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On the Theory of Ion Transport Across the Nerve Membrane, II. Potassium Ion Kinetics and Cooperativity (with $x = 4$)

(Hodgkin and Huxley/Cole and Moore)

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ABSTRACT **We use a tetrahedral model of four interacting protein subunits to represent the K^+ channel or gate in the squid nerve membrane. The kinetic predictions, with varying degrees of cooperativity, are compared with experimental observations, especially those of Hodgkin and Huxley (*J. Physiol. 117*, 500, 1952) and of Cole and Moore (*Biophys. J. 1*, 1, 1960). The tentative conclusion reached is that if there is any cooperativity present it must be rather weak. There is no indication here that cooperativity improves the Hodgkin–Huxley assumption of independent "subunits". Other related models will be discussed in Part III. We also find evidence against the suggestion that there is cooperativity *between* K^+ channels arranged in patches of a two-dimensional lattice.**

In our first work in this field (1, 2), we were concerned with K^+ steady-state negative resistance at high external $[K^+]$. We used a model which included cooperativity between K^+ channels, and K^+ transport across the membrane was presumed to occur via binding to sites. We now believe that both of these features are very unlikely, though perhaps not completely ruled out: both Na^+ and K^+ channels are apparently very far apart, on the average, and almost certainly independent of each other (3–5); if it is argued that Na^+ and K^+ channels might occur in widely separated small (cooperative) patches, then there appear to be serious problems with the K^+ kinetics (see below); and transport via binding to sites almost always leads to nonlinear (6) instantaneous current curves, whereas approximately linear experimental curves are found for both Na^+ and K^+ in the squid membrane under normal (7) and some other conditions (8).

We turned next (9; see also *Biophys. Soc. Abstracts*, 1971, p. 139a) to a model with independent and dispersed Na^+ and K^+ channels, but with possible cooperativity *within* (rather than *between*) channels. This seemed a reasonable hypothesis because: a channel (or the "gate", if there is a distinction) could very well be a protein complex (9), and such complexes usually exhibit cooperativity between subunits; and cooperativity might be an aid in understanding both the steep Hodgkin–Huxley, or HH (10) $n_\infty(V)$, $m_\infty(V)$, and $h_\infty(V)$ curves (V = absolute membrane potential) and also the familiar (10, 11) initial "induction" behavior observed in both K^+ and Na^+ conductance versus time curves on depolarization from the rest potential or from hyperpolarizing potentials.

The model is outlined in much more detail in Part I (9). Our main object in the present paper and in the forthcoming Part III is to compare, in a few special cases, the consequences of cooperativity within a channel (or gate) with the qualitative features of observed K^+ kinetic curves. Our results are pertinent also for models with cooperativity *between* channels (2, 12). The conclusion we reach (below, and confirmed in Part III) is that there is probably little, if any, cooperativity of either kind (for K^+)—though this cannot be considered a rigorous deduction. We have not done enough work on Na^+ kinetics yet to be able to make any comment about the Na^+ case.

Having at least tentatively abandoned a significant degree of cooperativity between or within channels (on the basis of results in this paper and in Part III), our present working hypothesis is that a K^+ channel (or gate) is a protein complex of essentially independent ($w \cong 0$, below) subunits, x in number. The channel opens or closes by means of a V-dependent subunit conformational change (9). In a formal way, this is of course identical with the original HH empirical analysis for K^+ ($x = 4$). In Part IV we will discuss the considerable improvements of the HH K^+ early induction fit which can be obtained simply by lowering the $n_\infty(V)$ curve near the rest potential (the small steady-state K^+ current thereby "lost" must be accounted for in another way). (We also consider subunit numbers x other than four.) At the same time, we will present a model which extends the HH approach into the hyperpolarization region and accounts for the Cole–Moore delay time as a function of initial V (13). The basic idea is that hyperpolarization brings into play a multi-step (of the order of 4–8 steps) process in each subunit which strongly reduces the equilibrium constant for the subunit conformational change ($i \rightarrow ii$, below) necessary for channel opening. The process might be, for example, binding of a positive ion to the subunit, desorption of a negative ion or disaggregation of the subunit. The Cole–Moore delay (on depolarizing after prior hyperpolarization) may then be attributed to the time required to reverse the above process so that the usual (HH) conformational change can take place. Thus the Cole–Moore (13) single-process exponent 25 is in effect generalized to a two-process 4 (HH) $\times$ 4, or 4 $\times$ 6, etc., with different rate constants in the two processes. This seems to provide an acceptable physical basis for the large exponents explored first by Cole and Moore.

We have already done some preliminary work on the theory of the V-dependence of a protein conformational equilibrium constant (2, 9), but to proceed further we need first to settle on a particular experimental $n_\infty(V)$ curve (in the K^+ case). This depends on conclusions reached in this paper and in Parts III and IV.

Abbreviation: HH, Hodgkin–Huxley (ref. 10).

Part I of this series is ref. 9.

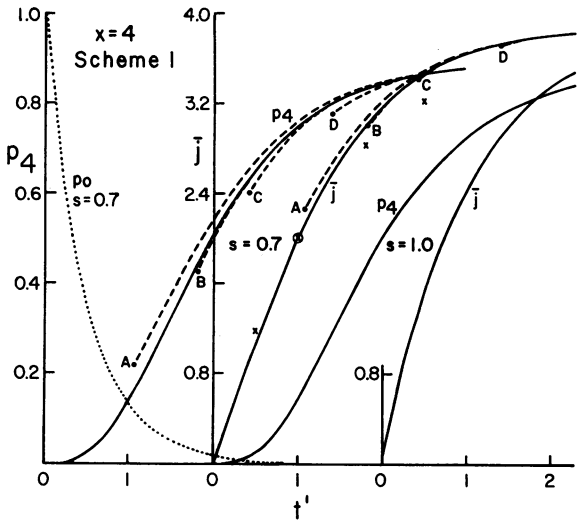

FIG. 1. Scheme 1: fraction of open channels $p_4(t')$, and $\bar{j}(t')$, for $s = 1$ (HH) and $s = 0.7$. Solid curves: depolarization $-\infty \rightarrow +50$ mV. Dotted curve $p_0(t')$ for $-\infty \rightarrow +50$ mV, $s = 0.7$. Dashed curves: depolarization to +50 mV from (*A*) −40 mV, (*B*) −25 mV, (*C*) −10 mV, (*D*) +15 mV (see text). The ×'s show a first-order process for comparison with $\bar{j}(t')$ (⊗ chosen on the curve).

COOPERATIVE KINETIC MODEL WITH $x = 4$ (TETRAHEDRAL)

We shall divide our rather negative report on cooperativity into two parts: in this paper we consider $x = 4$ with subunits arranged tetrahedrally; in Part III we consider $x = 4$ (square), models with $x > 4$, aggregation of subunits as a possible alternative to a conformational change, and related topics. These systems (except for aggregation) are small kinetic "Ising" (14) systems; if x is not too large (say $x \leq 10$), they can be treated exactly, as in this paper. For larger x, Monte Carlo methods may be used (15) to avoid approximations such as that of Bragg-Williams (BW) (1, 2, 12).

Let us suppose that a K^+ channel (or gate) contains four identical protein subunits, arranged in a tetrahedron. Each subunit can be in one of two conformations, *i* and *ii*. There is room for a K^+ ion to pass through the four subunits only if *all* are in conformation *ii* (as in HH). The conformational change for a hypothetical isolated (i.e., independent) subunit follows first-order kinetics with intrinsic rate constants α (for $i \rightarrow ii$)

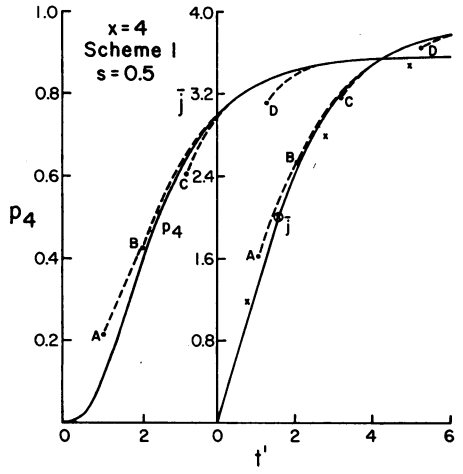

FIG. 2. Scheme 1, $s = 0.5$; otherwise as in Fig. 1.

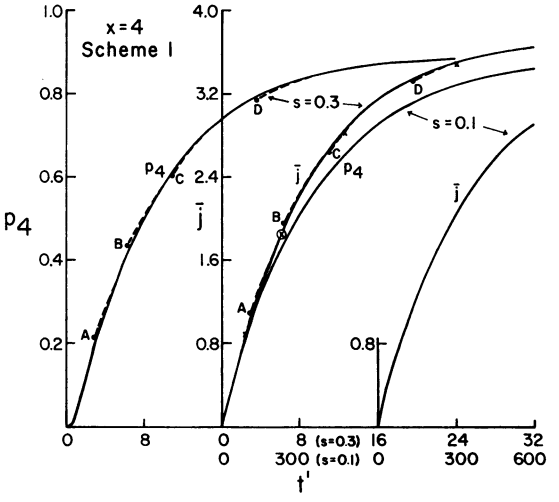

FIG. 3. Scheme 1, $s = 0.3$ and 0.1; otherwise as in Fig. 1.

and β (for $ii \rightarrow i$). (We drop the HH subscript n here because only K^+ is being considered.) Let w_{11} be the interaction energy (or free energy) between two subunits in state *i*, w_{12} between a *i* and a *ii*, etc. There are six pair interactions in the system (of four subunits). Let Q_1^4 be the (canonical) partition function for the system in state [0] and Q_2^4 for state [4], where $[j]$ denotes the state with j subunits in state *ii*. Then the probabilities (i.e., fractions of channels) p_j^e of the five states, at equilibrium, are

$$p_0^e = Q^4\xi^{-1},\ p_1^e = 4s^3Q^3\xi^{-1},\ p_2^e = 6s^4Q^2\xi^{-1},\ p_3^e = 4s^3Q\xi^{-1},\ p_4^e = \xi^{-1} \quad (1)$$

where

$$\xi = Q^4 + 4s^3Q^3 + 6s^4Q^2 + 4s^3Q + 1$$

$$Q = Q_1/Q_2 = \beta/\alpha$$

$$s = e^{w/2kT},\ w = w_{11} + w_{22} - 2w_{12}.$$

The interaction parameter s corresponds to y^2 in Part I (9).

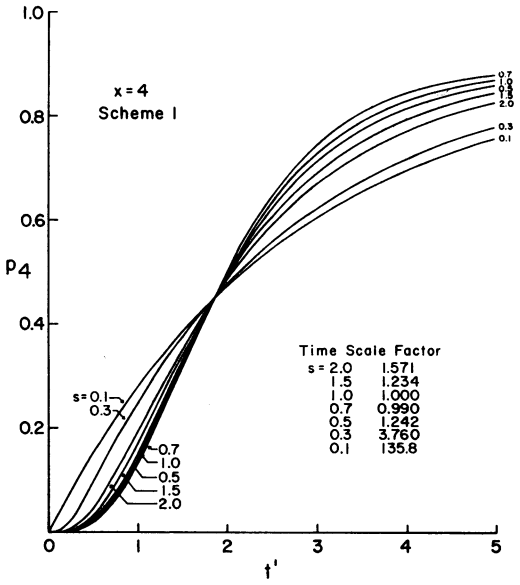

FIG. 4. Scheme 1: $p_4(t')$ for various values of s, with time scales adjusted so that curves cross at $p_4(\infty)/2$, $t'_{1/2} = 1.8385$.

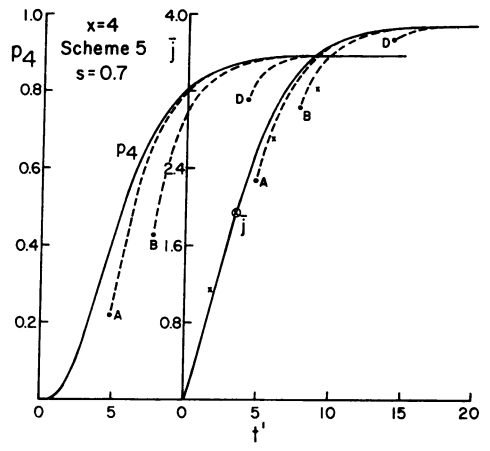

FIG. 5. Scheme 5, $s = 0.7$; otherwise as in Fig. 1.

Although α, β, and Q are functions of V, we assume for simplicity that w is not. For example, the predominant interaction might be a simple steric repulsion (poor fit) between a subunit in state i and another in state ii ($w_{12} > 0$). "Cooperativity" occurs when $w < 0$, $s < 1$. The HH case is $w = 0$, $s = 1$, $Q = (1 - n_\infty)/n_\infty$, $p_4^e = n_\infty^4$.

Q is the intrinsic i/ii partition function ratio, or equilibrium constant (for $ii \rightleftarrows i$). In a depolarization experiment, for example, we start with an ensemble of these independent systems (channels) at equilibrium with a given (initial) Q and observe, via $p_4(t)$—the fraction of open channels—the time evolution of the ensemble to a final equilibrium at a different (final) Q. Eq. 1 gives initial and final probability distributions.

An indefinite number of kinetic schemes can be consistent with this equilibrium model. One of the most likely is the "symmetrical" scheme

$$[0] \underset{\beta s^{-3/2}}{\overset{4\alpha s^{3/2}}{\rightleftarrows}} [1] \underset{2\beta s^{-1/2}}{\overset{3\alpha s^{1/2}}{\rightleftarrows}} [2] \underset{3\beta s^{1/2}}{\overset{2\alpha s^{-1/2}}{\rightleftarrows}} [3] \underset{4\beta s^{3/2}}{\overset{\alpha s^{-3/2}}{\rightleftarrows}} [4] \text{ (Scheme 1)} \tag{2}$$

which assumes that the activated complex between i and ii has intermediate interaction properties. An alternative is (only s factors shown—see Eq. 2)

$$\begin{matrix} s^3 & s & 1 & 1 \\ 1 & 1 & s & s^3 \end{matrix} \quad \text{(Scheme 2)} \tag{3}$$

If the predominant interaction is a ii–ii attraction ($w_{22} < 0$), it is convenient to redefine Q_1 and Q_2 so that the equilibrium probabilities are proportional to (ordered as in Eq. 1)

$$Q^4, 4Q^3, 6s^{-2}Q^2, 4s^{-6}Q, s^{-12}. \tag{4}$$

Then s^3Q here is equal to the Q of Eq. 1. Two possible kinetic schemes are then

$$\begin{matrix} 1 & s^{-1} & s^{-2} & s^{-3} \\ 1 & s & s^2 & s^3 \end{matrix} \quad \text{(Scheme 3)} \tag{5}$$

and

$$\begin{matrix} 1 & 1 & 1 & 1 \\ 1 & s^2 & s^4 & s^6 \end{matrix} \quad \text{(Scheme 4)} \tag{6}$$

Similarly, if a i–i attraction dominates ($w_{11} < 0$), we again redefine Q_1 and Q_2 so that Eq. 4 becomes

$$s^{-12}Q^4, 4s^{-6}Q^3, 6s^{-2}Q^2, 4Q, 1 \tag{7}$$

and Eq. 6 becomes

$$\begin{matrix} s^6 & s^4 & s^2 & 1 \\ 1 & 1 & 1 & 1 \end{matrix} \quad \text{(Scheme 5)} \tag{8}$$

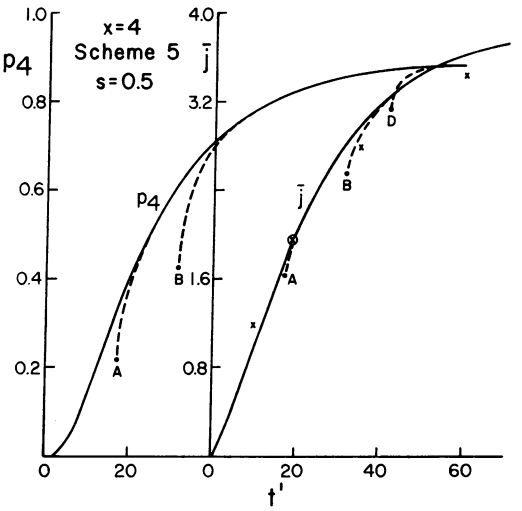

FIG. 6. Scheme 5, $s = 0.5$; otherwise as in Fig. 1.

The kinetic behavior of the ensemble of channels is governed by a set of five (four independent) linear first-order differential equations in the p_j's which can be solved on a computer by standard matrix methods. For example, in Scheme 1 we use for p_0 the dimensionless equation

$$dp_0/dt' = bs^{-3/2}p_1 - 4as^{3/2}p_0 \tag{9}$$

where

$$t' = (\alpha + \beta)t,\ a = \alpha/(\alpha + \beta) = (1 + Q)^{-1},\ b = Q(1 + Q)^{-1}. \tag{10}$$

The Q here is of course the *final* Q.

This formalism is also applicable to models with interactions between K^+ channels, as in Adam's work (12). A "system" is now a patch of x channels and the "ensemble" is a large collection of independent patches. Conformation i becomes a closed channel; conformation ii is an open channel. Whereas in our model (following HH) the fraction of open channels is p_4, in the Adam kind of model it would be $\bar{j}/4$, where $\bar{j} = \sum_j j p_j$. We shall note the behavior of $\bar{j}(t)$ as well as $p_4(t)$, below. Incidentally, tetrahedrally arranged subunits are identical topologically (in counting interactions) with a 2×2 hexagonal patch (of channels) with periodic boundary conditions (15) (to simulate a larger patch). Each interaction is doubled in the 2×2 case. Adam's work (BW approximation) corresponds to Scheme 5. When $s = 1$, $p_4(t) = n(t)^4$ and $\bar{j}(t) = 4n(t)$, where $n(t)$ is the HH function. We consider Scheme I first.

CALCULATIONS

We shall concentrate on Schemes 1 and 5, which represent the extremes of kinetic symmetry and asymmetry. We are especially concerned with two experimentally observed properties: the extremely good superposition of K^+-current versus time depolarization curves with various starting points but the

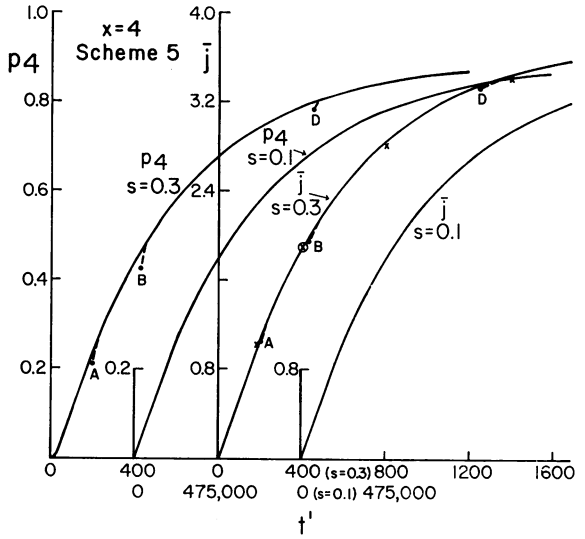

FIG. 7. Scheme 5, $s = 0.3$ and 0.1; otherwise as in Fig. 1.

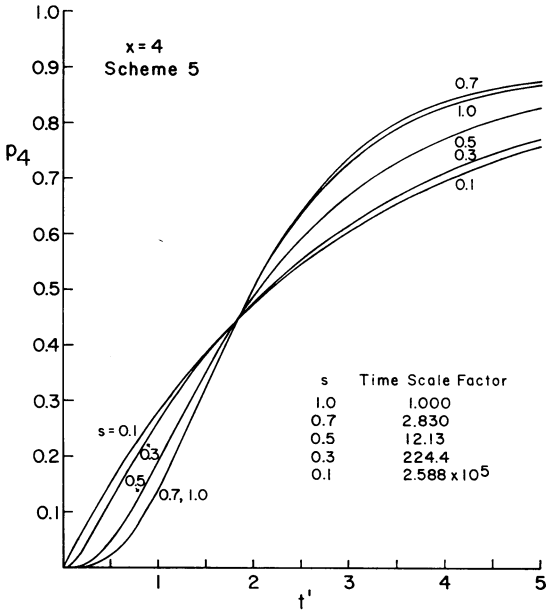

FIG. 8. Scheme 5; otherwise as in Fig. 4.

same *final* state ($V = E_{Na}$), found by Cole and Moore (13); and the initial K^+ induction behavior referred to in the introduction. We consider Scheme 1 first.

Figs. 1 to 3 show simulated Cole–Moore depolarization K^+-current curves in the form of $p_4(t')$ or $\bar{j}(t')$ (Adam), for various values of s. The final (equilibrium) state in all cases corresponds to $p_4(\infty) = 0.8945$, which is the HH value of n_∞^4 at $V = E_{Na} = +50$ mV. The solid curves all start from $p_0(0) = 1$ (saturation hyperpolarization for this model), and hence exhibit the best possible induction behavior (near $t' = 0$). The dashed curves start from *equilibrium* states with $p_4(0) = 0.2121$, 0.4228, 0.5960, and 0.7755, these being the HH n_∞^4 values corresponding to conditioning ($t' < 0$) potentials $V = -40$ mV, -25 mV, -10 mV, and $+15$ mV (rest potential $= -65$ mV). The dashed curves have been translated along the time axis to achieve superposition with the solid curves at large times. The early superposition in the $p_4(t')$ curves is seen to be bad for $s = 0.5$ and 0.7 ($s = 0.4$ is worse) but rather good for $s = 0.3$. The $\bar{j}(t')$ superposition is always better than the corresponding $p_4(t')$ superposition. Superposition is mathematically exact (13) for $s = 1$ (HH) and virtually exact (on this scale) for $s = 0.1$. The early induction behavior is missing for all s in the $\bar{j}$ curves and for $s = 0.1$ in p_4; the amount of induction in p_4 is inadequate for $s = 0.3$. The induction behavior for p_4 is summarized in Fig. 4, in which the time scales have been adjusted so that all curves pass through the point $p_4 = p_4(\infty)/2$, $t'_{1/2} = 1.8385$ ($s = 1$ scale). Cooperativity does not enhance the induction, as we had expected; the major effect (for moderate s) is on the time scale.

Incidentally, $s = 0.7$ corresponds to $w = -0.40$ kcal/mol (at 6°C) and $s = 0.1$ to $w = -2.56$ kcal/mol. Note that if, as is likely, $w \cong -2w_{12}$, then $-w_{12}$ is one-half of the above values. Hence $s = 0.7$ represents a rather weak interaction.

Figs. 5–8 show corresponding results for Scheme 5. Note the more extreme time scales. The $s = 1$ curves are, of course, the same as in Fig. 1. Superposition is generally much worse, but it is again virtually exact for $s = 0.1$. There is a slight inflection in $\bar{j}$ for intermediate s, but no induction.

DISCUSSION

In these examples (and in others in Part III), the model with interactions *between* channels does not produce the required induction behavior in $\bar{j}(t')$. Where there is a hint of induction (i.e., an inflection at small t' for intermediate s values in Scheme 5), superposition in $\bar{j}$ is not good. These results bespeak difficulties for the Adam model, but we reserve further comment until Part III where we can compare several values of x.

Concerning our model with cooperativity *within* a channel, the $p_4(t')$ curves indicate (and this is confirmed in Part III) that a moderate amount of cooperativity ($s = 0.7$, say) leads to failure of superposition (beyond experimental error) while more extreme cooperativity ($s = 0.1$ or 0.3, say) restores superposition but eliminates all or most of the induction. It appears, therefore, that if there is cooperativity in this kind of model, it must be rather weak (additional calculations on Scheme 1 indicate that $s = 0.8$, $w = -0.25$ kcal/mol would be acceptable). Thus one is led back to the original HH assumption of essentially independent subunits.

It is interesting that Hoyt (16) found that Cole–Moore superposition argues against Na^+–K^+ coupled models; we find that the same data argue against significant cooperativity.

The reason for the failure of superposition (with moderate cooperativity) is straightforward. Exact superposition will be a property of any model in which the ensemble, in relaxing from an initial equilibrium state to a different final equilibrium state, passes on the way *through equilibrium states only*. This occurs in the HH case ($s = 1$): an equilibrium distribution of the p_j's is a binomial distribution (Eq. 1); the ensemble starts in a binomial distribution, ends in another binomial distribution, and passes through intermediate binomial distributions only (17). An equivalent statement: initial, final, and intermediate states can all be specified by the value of a single time-dependent parameter [$n(t)$ in the HH case, or the "equilibrium constant" $Q(t) = (1 - n)/n$] (13). Incidentally, simple BW approximate kinetics (12) will (incorrectly) predict superposition because only a single parameter is involved in the rate equation.

However, when cooperativity is included, an examination of the computed $p_j(t)$'s shows immediately that, in general, although the system starts and finishes with equilibrium sets of p_j's (no longer binomial; Eq. 1), intermediate sets of p_j's—

i.e., at arbitrary times—do not correspond to *any* equilibrium distribution. That is, a single parameter $Q(t)$ no longer suffices to determine all the $p_j(t)$'s and thus to characterize an intermediate state of the ensemble: we need to specify the whole set of p_j's to do this. Thus the solid curves in the figures are nonequilibrium kinetic "tracks"; each dashed curve starts at an equilibrium *point* but itself goes onto a nonequilibrium track which merges in due course with the solid track. This behavior of small kinetic Ising systems, treated exactly, and especially the time dependence of fluctuations and of the extent of deviation of the p_j set from the nearest equilibrium set, is an interesting statistical mechanical problem in its own right—and will be reported on separately by Dr. Edward Paul and one of us (T.L.H.).

Superposition (in p_4 and $\bar{\jmath}$) is recovered with strong cooperativity (e.g., $s = 0.1$) because, in effect, a phase transition is now involved: p_1, p_2, and p_3 are all very small; $p_0(t') + p_4(t') \cong 1$; as the ensemble evolves with time, "phase" [0] is converted into "phase" [4] by slow first-order kinetic "leakage" through intermediate states, [0] $\rightleftarrows$ [4]; the single parameter which characterizes the state of the ensemble and which "produces" superposition is, say, p_0/p_4 (analogous to Q when $s = 1$). Note in Figs. 3 and 7 ($s = 0.1$) that $\bar{\jmath}(t') \cong 4p_4(t')$. These computed curves are easily verified to be first-order; their time constants can be independently and accurately calculated, as a check, from the mean first passage times (18) for [0] $\rightarrow$ [4] and [4] $\rightarrow$ [0] using rate constants from Eqs. **2** and **8**. A single system of the ensemble has a "nucleation" delay, or induction period, before the transition $j = 0 \rightarrow j = 4$ occurs (mean delay = mean first passage time, above). But the ensemble average of j is what would be observed (Adam model), and this exhibits first-order kinetics (the decay of a radioactive sample is very similar). Much faster first-order kinetics ($i \rightleftarrows ii$) are of course obtained for $\bar{\jmath}(t') = 4n(t')$ when $s = 1$ (Fig. 1). In Figs. 1–3 and 5–7, the $\bar{\jmath}$ curves for intermediate s depart somewhat from first order (the $\times$'s are first-order points, for comparison, based on $\bar{\jmath}(\infty)$ and a midpoint $\otimes$ on the actual curve).

We have supplemented the above with a number of other calculations on the tetrahedral model, especially repolarizations using Schemes 1, 2 and 5, and depolarization from $-65 \rightarrow -15$ mV using Schemes 1 to 4. We omit details because no really new features emerge. In the repolarizations, p_0 and p_4 merely exchange roles (compared to depolarizations; see Fig. 1), as do $\bar{\jmath}$ and $4 - \bar{\jmath}$. Typically, in a repolarization, $p_4(t')$ decreases following a more or less first-order curve, while $p_0(t')$ increases after an early induction period (if $p_0(0)$ is at or near zero and s is not too small). In the depolarizations: Scheme 1 gives results as expected from Fig. 4; Schemes 2 and 3 are very similar to Scheme 1, except for the time scale; Scheme 4 is somewhat different (it is the "inverse" of Scheme 5).

We are indebted to Drs. Gerold Adam and Max Delbrück for stimulating discussions. This work was supported in part by grants from the National Science Foundation and from the General Medical Sciences Institute of the U.S. Public Health Service.

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