Addendum

While this book was in press some new important data appeared concerning the molecular mechanisms of the ionic permeability changes.

1. An agent was found which is able to increase specifically the resting sodium permeability of the nerve and muscle fibers. This agent is batrachotoxin(BTX)-steroidal alkaloid extracted from the skin secretion of the Colombian arrow poison frog. The increase of g_{Na} (to about 10% of $\bar{g}_{Na}$) leads to irreversible membrane depolarization. In squid giant axon the resting potential was eventually reversed by as much as 15 mV. The depolarization progressed more rapidly with internal application than with the external application of BTX to the membrane (550-1100 nM). External application of 1000 nM tetrodotoxin (TTX) completely restored the BTX depolarization. Despite the increase of resting P_{Na}, the BTX-poisoned membrane was still capable of undergoing a large permeability increase of normal amplitude upon depolarization provided the membrane potential was brought back to the original level. The action potential induced in such condition has a large and long-lasting negative after-potential (Narahashi, Albuquerque, and Deguchi, 1971). The presence of certain sulfhydryl and disulfide groups in the lobster axon membrane is essential for the action of BTX: previous treatment of the axon membrane with different sulfhydryl reagents resulted in a reduction of BTX depolarizing action (90%). These data strongly suggest the idea that BTX interacts with membrane protein.

The possible site of action of BTX is not the same as the site of action of TTX. This follows from the fact that pretreatment of the axon with sulfhydryl reagents does not change the effect of TTX.

2. The role of the protein components in the mechanism of membrane permeability changes was revealed quite distinctly in the experiments of Rojas and Armstrong (1971) on internally perfused squid axons (Dosidicus gigas). They found that the addition of 0.3-1.0 mg/ml pronase to the perfusion medium completely eliminates the sodium inactivation. Thus, after pronase treatment there is an abnormal sodium current that lasts as long as depolarization is maintained. TTX eliminates this current.

The results support the suggestion that inactivation depends on the intactness of a membrane protein.

3. Fox and Stämpfli (1971) have shown that ultraviolet radiation (wavelength 280 nm) induces an irreversible blockage of sodium channels . At 280 nm the sodium channels seem to be at least six times more sensitive than the potassium channels. The decrease of I_{Na} follows an exponential relation to the irradiation dose. In terms of target theory the single exponential relation of the dose–effect curve means that the irradiation effect is a one-hit event restricted to one target area. The volume of this area is estimated to be 200 $Å^3$. The spectral sensitivity of the sodium channels to ultraviolet radiation is expressed by a curve which is very similar to the ultraviolet absorption curve of the protein (maximum at λ = 280 nm) (Fox, 1972).

The above-mentioned data of Rojas and Armstrong (1971) and of Fox (1972) favor the view of separate sodium and potassium pathways in excitable membranes.

4. The relative permeabilities of sodium channels to different metal and organic cations were studied in medullated nerve fibers (Hille, 1971, 1972). The measured reversal potential of the early ionic current and the Goldman equation were used to calculate permeability ratios. The main results are summarized in Table 4. Special experiments have shown that methyl and methylene groups render organic cations impermeant. This fact is explained on geometrical grounds assuming that the sodium channel is an oxygen-lined pore about 3 Å by 5 Å in cross section; one pair of oxygens is assumed to be an ionized carboxylic acid.

TABLE 4. Permeability Ratios for All Measurably Permanent Monovalent Cations in Sodium Channel of Frog Node (Hille, 1972a)

Ion	P_{ion}/P_{Na^+}
Sodium	1.0
Hydroxylamine	0.94
Lithium	0.93
Hydrazine	0.59
Thallium	0.33
Ammonium	0.16
Formamidine	0.14
Guanidine	0.13
Hydroxyguanidine	0.12
Potassium	0.086
Aminoguanidine	0.06

5. Protonation of an anionic group in the sodium channel at low pH (<6) induces a selective blockage of this channel. In the voltage-clamp experiments performed on Ranvier nodes Woodhull and Woodbury (1972) found that this blockage is voltage-dependent, being much stronger at E = 0 mV than at E = +160 mV. They assumed that H^+ ions can bind to sites within open sodium channels with voltage-dependent binding and unbinding rates obeying Eyring's rate theory. Positive internal potentials decrease H^+ binding by repelling H^+ ion binding sites. A new model has been proposed to account for this blockage of the sodium channel (Woodhull, 1972).

6. The ionic permeability of potassium channels of the Ranvier node membrane was investigated by Hille (1972). Relative permeabilities to test cations were calculated from reversal potentials using the Goldman equation. The main results are summarized in Table 5. The potassium channel, according to Hille, is much narrower than the sodium channel and therefore requires more fully dehydrated ions. The pore is physically too small for Cs^+ and too large for the close contact with Li^+ and Na^+ needed to offset the work of dehydration of these small ions.

TABLE 5. Permeability Ratio for Monovalent Cations in Potassium Channel (Hille, 1972b)

Ion	P_{ion}/P_{K^+}
Lithium	< 0.1
Sodium	< 0.05
Potassium	1.0
Thallium	2.80
Rubidium	2.96
Ammonium	0.13
Cesium	< 0.1

7. The specific late Ca^{++} channels in squid axon membrane were discovered by Baker, Hodgkin, and Ridgway (1971). These channels are sensitive to the blocking action of Mn^{++} ions and insensitive to TTX, just like other calcium channels in the presynaptic ending, myocardial tissue, slow muscle, some molluscan neurons, etc.