DETERMINATION OF REACTION RATE CONSTANTS IN THE MAMMILLARY SYSTEM*

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It is shown that the individual rate constants can be determined for the composite chemical system:

\[ A + B_i \rightleftharpoons C_i; \quad i = 1 \ldots N \]

with only measurements of the unbound species, \( A(t) \), required. The dissociation rate constants can be determined by direct analysis of a single steady state tracer study. The association constants then follow from the analysis of stable equilibrium determinations reported earlier (Hart, 1965). An approximate solution when tracer methods are inapplicable is also given.

I. Introduction. Determination of the stability constants and maximum binding capacities in mammillary systems making use of unbound species determinations alone has been reported previously (Hart, 1965).† The methods outlined involve analysis of the equilibrium values obtained in a series of experiments. In many cases of interest, however, it may also be desirable to evaluate the association and dissociation rate constants separately.

In Section II, it will be shown that complete determination of the individual

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‡ If explicit knowledge of \( A(t) \), \( B_i(t) \) and \( C_i(t) \) is available then, of course, the system of equations

\[ \frac{dC_i(t)}{dt} = K_{at} \cdot A(t) \cdot B_i(t) - K_{Dt} \cdot C_i(t); \quad t = t_1, t_2 \]

permits an immediate solution for \( K_{at} \) and \( K_{Dt} \) by determinants.
reaction rates is possible by appending a single kinetic tracer study. In Section III an approximate solution for three species or less is outlined for those systems where kinetic studies on non-tracer concentrations can be carried out but where sufficiently rapid kinetic tracer determinations are not feasible.

Notations

- $K_{ai}$ = association constant of the $i$th type site; $i = 1 \ldots N$.
- $K_{Di}$ = dissociation constant of the $i$th type site; $i = 1 \ldots N$.
- $K_i$ = stability constant of the $i$th type site $= \frac{K_{ai}}{K_{Di}}$; $i = 1 \ldots N$.
- $M_i$ = mols·liter$^{-1}$ of total binding sites of the $i$th type.
- $m_i(t)$ = mols·liter$^{-1}$ of binding sites of the $i$th type unoccupied at time $t$.
- $M_i - m_i(t)$ = mols·liter$^{-1}$ of occupied sites of the $i$th type at time $t$.
- $T_j$ = equilibrium molar concentration of the unbound reacting molecule under condition $D_j$.
- $T(t)$ = mols·liter$^{-1}$ at time $t$ of the unbound reacting molecule.
- $T^*(t)$ = concentration at time $t$ of the labelled unbound reacting molecule.

II. Tracer Kinetic Method. Consider the reversible reactions:

$$T + m_i \rightleftharpoons (Tm_i); \ i = 1 \ldots N.$$ (1)

It follows directly from chemical kinetics that:

$$\frac{dm_i}{dt} = -[K_{Di} + K_{ai}(T)]m_i + K_{Di}M_i; \ \ \ i = 1 \ldots N.$$ (2)

and

$$\frac{dT}{dt} = - \sum_{i=1}^{N} K_{ai}(T)(m_i) + \sum_{i=1}^{N} K_{Di}(M_i - m_i); \ \ i = 1 \ldots N.$$ (3)

At equilibrium, (2) becomes:

$$\frac{M_i}{m_{ij}} = 1 + K_iT_j.$$ (4)

Since the $T_j$ are measured and $M_i$ and $K_i$ can be calculated, it follows that $m_{ij}$, the mols·liter$^{-1}$ of available sites of species $i$ under equilibrium condition $D_j$, can be determined.

Consider the effect of introducing a tracer $T^*$ into any equilibrium state $j$.

§ The notation conforms to that previously employed in Hart (1965).