SHORT NOTE

THE MECHANISM OF CELLULAR RESTING POTENTIAL ACCORDING TO THE ASSOCIATION-INDUCTION HYPOTHESIS AND THE PERFUSED SQUID AXON: CORRECTING A MISREPRESENTATION

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In 1968, in the lead article of the 48th volume of *Physiological* Reviews,' P. C. Caldwell cited work on internally perfused squid axon as creating "difficulties for theories due to . . . Ling (185-187 [in this paper, ref. 2-41)." Caldwell stated in his article: "The difficulties raised for Ling's fixed-charge hypothesis (185-187) are . . . fairly fundamental. This hypothesis implies that ions are held in cells in concentrations differing from those in the external medium by selective interaction with fixed, charged sites on cytoplasmic proteins rather than by selective permeability processes and active transport in the cell membrane. It is very difficult to see how this hypothesis could be made to account for the apparently normal resting and action potentials of perfused squid axons from which the natural axoplasm has been removed, since the perfusion fluids are simple salt solutions containing no large polyanions capable of selective adsorption of cations."

In the years following the publication of his article, Caldwell's criticism has often and sometimes quite faithfully been echoed by other reviewers and scientists (e.g., ref. 5 and 6) and has, therefore, been considerably influential in the adverse opinions about the Association-Induction Hypothesis. It is the purpose of this note to point out that these difficulties found by Caldwell arose entirely from his failure to grasp the essence of the theory he criticized.

The three publications bearing my name and cited by Caldwell in his review were presented over a period of ten years. The first, published in 1952, introduced the hypothesis that later gained the name of the "Fixed-Charge Hypothesis"; the second, published in 1960, gave the hypothesis a revised name, the "Fixed-Charge-Induction Hypothesis"; in the last cited publication, my 1962 monograph, the hypothesis assumed its final title, the "Association-Induction Hypothesis." The name "Fixed-Charge Hypothesis" had been abandoned for eight years by the time Caldwell published his review. However, his misunderstanding was not limited to the use of an outdated name.

The Fixed-Charge Hypothesis was primarily concerned with the mechanism of selective accumulation of K^+ in living cells. The subsidiary theory of cellular electric potential was

not published until 1960 in the second article cited by Caldwell; its full details were also given in the last publication Caldwell cited. It is extremely difficult to understand how Caldwell, having cited all three publications, should fail to know where the theory was presented and, more seriously, what the theory is.

The electric potential (ψ) according to the Association-Induction Hypothesis is fully expressed as (see ref. 3 and **4,** also ref. 7-9):

$$\psi = \text{constant} - \frac{RT}{F} \ln \sum_{i}^{n} K_i [C_i]_{ex} , \qquad (1)$$

or more specifically,

$$\psi = \text{constant} - \frac{RT}{F} (K_{K^+}[K^+]_{ex} + K_{Na^+}[Na^+]_{ex}) , \qquad (2)$$

where R, F and T are the gas constant, Faraday constant, and absolute temperatures, respectively. $[C_i]_{ex}$ and K_i are the concentration of the ith cation in the external environment and its adsorption constant on anionic sites on a microscopically thin layer of the cell surface. $[K^+]_{ex}$, $[Na^+]_{ex}$, K_{K^+}, K_{Na^+} refer more specifically to the external K^+, Na^+ concentrations and their adsorption constants on cell surface anionic sites, respectively.

Equations 1 and *2* unequivocally state that the only fixed anionic sites in the living cell involved in the generation of the cellular electrical potential are those on the microscopic outer surface of the cell. In this theory, the electrical potential is most emphatically *not* directly related to the concentration of K^+, Na^+, and other ions in the cytoplasm and, therefore, it is also most emphatically unrelated to the fixed anionic sites in the bulk of cytoplasm on which most cell K^+ and some cell Na^+ are adsorbed.

To substantiate this new theory and to refute the membrane theory, which relates the potential to the ionic concentration both within and outside of the cell, I have cited a massive body of evidence that the resting potential does not depend on the internal K^+ concentration in the cell (ref. 3 and 4, see also ref. 10). The maintenance of normal resting and action potential in the perfused squid axon, whose cytoplasm has been previously removed, adds another piece of evidence in support of the Association-Induction Hypothesis but ***against*** the membrane theory rather than vice versa, as Caldwell's review incorrectly represented.

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