Membrane Potentials of the Squid Giant Axon Recorded with an Inserted Antimony Microelectrode

Direct measurements of cellular membrane potentials are usually made with KCl-filled microcapillaries in preference to metallic insertion electrodes. The former besides being easy to prepare[1] are not subject to variations in contact potentials which often develop with the latter. Nevertheless, some metallic electrodes have a realm of special usefulness. Thus, a reversible Ag-AgCl electrode can serve to determine the electrochemical activity of Cl^- in an appropriate system. For the squid giant axon it has been shown[2] that Cl^- inside and outside the excitable membrane is not in thermodynamic equilibrium. This note reports measurements of intra-axonal potentials by means of antimony electrodes which, when properly used, are reversibly sensitive to H^+.

Glass-insulated Sb microelectrodes[3] with overall tip diameters of 5–10μ were connected to an amplifier which, because of the low magnitude and polarity of its grid current did not affect the H^+ sensitivity of the electrodes. The electrodes were checked before insertion into and after removal from the axon. Against a Ag-AgCl reference, a standard phosphate buffer of pH 7 was 170–180 mV more negative than a buffer of pH 4.

Results. Measurements were obtained on six nerves. The axon was first impaled with a KCl-filled microcapillary. The resting potential (– 60 mV) and the spike (110 mV) recorded in this way are shown in Figure 1. Subsequent insertion of the Sb electrode less than 1 mm from the site of the microcapillary did not affect the potentials at the latter. The initial potential in sea water between the Sb electrode and the reference electrode (considered as the zero potential) changed abruptly and violently in a positive direction when the Sb electrode touched, but did not penetrate the axon. The shift, which varied between 100 and 200 mV, may have been caused by a temporary increase in the resistance as the Sb surface came into contact with lipid material of the axonal sheath. Small potential variations, usually not more than 5 mV, are frequently obtained when a KCl microcapillary is pressed against the axon.

As soon as the Sb electrode penetrated the axonal membrane, however, it registered a value closely approximating the resting potential recorded with the KCl microcapillary. The small difference (4 to 7 mV, Fig. 1 and 2) is ascribable to junctional potentials. Penetration of the Sb electrode into the axon was further signalized by the appearance of transmembrane spikes almost identical with those recorded by the KCl microcapillary.

In two experiments, 0·54 *M* KCl was added to the sea water surrounding a 1·2 cm stretch of the axon located in a well separated from the rest of the nerve chambre by vaseline seals. Both electrodes impaled the central portion of the axon lying within this well. One end of the axon was stimulated. Externally recording electrodes, straddling the well, monitored the propagation of the impulse into the well and out of it.

Both internal electrodes recorded identically the depolarization and decrease of spike amplitude caused by the addition of KCl (Fig. 1 and 2). The depolarization began immediately and continued to develop while the KCl was again replaced with sea water, since removal of the excess KCl by dilution was a relatively slow process. The response lost is overshoot and propagation

[1] G. LING and R. W. GERARD, J. cell. comp. Physiol. *34*, 383 (1949). – W. L. NASTUK and A. L. HODGKIN, J. cell. comp. Physiol. *35*, 39 (1950). – C. Y. KAO, Science *119*, 846 (1954).

[2] A. MAURO, Fed. Proc. *13*, 96 (1954).

[3] We are indebted to Dr. J. Y. LETTVIN, Department of Electrical Engineering, Massachusetts Institute of Technology, for providing the electrodes and for his kind help.

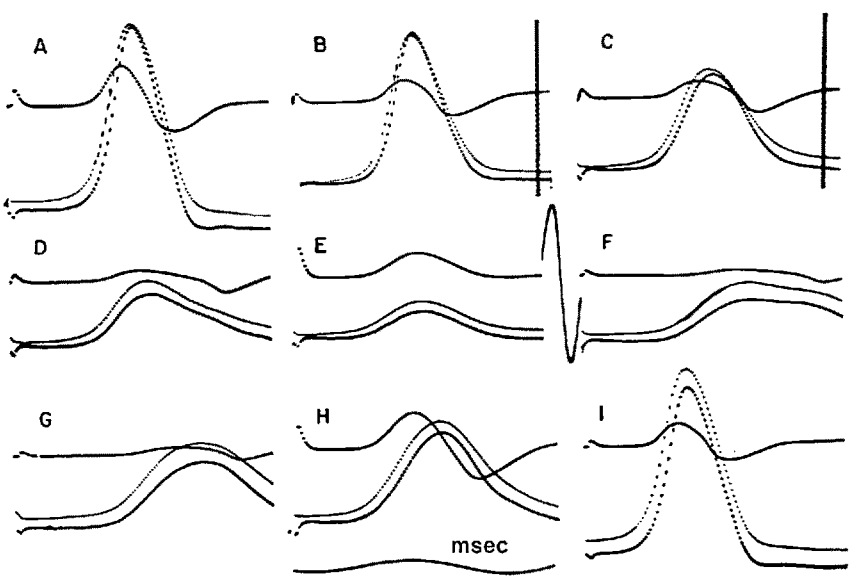

Fig. 1.—Membrane potentials simultaneously recorded with KCl and Sb microelectrodes inserted into loci less than 1 mm apart of squid giant axon. *Upper trace:* Zero potential base line for both microelectrodes prior to impalement; also carries the record of diphasic spike, propagating into and out of impaled region. *Two traces below:* KCl and Sb electrodes, respectively. *A*, initial record. *B*, within first second after adding 0·54 *M* KCl to impalement region. Signal marker indicates the addition of the KCl. *C*, fifth second. *D*, at 10 s, prior to block of conduction; *E*, at 15 s, maximum block. *F* to *I*, recovery at 25, 30, 35 and 65 s, respectively. Calibration, 100 mV, between *E* and *F*.

ceased when the resting potential in the recording region fell to 40 mV (Fig. 1 *D*, *E*). Recovery of response height and of conduction (Fig. 1 *F*, *G*) were associated with a repolarization of only 2 mV (Fig. 2). The spike amplitude increased more rapidly than did the resting potential, except toward the end of recovery, when rapid repolarization brought both the resting potential and the spike almost fully back to their initial values.

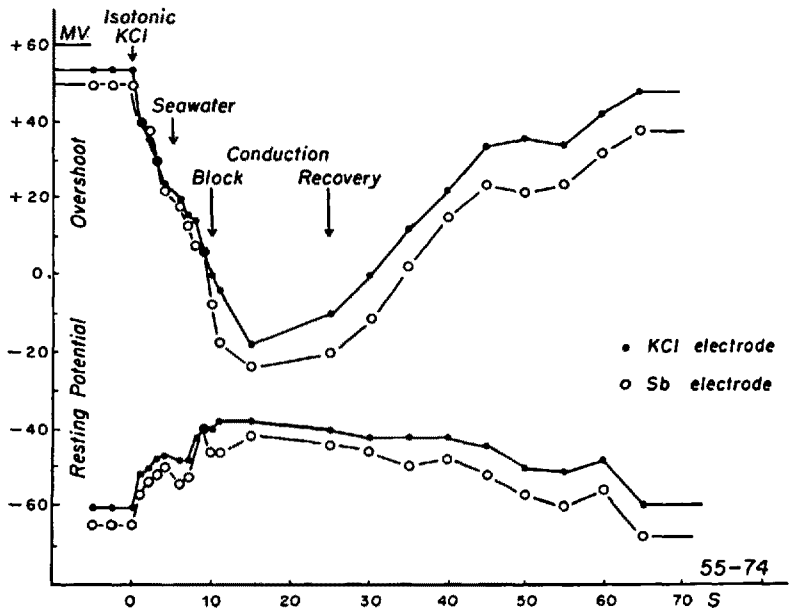

Fig. 2. – Time course of membrane potential changes and character of the response produced by changing KCl concentration of the external medium. Same experiment as in Figure 1.

Discussion. If the internal H^+ concentration were determined by a Donnan distribution, a potential of 60 mV, inside negative, recorded with the Sb microelectrode would indicate that the interior of the axon should have a pH of about 9, since that of the sea water was about 8. However, colorimetric determinations disclose an axoplasmic pH of 6·6 and 6·8 for giant axons of *Sepia* and *Loligo* respectively[4], values which should correspond to a potential of about 70 mV, inside positive. These results are not in agreement with a Donnan distribution even assuming that the potential recorded with the Sb electrode were determined by an algebraic summation of the potential of the H^+ distribution ratio and of the resting potential (of whatever independent origin) as determined by the KCl-filled microcapillary. In crab and frog muscle fibers[5], the internal pH is likewise different from that calculated on the basis of the Donnan ratios.

Conceivably, in the axoplasmic medium, the Sb electrode failed to serve as a pH electrode, despite the fact that before insertion into and after removal from the axon it recorded H^+ activity reversibly. An alternative interpretation[6], made more likely by the nearly identical potentials recorded with the different electrodes, is that the resting potential is not due to a Donnan potential resulting from the asymmetrical distribution of ions, but is a consequence of active processes in the membrane.

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Résumé

Les enregistrements pris simultanément des axones géants de *Loligo* au moyen de microélectrodes de KCl et de l'antimoine donnent à peu près les mêmes valeurs pour le potentiel de repos et pour la pointe. Les deux microélectrodes enregistrent d'une façon identique les changements de potentiel de la membrane pendant la dépolarisation avec KCl et pendant la restitution. Bien que les microélectrodes de Sb restent toujours réversibles à H^+, les potentiels enregistrés ne correspondent pas à la distribution de H^+. Ces données, comme d'autres, indiquent que le potentiel de repos n'est pas une conséquence des distributions de Donnan, mais plutôt, selon toute probabilité, un effet des propriétés de la membrane électrogène.

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