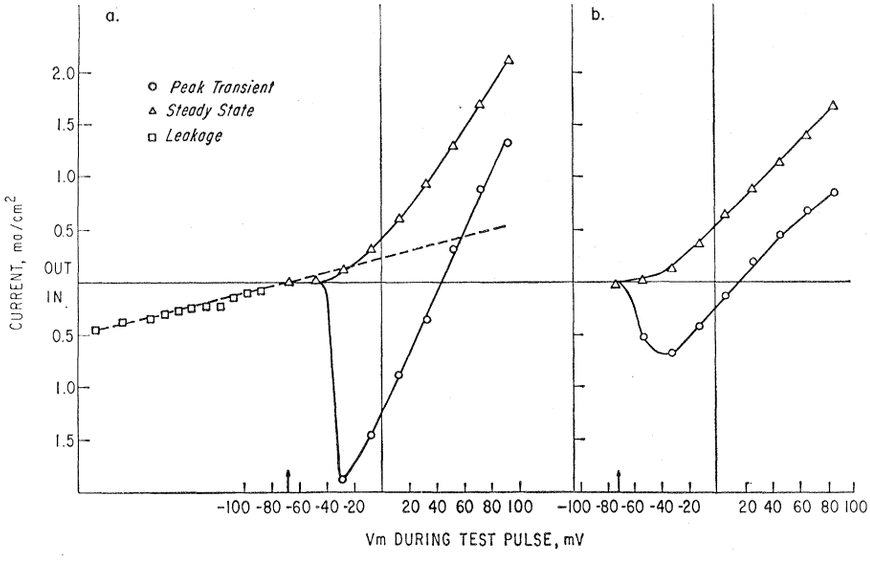

Fig. 2. (a and b) Current-voltage relations from two clamped *Myxicola* giant axons. Circles and triangles were taken from the peak transient and steady-state delayed currents, respectively. Squares and dashed line: leak current.

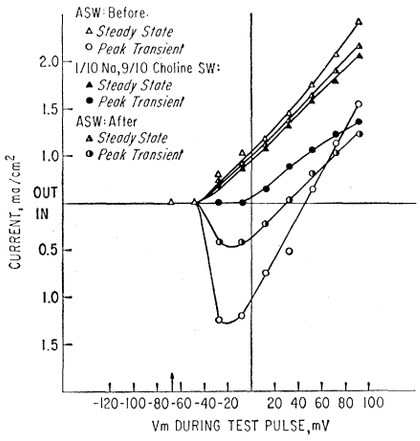

Fig. 3. Current-voltage relation for the giant axon of *Myxicola* in ASW (open symbols), ASW with low sodium concentration (filled symbols), and back in ASW (half-filled symbols). In each case circles represent the peak transient currents, and triangles represent the steady-state delayed currents. All currents were corrected for leak.

Figure 1, c and d, is a series of current records obtained under voltage clamp. For depolarizing pulses somewhat above threshold, after a capacitive surge, two components of the membrane current are seen—an early transient inward current and a delayed outward current which rises to a steady state. For stronger suprathreshold depolarizing pulses, the early transient current is first reduced in amplitude, then reverses, becoming outward. Figure 1d shows a series of voltage-clamp records near this reversal point. These records are very similar to those obtained from the squid giant axon (*3*, *4*), amphibian myelinated axons (*10*), and *Homarus* axons (*11*). The peak transient current is shifted in time when the current is outward in direction relative to when it is inward (*3*, Fig. 14; *2*, Fig. 3).

Current-voltage relations were observed in four axons. Values were taken from peak transient and steady-state delayed currents, after correction for leak. Leak currents were determined from the currents obtained as a response to hyperpolarizing pulses. This current-voltage characteristic was then taken to be linear for all values of membrane potential. Figure 2a is a representative example of such current-voltage curves for the axon of Fig. 1, a and b. The peak transient current component exhibits a negative conductance region. In this axon no points were actually recorded from the negative conductance region because of the

steepness of the curve. Figure 2b shows another current-voltage curve taken from another, less vigorous, axon showing a point in the negative conductance region. These curves are very similar in all essential features to those observed in the squid giant axon (*3*) and *Homarus* axons (*11*).

In three cases, membrane currents were also recorded in ASW with a low sodium concentration. In each case, the action potential was abolished. Figure 3 shows the current-voltage relation of an axon in ASW, in ASW with low sodium concentration and back again to ASW. In this axon, recovery was incomplete. The action potential before sodium replacement was 82 mv, and in ASW again after exposure to the ASW with a low sodium concentration it was 57 mv. In this and in every other case, there was no inward current for any values of depolarizing pulses in a low sodium concentration. There was very little effect on steady-state currents. The transient current therefore may be identified at least tentatively as being carried by sodium ions. Sodium, then, would seem to carry the current responsible for the depolarization phase of the action potential.

The membranes of *Myxicola* giant axons, then, seem to be very similar in essential features to those of the squid giant axons.

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4 October 1967

Cis-Trans Isomerism in Naphthoquinones: Interconversion and Participation in Oxidative Phosphorylation

Abstract. *Synthetic phylloquinone was resolved into* cis *and* trans *isomers by thin-layer chromatography. The two isomers had identical ultraviolet spectra characteristic of vitamin K_1 and were differentiated by nuclear-magnetic-resonance spectroscopy on the basis of the displacement of the peak corresponding to the olefinic methyl group in the naphthoquinone side chain. Studies on the restoration of electron transport coupled to phosphorylation in irradiated preparations of* Mycobacterium phlei *showed that only the* trans *isomer was active with substrates linked to nicotinamide-adenine dinucleotide. The purified* trans *phylloquinone was enzymatically converted to the* cis *isomer. Under similar conditions,* cis *vitamin K_1 gave rise to the* trans*-naphthoquinone. The natural naphthoquinone of* M. phlei *vitamin MK_9(II-H) was similarly resolved into* cis *and* trans *isomers.*

Restoration of oxidative phosphorylation in a quinone-depleted system from *Mycobacterium phlei* requires the addition of the natural naphthoquinone [MK_9(II-H)], vitamin K_1, or its homologues (*1*). Examination of the specificity for restoration of both oxidation and phosphorylation revealed a requirement for the 1,4-naphthoquinone nucleus with a methyl group in the carbon 2-position and at least one isoprenoid unit in the carbon-3-position (*2*). A number of compounds, such as dihydrophytyl vitamin K_1 and lapachol, which have been shown to restore oxidation by the main respiratory pathway, fail to restore phosphorylation (*2*, *3*). The requirements for restoration of phosphorylation by quinones appears to be more specific than that required for restoration of oxidation. Synthetic vitamin K_1 contains a mixture of *cis* and *trans* isomeric forms (*4*, *5*). The resolution of the synthetic quinone into its geometric isomers has permitted a further definition of the stereospecific requirements for restoration of coupled phosphorylation. Of particular interest was the finding of an enzymatic interconversion of the isomers of vitamin K_1. The ability of the *trans* isomer to restore oxidative phosphorylation and a consideration of the energetic processes involved in *cis-trans* isomerization may provide knowledge of the bioenergetic process and of the role of quinones in oxidative phosphorylation.

Chromatography of synthetic vitamin K_1 (*6*) was performed on a thin layer of silica gel G containing 0.1 percent rhodamine 6G with 10 percent butyl ether in hexane as solvent. The rhodamine plates fluoresced in ultraviolet light while ultraviolet-absorbing material quenched the fluorescence and gave rise to a dark band (*7*). Synthetic vitamin K_1 could be resolved into two bands, with R_F being 0.44 and 0.51, respectively (Fig. 1). Both of these compounds were identified as phylloquinones by ultraviolet spectroscopy, reverse-phase thin-layer chromatography, and infrared spectroscopy. With the molecular extinction coefficient reported for the absorption maximum at 269 mμ of phylloquinone in isooctane (*8*), the ratio of the amount of the slow- to the fast-moving band was about 10 : 1. A further identification of both bands as isomers of the same synthetic compound was obtained by nuclear-magnetic-resonance (NMR) spectroscopy (*9*). Both compounds yielded identical NMR spectra except for a difference in chemical shift in the region of 8.2 to 8.4 (Fig. 2). The peak in this area

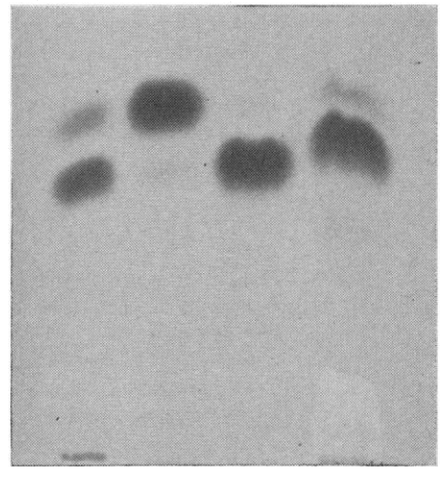

Fig. 1. Thin-layer chromatographic separation of geometrical isomers of phylloquinone and vitamin K_9(II-H). Chromatography was done on a thin layer (250 μ) of silica gel G impregnated with rhodamine 6G and developed in butyl ether: hexane (1 : 9 by volume). Spots visualized as dark bands under ultraviolet light (left to right): a sample of synthetic vitamin K_1; *cis* K_1, and *trans* K_1, the repurified bands from the synthetic K_1 (R_F *cis* K_1, 0.51, R_F *trans* K_1, 0.44); K_9(II-H), the natural naphthoquinone prepared from whole cells of *Mycobacterium phlei*. The quinone was purified by column chromatography prior to thin-layer chromatography according to the procedure of Dunphy *et al.* (*13*).