Free Energy Transduction in Biology

The Steady-State Kinetic and Thermodynamic Formalism

TERRELL L. HILL

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BETHESDA, MARYLAND

ACADEMIC PRESS New York San Francisco London 1977

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

Library of Congress Cataloging in Publication Data

Hill, Terrell L
Free energy transduction in biology.

Includes bibliographical references.
1. Bioenergetics. 2. Thermodynamics.
I. Title.
QH510.H54 574.1'9121 77-1228
ISBN 0-12-348250-X

PRINTED IN THE UNITED STATES OF AMERICA

Preface

The subtitle of this book is very important. The book does not cover the entire field of free energy transduction in biology but rather only one special topic: the steady-state kinetic and thermodynamic formalism related to free energy transduction. As the word "formalism" implies, the discussion concerns general principles and methods and not details of proposed mechanisms in the various special cases. Although the main argument is put forward in terms of examples, the examples are chosen, for the most part, for pedagogical reasons rather than as serious models.

The advantage of this kind of approach is that one can attain a kind of overview of how free energy transduction is accomplished. The disadvantage is that most current research activity quite naturally lies elsewhere: in attempts to establish the molecular mechanism in particular cases. But as the latter work progresses and detailed models are suggested, the formalism presented here should prove useful in the calculation and understanding of the steady-state kinetic and thermodynamic properties implicit in such models.

Perhaps the main theme of the present book is that, with respect to general principles, free energy transduction can be quite simply understood in terms of conventional kinetics and thermodynamics—suitably related to each other. In particular, it will be argued that free energy transduction is accomplished by complete biochemical cycles and not by individual steps or transitions (as often assumed). A brief abstract of this argument is to be published in *Trends in Biochemical Sciences* (1977).

The book attempts to bring together, in a single coherent account, work published over the past ten years in various research papers. In addition, many new examples and much new material have been added.

Additional introductory comments are included in the first section of Chapter 1.

The writing of this book was greatly aided by the skill and patience of Mrs. Alma Martinson, who typed the manuscript.

I am indebted, for very valuable comments on the manuscript or its subject matter, to Drs. Britton Chance, Elliott Charney, Don DeVault, John Gergely, Joel Keizer, David Kliger, Robert Simmons, Eugene Switkes, and Peter von Hippel.

Note on Notation

The symbol A' was originally introduced for the "basic free energy" [*Progr. Biophys. Mol. Biol.* **28**, 267 (1974)] because of its relationship to the canonical partition function Q. This partition function is a practical one to use in macromolecular statistical mechanics, whereas the partition function Δ (related to the Gibbs free energy G) is not. However, for formal thermodynamic and kinetic purposes, as in this book, one might as well use G' instead of A' because G' is the exact quantity for a constant pressure system while A' is a close approximation of it. The reader should feel free, if he wishes, to make a mental substitution of G' for A' throughout the book.

Contents

Chapter 1 The Diagram Method: States

1.1 Introduction

There are many examples in biochemistry in which a macromolecule, usually an enzyme or enzyme complex, can exist in a finite number of discrete states and such that the macromolecule undergoes continuous cycling among these states at steady state. Ligands, substrates, and products (at fixed concentrations) are involved in some of the transitions between states, but in the formal kinetics the macromolecule plays the central role because it is present in every state. Although simple steady conversion of substrate into product by an enzyme is an example, the more interesting cases involve transduction of one form of free energy into another, as in various kinds of active transport, oxidative phosphorylation, phototranslocation coupling, muscle contraction, etc. Analysis of systems of this type provides a foundation for the understanding of the general principles involved in many, if not most, bioenergetic transformation problems.

Let us try to be quite clear at the outset about the level and the generality of the theory to be summarized in this book. What will be presented here is *not* a theory at the most fundamental molecular or atomic level. In fact, at the present time there is no single example of free energy transduction about which sufficiently detailed experimental information is available to allow the construction (with confidence) of a complete molecular model or theory. Such models can be expected to come along, one at a time, in the future. [Because of its relative simplicity, phototranslocation coupling (1, 2) in the purple membrane of *Halobacterium halobium* is likely to provide the first example.] In fact, we can never expect a completely *general* theory of free

energy transduction at the *molecular–atomic* level because of the vast variety of detail that must be encompassed. Rather, molecular models must be approached on an ad hoc basis, though undoubtedly many of them will prove to be closely related to one another.

On the other hand, in this book, we do not go to the opposite extreme of presenting a discussion of free energy transduction in completely phenomenological terms—for example, in the language of Onsager's nonequilibrium thermodynamics suitably generalized, as would be required for most of these problems, to apply very far from equilibrium.

Instead, we follow an intermediate course. We deal with macromolecules (proteins, enzymes, complexes), their interstate transitions (including the smaller molecules with which they interact), and the rate constants governing the probabilities of these transitions. But, as suggested above, we do not attempt, in any example, to furnish an ab initio theory of the rate constants per se. We take the macromolecular states, transitions, and rate constants as *given*, and then examine the nature of free energy transduction, and a number of related topics, in these terms and at this level of detail. This approach seems to provide the clearest possible overview of the *general* theoretical principles involved in free energy transduction; it does not lose sight of the forest for the trees, as must almost inevitably be the case in a completely detailed molecular analysis of various special cases. Thus the level we adopt permits of very considerable generality and allows further details to be incorporated into particular examples, as the details become available, without disturbing the validity of the general kinetic formalism to be presented here.

Incidentally, exactly this same level of detail—neither ab initio molecular nor phenomenological—is used with great effect by Joel Keizer (3) in his recent very general treatment of dissipation and fluctuations in far-from-equilibrium thermodynamic systems.

The analysis of steady-state enzyme action and free energy transduction in terms of the rate constants operating between discrete macromolecular states is, of course, routine rather than novel in special cases (1, 4–14). But our object in the present monograph is to present a single systematic formalism that is applicable to a wide variety of such examples. We are interested here in analytical methodology rather than in particular models for particular problems. This same point of view was adopted, incidentally, in two recent papers (15, 16) on the theory of muscle contraction; but, of course, experimentally founded "particular models" are the *ultimate* goal.

The main analytical tool in the study of these discrete-state, cycling systems is a diagram method introduced by King and Altman (17) in 1956 and rediscovered and extended in several ways (cycles, cycle fluxes, coupling, reciprocal relations, membrane transport) by Hill (18, 19) in 1966. In fact, it

is the extensions of the King–Altman method that will be found of most use in the present book. More recently, the different kinds of free energy levels of the macromolecular states, especially at steady state, have been discussed by Hill (15, 20), Hill and Simmons (21, 22), and Simmons and Hill (23). Also, fluctuations and noise in the steady-state probabilities of states and in cycle fluxes have been investigated by Hill (20, 24), Hill and Chen (25), Chen and Hill (26), and Chen (27–29). Finally, multienzyme complexes have received some attention (30). Thus, a substantial theoretical foundation is now available as an aid in the study and understanding of these systems.

Although the object of this book is to provide a unified account of these subjects, we shall use, for this purpose, illustrative examples rather than abstract generalities as much as possible. This should make much of the material here—especially the essentials—easily accessible to nontheoretical biochemists and biophysicists. Furthermore, most of the numerous examples will be chosen strictly for their pedagogical value rather than as models to be taken seriously. One aim is to provide sufficient and suitable examples so that the interested reader will be able to analyze his own models by these methods.

Although expressed in quite different language (18, 19), Chapter 1 has for its foundation the King–Altman (17) diagram method for the calculation of steady-state probabilities of states. Chapter 2 then introduces the essential topic of cycles, cycle fluxes, etc. Chapters 3–5 contain a discussion of the more important bioenergetic principles that emerge from the diagram approach. These are the most important chapters in the book. Chapters 6 and 7 are concerned with somewhat more specialized aspects of the subject: stochastics and fluctuations (Chapter 6); and interacting subsystems and multienzyme complexes, including oxidative phosphorylation (Chapter 7). Incidentally, Chapter 7 does not depend at all on Chapters 5 and 6.

Certain important special topics are treated briefly in the appendices: "reduction" of diagrams; membrane potential and charge carriers; and systems that make use of photon absorption. As will be explained in Appendix 5, systems that absorb (or produce) radiant energy are exceptional and do not fully fit into the formalism of this book: Chapters 1, 2, and 6 are applicable to such systems, but not Chapters 3 and 4 as they stand.

A chapter on noise theory, which would be a logical extension of Chapter 6, is omitted because it would have to be relatively sophisticated mathematically, and because a review of this topic has just been written by Chen (31).

Actually, much of the discussion in this book (all but Chapters 4 and 5) is more general than implied above. That is, some of the analysis applies to *any* first-order discrete-state kinetic system at steady state (19, 32). However, for definiteness and because the motivation here is biochemical, we shall introduce and maintain a macromolecular or enzymatic context throughout.

1.2 Diagrams for Steady-State (and Equilibrium) Systems

We consider a large number N (an ensemble) of equivalent and independent macromolecular units or systems (e.g., one unit equals one enzyme molecule or complex), either free in solution or immobilized (e.g., in a membrane or in a myofilament). Each unit may exist in any one of n discrete states, $i = 1, 2, \ldots, n$. Transitions are possible between some pairs of these states (possibly all pairs). Ligands, substrates, and products may be involved in some transitions but, if they are, their concentrations are assumed to be essentially constant over the time scale of interest here. All transitions are treated as first-order processes; the first-order rate constant for the transition $i \to j$ is denoted by α_{ij}. For example, for the binding transition E (enzyme) + S (substrate) → ES, we would use $\alpha = \alpha^* c_S$, where α is the first-order rate constant, α^* the second-order rate constant, and c_S the concentration of substrate (see Section 3.1 for further details).

The n possible states for each unit can be represented by points in a diagram, with a line between two points indicating possible inverse transitions. For example, Fig. 1.1b is the diagram representing the kinetic scheme in Fig. 1.1a (where P means product).

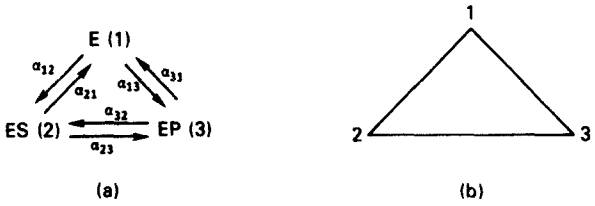

FIG. 1.1 (a) First-order rate constant notation. (b) "Diagram" corresponding to (a).

A few other examples of diagrams are shown in Fig. 1.2 (many others will be encountered later in the book). Figure 1.2a could represent a condensation of Fig. 1.1b if state 3 in Fig. 1.1b is a transient intermediate (see Appendix 1). Another possibility would be: state 1 = E in a membrane; state 2 = EL in the membrane; L is a ligand present in both baths (A and B), on either side of the membrane (usually $c_A \neq c_B$), with binding of L on E possible from either bath. The left-hand line in Fig. 1.2a would then represent, say, transitions involving adsorption–desorption from bath A while the right-hand line relates in the same way to bath B.

Figure 1.2b is the diagram of a common type of system, one with consecutive reactions. For example, the Hodgkin–Huxley potassium channel (four subunits) in the squid giant axon membrane has this kind of diagram but with five consecutive states (33).

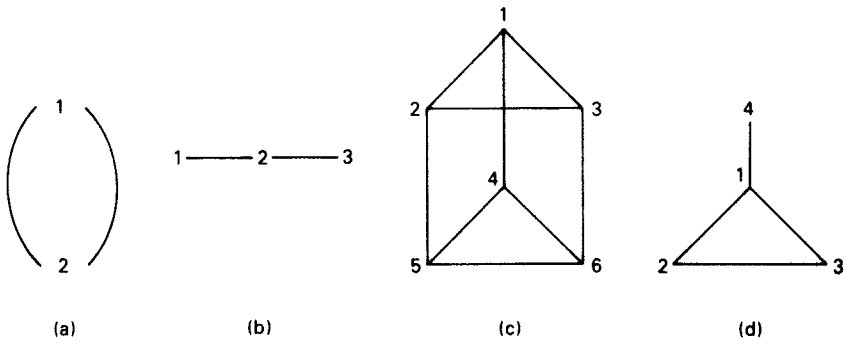

FIG. 1.2 Examples of diagrams.

Figure 1.2c might represent an expansion of Fig. 1.1b if the enzyme can exist in two conformations, E and E*, with state 4 = E*, state 5 = E*S, and state 6 = E*P. The same diagrams would obtain if E binds, on a separate site, a ligand L that may or may not modify the enzyme kinetics. In this case, 4 = LE, 5 = LES, and 6 = LEP. An example of this would be E = myosin, S = ATP, P = ADP + P_i, and L = actin. Figure 1.2d is a special case of Fig. 1.2c in which states 5 and 6 are unstable (i.e., S and P do not bind appreciably to E* or to LE in the two examples above).

If $N_i(t)$ is the number of units of the ensemble in state i at t, the kinetic equations for the diagram in Fig. 1.1b (as an example) are

$$dN_1/dt = (\alpha_{21} N_2 - \alpha_{12} N_1) + (\alpha_{31} N_3 - \alpha_{13} N_1), \tag{1.1}$$

with similar relations for dN_2/dt and dN_3/dt, together with the conservation relation

$$N_1 + N_2 + N_3 = N. \tag{1.2}$$

However, one of the three differential equations is redundant (i.e., not independent). In any example the differential equations are automatically implied by the diagram: each line in the diagram leading into state i will provide a pair of terms in the expression for dN_i/dt, as in Eq. 1.1.

The probability of state i, that is, the fraction of units in state i, is $p_i = N_i/N$. Fluctuations in the N_i, and related topics, will not be considered until Chapter 6.

At $t = \infty$, all $dN_i/dt = 0$ and the ensemble of N units will be either at equilibrium or at a nonequilibrium steady state. A steady state is possible only if the diagram contains at least one cycle (a single closed path in the diagram, not including any appendages). Thus Fig. 1.2b, with no cycle, can only lead to equilibrium at $t = \infty$. The other diagrams in Figs. 1.1 and 1.2 contain cycles (indeed, Fig. 1.2c has 14 different cycles—see Fig. 4.13). If a

diagram possesses one or more cycles, the rate constants *might* have values such that the ensemble reaches equilibrium at $t = \infty$, but in general a steady state is to be expected. If the product of rate constants in a particular direction around any given cycle κ of a diagram is designated by $\Pi_{\kappa+}$, and the product in the opposite direction is designated $\Pi_{\kappa-}$, then the condition for equilibrium is $\Pi_{\kappa+} = \Pi_{\kappa-}$ for every cycle in the diagram. (Ordinarily we take + to be counterclockwise for each cycle.) For example, in Fig. 1.1a, the condition is $\alpha_{12}\alpha_{23}\alpha_{31} = \alpha_{21}\alpha_{32}\alpha_{13}$. This requirement is a straightforward consequence of the application of detailed balance at equilibrium (e.g., $\alpha_{21} N_2^e = \alpha_{12} N_1^e$ in Fig. 1.1a and Eq. 1.1) to each line in the cycle being considered.

The individual transitions in each of the N systems (units) of an ensemble are stochastic in nature. Therefore, in a collection of identically prepared ensembles, or if the same experiment is repeated over and over on a single ensemble, we would encounter fluctuations in the quantities $N_i(t)$ about mean values $\bar{N}_i(t)$. It is actually the mean values that appear in equations such as 1.1. But, for simplicity of notation, we shall omit mean value overbars on the N_i (and on the fluxes, below) until needed explicitly in Chapter 6.

1.3 Directional Diagrams and the Steady-State Populations of States

We shall introduce this subject by means of a hypothetical model for active transport of one ligand by another across a membrane. As indicated in Fig. 1.3a, a protein E has a site (·) for binding a ligand L_1 and another site (×) for binding a second ligand L_2. Both baths contain both ligands at the concentrations indicated in the figure. However, the L_2 site on E is "activated" only if L_1 is already bound. The protein, with ligand L_1 bound, can undergo a conformational change ($2 \rightleftarrows 3$ or $4 \rightleftarrows 5$) that has the effect of switching the bath to which the binding sites are accessible. The diagram is shown in Fig. 1.3b. It can be thought of, for example, as a reduced form of the diagram in Fig. 1.3c, if there is a fast equilibrium between states 1 and 1′ (i.e., a fast conformational change in E in the absence of ligands); see Appendix 1 in this connection.

As the system (i.e., $E + L_1 + L_2$) moves via transitions around the diagram (Fig. 1.3b), completing cycles of the three types possible, the net effect is to transport L_1 and L_2 from one bath to the other. At equilibrium $c_{1A} = c_{1B}$, $c_{2A} = c_{2B}$, and there is no average net transport of either ligand. But at steady state, where we have concentration inequalities rather than equalities, a sufficient concentration difference in one ligand can cause a net flux in the other ligand *against* its own concentration gradient. That is, a free

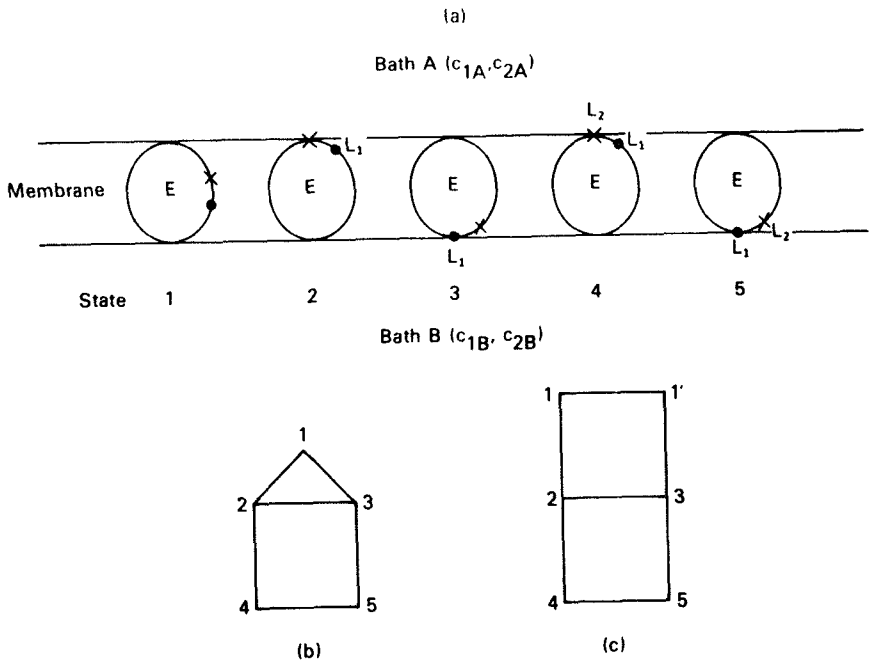

FIG. 1.3 (a) Illustrative model for membrane transport of two ligands L_1 and L_2 between two baths A and B: •, site for L_1; ×, site for L_2; E, macromolecule or enzyme. (b) Diagram corresponding to (a). (c) Expanded diagram if state 1 is expanded into two states.

energy decrease in one ligand can be partially converted into a free energy increase in the other, with a certain nonzero efficiency. This aspect of the model is pursued in Chapter 3. But we turn now to the algebraic problem.

Our main concern is with steady-state fluxes, but we are interested also in the steady-state values of the N_i, denoted by N_i^{ss} (equilibrium values of the N_i will be indicated by N_i^{e}). Of course, the N_i^{ss} may be found in a straightforward way, in any particular case, by solution of a set of linear algebraic equations (see Eq. 1.4, below). But if the model is at all complicated, this may involve a great deal of tedious labor. One of our main objects in this chapter is to show how the solution of the algebraic equations can be found, alternatively, from an enumeration of a certain class of diagrams (17–19). Furthermore, the solution in terms of diagrams has a certain intuitive appeal, and leads directly to the net flux between any transition-pair of states in the diagram.

In the above paragraph, we are referring to a solution of the linear equations for the N_i^{ss} as *explicit functions* of all the rate constants of the

model and N. Of course if one needs only *numerical* solutions for the N_i^∞ or $p_i^\infty = N_i^\infty/N$ in particular cases, the job is most simply done by computer without reference to diagrams.

The directional diagrams (17–19) introduced in this chapter, then, are valuable in providing explicit solutions for the p_i^∞. But the flux diagrams (18, 19) defined in Chapter 2 play a more fundamental role: they furnish the basis for a comprehension of the various *components* of flux present in a steady-state system with a multicycle diagram, and therefore of such properties as thermodynamic "coupling," free energy transfer, reciprocal relations (near equilibrium), rate of entropy production, fluctuations and noise in fluxes, etc.

The differential equation for N_1 is

$$\frac{dN_1}{dt} = (\alpha_{21} N_2 - \alpha_{12} N_1) + (\alpha_{31} N_3 - \alpha_{13} N_1), \tag{1.3}$$

with similar expressions for dN_2/dt, etc. Each pair of terms on the right corresponds to a line in the diagram, Fig. 1.3b. Thus dN_2/dt is equal to three pairs of terms, and there are three lines emanating from state 2 in the diagram; etc. At steady state, Eq. 1.3 becomes

$$(\alpha_{21} N_2^\infty - \alpha_{12} N_1^\infty) + (\alpha_{31} N_3^\infty - \alpha_{13} N_1^\infty) = 0. \tag{1.4}$$

At equilibrium, each pair of terms is *separately* equal to zero (detailed balance).

We obtain an equation like Eq. 1.4 for each state. Thus, in this example, we have a set of five linear equations in the five N_i^∞. But only four of these equations are independent. The fifth independent equation, necessary to solve for the N_i^∞, is $\sum_i N_i^\infty = N$. The solution will give each $p_i^\infty = N_i^\infty/N$ as a function of rate constants.

However, instead of solving for five unknowns in the conventional way, as an alternative we can write the solution using diagrams as follows. [The proof (18) is given in Appendix 2.] The first step is to construct the complete set of *partial diagrams*, each of which contains the maximum possible number of lines (four here) that can be included in the diagram without forming any cycle (closed path). There are eleven such partial diagrams in this case, shown in Fig. 1.4.

If one more line is introduced into any vacant position in any of these partial diagrams, a cycle is produced.

At least one line goes to each vertex (state) in a partial diagram (otherwise more lines could be introduced without forming a cycle).

The next step is to introduce arrows (i.e., a directionality for each line) into the partial diagrams of Fig. 1.4 in five different ways, one way for each state (vertex). For example, consider state 2. Figure 1.5 shows the eleven

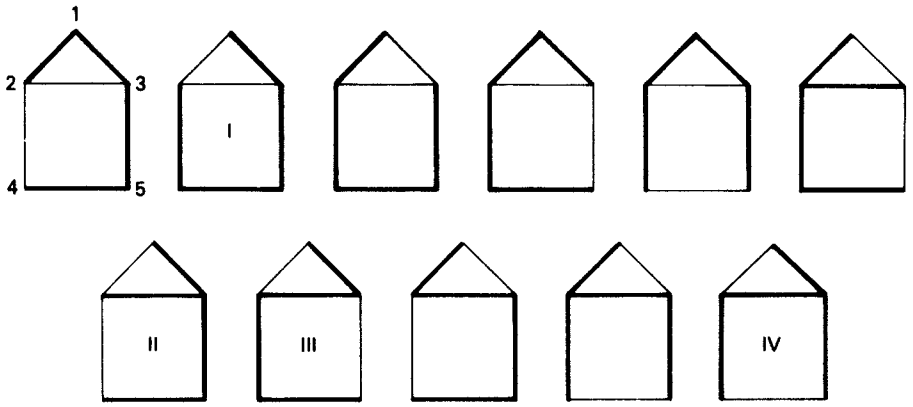

FIG. 1.4. Partial diagrams for Fig. 1.3b. I, II, III, and IV are referred to in text (Chapter 2).

directional diagrams for this state, as obtained from Fig. 1.4. The recipe for introducing arrows is simple: all connected paths in Fig. 1.5 are made to "flow" *toward* and *end at* vertex 2. It will be noted that in the flow toward the ultimate vertex (vertex 2 in Fig. 1.5), "streams" may converge but they never diverge (for this would require a cycle in the partial diagram).

There is a set of eleven directional diagrams for each of the five states. In each case, all streams flow toward—and end at—the particular state being considered.

Now each directional line or arrow in Fig. 1.5 corresponds to a rate constant; the key for assigning rate constants to directional lines is provided

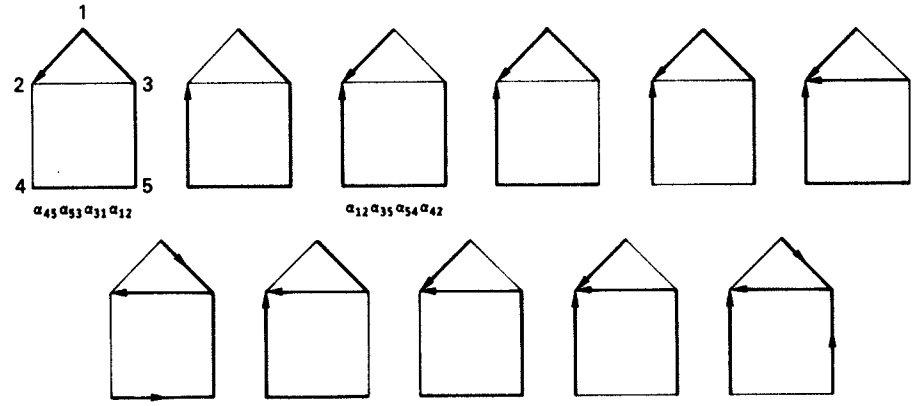

FIG. 1.5 Directional diagrams for state 2. Algebraic values of first and third directional diagrams are given.