

# THE INDEPENDENCE PRINCIPLE

## A RECONSIDERATION

MICHAEL C. MACKEY *and* MILDRED L. MCNEEL

*From the Physical Sciences Laboratory, Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland 20014. Dr. Mackey's present address is the Department of Physiology, McGill University, Montreal, Quebec, Canada.*

**ABSTRACT** The electrodiffusion model presented in the previous paper, which specifically excludes ion-ion interactions, is analyzed for the ratio of one-way fluxes (flux ratio) as a function of the ionic driving force across the membrane. Significant deviations from the behavior expected on the basis of the Ussing relation are found. These are sufficient to explain the "nonindependent" ion movement noted in some biological flux ratio data. One-way fluxes are dependent on the ionic concentration on both sides of the membrane. The coupling of these fluxes to ionic concentrations comes from the dependence of ionic mobility and the diffusion coefficient on the equilibrium potential. It is concluded that nonindependent behavior in experimental data is not sufficient to implicate ion-ion interaction as the source of the discrepancy.

## INTRODUCTION

In a classical study Hodgkin and Keynes (1955) examined one-way ionic fluxes through the potassium channel of the squid giant axon membrane with radioactive tracers. They noted that the experimental flux ratios did not fit the form expected from the Ussing (1949) equation. Their data was empirically described by an equation of the form

$$|I_{12}/I_{21}| = \exp [n\delta e(\phi - \phi_e)/kT], \quad (1)$$

where  $\phi(\phi_e)$  is the membrane (equilibrium) potential,  $\delta$  is the membrane thickness,  $I_{12}(I_{21})$  is the ionic current from side 1(2) to side 2(1), and  $n$  is a constant. For their data,  $n \sim 3$  ( $n = 1$  from electrodiffusion theory).

The Ussing relation is obtained by integrating the Nernst-Planck equation. Customary derivations of this equation do not explicitly consider the effects of ion-ion interaction. The discrepancy between the predictions of the Ussing relation and experimental findings has been widely interpreted as evidence for the interaction of ions with each other as they cross the membrane, and as evidence against the

importance of electrodiffusion mechanisms in determining movement through the potassium channel.

Mackey (1971 *a, b*) and Mackey and McNeel (1971) have examined an extended electrodiffusion model for ion movement through the plasma membrane. This model considers the effects of large electric field strengths and specific types of ion-membrane molecule elastic collisions on ionic energy. It is capable of resolving many of the inconsistencies between previous electrodiffusion theories of membrane ion transport and experimental data (Cole, 1968). This model excludes interactions between ions.

In this paper we have examined the theoretical flux ratios for this extended electrodiffusion model. We find significant deviations in our analysis from the behavior predicted by the classically derived Ussing relation. For small values of the ionic driving potential ( $\phi - \phi_s$ ), these deviations can be related to the parameter  $n$  in equation 1. For some ranges of parameters our formulation gives results consistent with  $n < 1$ . This is in contrast to the behavior noted by Hodgkin and Keynes. Other parameter values yield results characterized by  $n > 1$ , and qualitatively allow us to account for the flux ratio characteristics of the potassium channel. The concept of the independence principle is discussed in light of these results obtained from a model without ion-ion interaction present.

All assumptions and symbols used herein are defined and discussed in Mackey and McNeel (1971).

### *Analysis of Ionic Flux Ratios*

Mackey and McNeel (1971) have shown that the net dimensionless ionic current ( $\bar{I}$ ) flowing through a model membrane, separating solutions 1 and 2, is given by

$$\bar{I} = E\bar{\mu}[\exp(\bar{\mu}E\bar{\delta}/\bar{D}) - 1]^{-1}[N_1 \exp(\bar{\mu}E\bar{\delta}/\bar{D}) - N_2]. \quad (2)$$

In equation 2,  $E$  is applied electric field strength,  $\bar{\delta}$  is membrane thickness,  $N_1$  and  $N_2$  are ionic concentrations, and  $\bar{\mu}$  and  $\bar{D}$  are the mobility and diffusion coefficient respectively.<sup>1</sup> Both  $\bar{\mu}$  and  $\bar{D}$  depend on  $E$ ,  $N_1$ , and  $N_2$ , as given in the Appendix.

From equation 2, the one-way ionic currents are given by

$$\bar{I}_{12} = E\bar{\mu}N_1 \exp(\bar{\mu}E\bar{\delta}/\bar{D})[\exp(\bar{\mu}E\bar{\delta}/\bar{D}) - 1]^{-1} \quad (3)$$

and

$$\bar{I}_{21} = -E\bar{\mu}N_2[\exp(\bar{\mu}E\bar{\delta}/\bar{D}) - 1]^{-1}, \quad (4)$$

and the flux ratio,  $R_r = |\bar{I}_{12}/\bar{I}_{21}|$ , is therefore

$$R_r = \exp\{\bar{\delta}[(\bar{\mu}E/\bar{D}) - A]\}, \quad (5)$$

<sup>1</sup> All variables and derived quantities in this section are in dimensionless form.

where

$$A = \bar{\delta}^{-1} \ln (N_2/N_1).$$

As we have shown previously, the dimensionless equilibrium field ( $E_e$ ) is given by  $E_e = A/3$ . Finally, we note that the flux ratio predicted by classical electrodiffusion theory is, in terms of the dimensionless variables used here,

$$R_F = \exp [\bar{\delta}(3E - A)]. \quad (6)$$

The dependence of  $\bar{\mu}$  and  $\bar{D}$  on  $N_1$  and  $N_2$  implies that the one-way ionic currents will be functions of ionic concentration on both sides of the membrane, which has been found experimentally by Hodgkin and Keynes (1955).

In Fig. 1, we show the flux ratio as a function of  $(E - E_e)$  for  $p = 3/4$ ,  $\bar{\delta} = 1$ ,

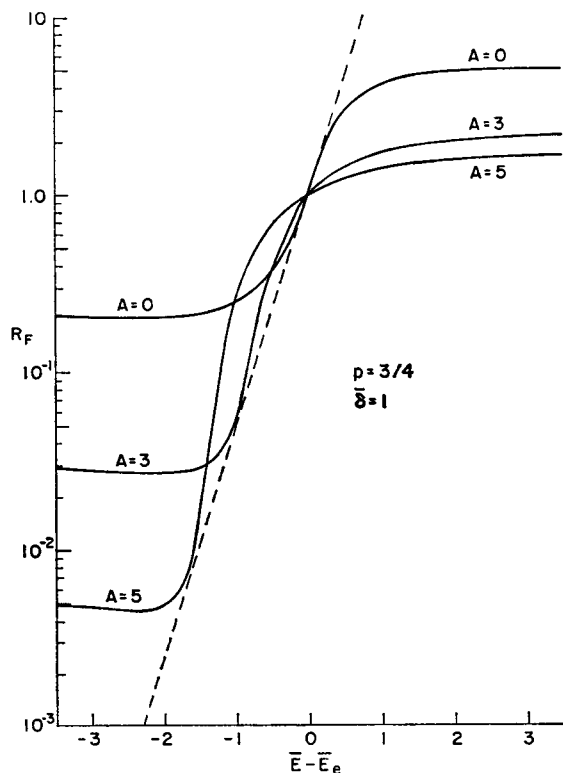


FIGURE 1 The flux ratio ( $R_F$ ) as a function of ionic driving force ( $\bar{E} - \bar{E}_e$ ) across the membrane for  $\bar{\delta} = 1$  and  $p = +3/4$ . The computations, based on equation 5, are shown for no concentration gradient ( $A = 0$ ) and concentration ratios of  $\sim 20.1$  ( $A = 3$ ) and  $\sim 148$  ( $A = 5$ ). The  $R_F$  vs.  $(\bar{E} - \bar{E}_e)$  curve predicted by the Ussing relation is shown as a dashed line for comparison.

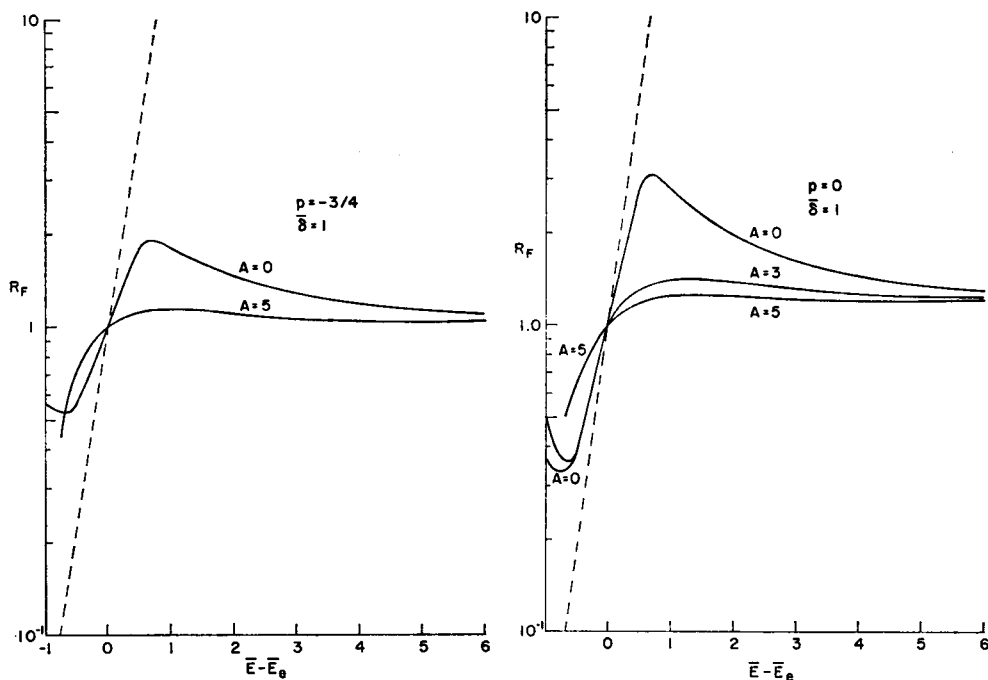


FIGURE 2 *a*

FIGURE 2 *b*

FIGURE 2 As in Fig. 1, but with  $p = -3/4$  and  $p = 0$  and only two values of the concentration ratio.

and three different values of  $A$ . For comparison the classical result, given by equation 6, is shown as a dotted line. The predictions of the electrodiffusion model we consider are quite different from those of classical electrodiffusion theory. For small departures of  $(E - E_0)$  from zero, the computed results may be fit by an equation of the form of equation 1, with  $n < 1$ . This is opposed to the result obtained by Hodgkin and Keynes.

In Figs. 2 *a* and 2 *b* we illustrate theoretical flux ratios as functions of  $(E - E_0)$ , with  $\delta = 1$ , several values of  $A$ , and  $p = -3/4$  and  $p = 0$  respectively. The curves are generally similar to those found with  $p = 3/4$ . The comments about fitting the theoretical curves with equation 1 apply again.

The computed results presented in Figs. 1, 2 *a*, and 2 *b* show that flux ratios obtained from an electrodiffusion model of ion transport need not have the form predicted by equation 6 if interaction between ions is negligible. Our model shows behavior departing significantly from the theoretical expectations of the classical formulation. Yet, as pointed out above, ion-ion interactions are excluded.

What is the significance of the fact that, with  $\delta = 1$ , all of the flux ratio vs.  $(E - E_0)$  curves computed for our model have a slope about  $E = E_0$  that is less than the classical prediction? That is, the curves in the neighborhood of  $E = E_0$  can be fit by

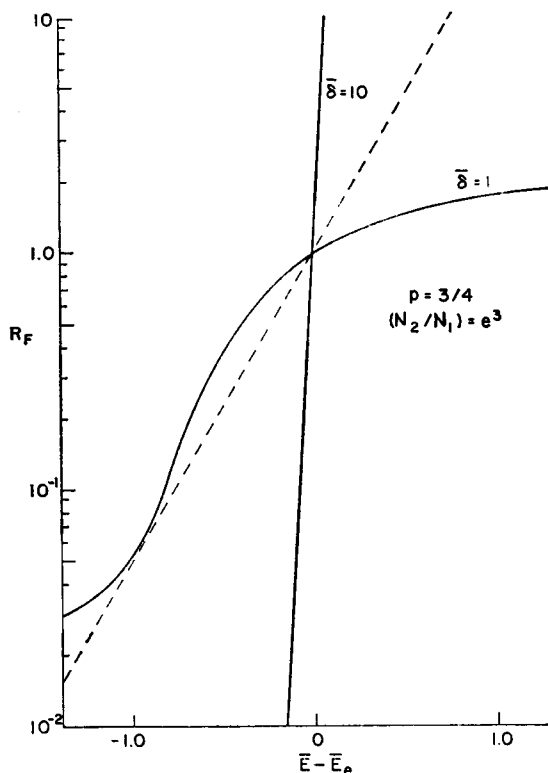


FIGURE 3 The influence of the parameter  $\bar{\delta}$  on computed  $R_F$  vs.  $(\bar{E} - \bar{E}_e)$  curves with a fixed concentration ratio of  $\sim 20.1$  ( $= e^3$ ). Alterations of  $\bar{\delta}$  rotate the curve about the origin, and are capable of qualitatively matching experimentally observed behavior.

equation 1 with  $n < 1$ . In Fig. 3, we illustrate the effect of changing  $\bar{\delta}$  on the flux ratio vs.  $(\bar{E} - \bar{E}_e)$  curves for  $p = 3/4$  and  $(N_2/N_1) = 20.09$  (corresponding to  $A = 3$  when  $\bar{\delta} = 1$ ). By varying the value of  $\bar{\delta}$  sufficiently we are able to overcome the discrepancy noted above and produce curves that, around  $\bar{E} = \bar{E}_e$ , can be fit by equation 1 with  $n > 1$ . Similar results are found for all values of  $p$ , but are not reproduced here.

Therefore, flux ratio characteristics similar to those noted in squid giant axon membrane may be qualitatively accommodated by our model. Although interionic interaction during ion movement through the plasma membrane may be sufficient to yield flux ratio data deviating from the Ussing relation, it is not necessary. Such deviations may be a result of the nonlinear way in which the driving field  $(\bar{E} - \bar{E}_e)$  alters the Einstein relation and ionic mobility, and thus affects one-way fluxes.

## APPENDIX

### *Expressions for $\bar{\mu}$ and $\bar{D}$*

We have shown previously (Mackey and McNeel, 1971) that for our model the mobility and

diffusion coefficient are given by

$$\mu = \left( \frac{3-p}{3} \right) \left[ \int_0^\infty duu^2 \exp(-W) \right]^{-1} \int_0^\infty duu^{2-p} \exp(-W)$$

and

$$D = \left[ 3 \int_0^\infty duu^2 \exp(-W) \right]^{-1} \int_0^\infty duu^{4-p} \exp(-W)$$

respectively. In these expressions  $p$  is a parameter characteristic of the ion-membrane molecule collision frequency and

$$W = 3 \int_0^u ds \frac{s^{2p} + 2EE_e}{s^{2p} + 2E^2}.$$

$\bar{E}_e = (3\delta)^{-1} \ln(N_2/N_1)$  is the equilibrium field.

Received for publication 5 January 1971 and in revised form 16 April 1971.

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