ADVANCES IN ENZYMEOLOGY
AND RELATED AREAS OF
MOLECULAR BIOLOGY

Volume 32
CONTRIBUTORS TO VOLUME 32

K. ALTLAND, Institute of Human Genetics, University of Hamburg, Hamburg, Germany

E. BOYLAND, Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, England

L. F. CHASSEAUD, Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, England

H. N. CHRISTENSEN, University of Michigan, Ann Arbor, Michigan

H. WERNER GOEDDE, Institute of Human Genetics, University of Hamburg, Hamburg, Germany

PAUL GRAFEN, Institute of Organic Chemistry, University of Vienna, Vienna, Austria

G. A. HAMILTON, Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania

H. HOLZER, Biochemisches Institut der Universitat Freiburg im Breisgau, Germany

V. I. IVANOV, Institute of Molecular Biology, Academy of Sciences of USSR, Moscow, USSR

M. YA. KARPEISKY, Institute of Molecular Biology, Academy of Sciences of USSR, Moscow, USSR

E. LEETE, Department of Chemistry, University of Minnesota, Minneapolis, Minnesota

R. B. MERRIFIELD, Rockefeller University, New York, New York

R. A. MORTON, Department of Biochemistry, University of Liverpool, Liverpool, England

G. A. J. PITT, Department of Biochemistry, University of Liverpool, Liverpool, England

H. SACHS, Department of Physiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio

U. SCHMIDT, Institute of Organic Chemistry, University of Vienna, Vienna, Austria
ADVANCES IN ENZYMOLYOGY
AND RELATED AREAS OF MOLECULAR BIOLOGY

Edited by F. F. NORD
FORDHAM UNIVERSITY, BRONX, NEW YORK

VOLUME 32

1969
INTERSCIENCE PUBLISHERS
a division of John Wiley & Sons
New York • London • Sydney • Toronto
The paper used in this book has pH of 6.5 or higher. It has been used because the best information now available indicates that this will contribute to its longevity.

Copyright © 1969 by John Wiley & Sons, Inc.

All Rights Reserved. No part of this book may be reproduced by any means, nor transmitted, nor translated into a machine language without the written permission of the publisher.

Library of Congress Catalog Card Number: 41-9213

SBN 470 64961
CONTENTS

Some Special Kinetic Problems of Transport. By Halvor N. Christensen.......................... 1

Dynamic Three-Dimensional Model for Enzymic Transamination. By V. I. Ivanov and M. Ya. Karpeisky........... 21


The Role of Glutathione and Glutathione S-Transferases in Mercapturic Acid Biosynthesis. By E. Boyland and L. F. Chasseaud........................................ 173

Solid-Phase Peptide Synthesis. By R. B. Merrifield................................. 221

Regulation of Enzymes by Enzyme-Catalyzed Chemical Modification. By H. Holzer............................... 297

Neurosecretion. By Howard Sachs.................................................. 327

Alkaloid Biosynthesis. By Edward Leete........................................... 373


Author Index.................................................. 471

Subject Index.................................................. 505

Cumulative Indexes, Volumes 1–32........................................... 519
SOME SPECIAL KINETIC PROBLEMS OF TRANSPORT*

By HALVOR N. CHRISTENSEN, Ann Arbor, Michigan

CONTENTS

I. Introduction................................................................. 1
   A. General................................................................. 1
   B. Types of Interactions between Analogs.............................. 3
      1. Complete and Homogeneous (Case I).............................. 3
      2. Homogeneous, but Complete in Only One Direction (Case II) 3
      3. Homogeneous, but Complete in Neither Direction (Case III) 4
      4. Complete, but Homogeneous in Only One Direction (Case IV) 4
      5. Complete, but Heterogeneous in Both Directions (Case V).... 4

II. Problems in Discrimination............................................. 4
   A. General................................................................. 4
      1. The Nonsaturable Component...................................... 4
      2. Low Acuity of Simple Kinetic Study............................. 5
      3. Consequences of Overlooked Heterogeneity..................... 5
      4. Definition of $K_i$ for Transport............................... 6
   B. Examples of the Demonstration of Case V Interactions.......... 7
      1. The $A$ and $L$ Systems of the Ehrlich Cell................... 7
      2. The $A$ and ASC Systems of the Ehrlich Cell.................. 10
      3. Biological Support for Kinetic Discriminations............... 12
   C. Example of Partial Elucidation of a Case III Interaction...... 13
   D. Description of Heterogeneity in Mode of Exodus from the Cell 15
      1. General............................................................. 15
      2. Countertransport Phenomena..................................... 15
      3. Special Importance of Substrates Specific to a Single System
         for the Study of Exodus......................................... 17

III. Summary............................................................................ 19

References.............................................................................. 19

I. Introduction

A. GENERAL

The complications in the kinetics of transport that I want to discuss
are not difficult problems in the sense of representing unusual inter-

* The experiments discussed here that derive from the authors laboratory were
  supported in part by a grant (HD01233) from the Institute for Child Health and
  Human Development, National Institutes of Health, U.S. Public Health Service,
  and in part by The University of Michigan.

1
actions between the catalyst and substrate or analogs of the substrate. Instead the complexities arise from the circumstance that we deal with the kinetics of mixed catalytic systems. The anisotropic nature of the process catalyzed contributes difficulties, but the principal difficulty is that we are generally forced to study the process in the natural, unresolved state. We might insist on delaying kinetic study until the pure catalyst can be isolated. For transport, the trouble is that if we were to insist on first isolating the effector molecules from the membrane, or even if we were to only interrupt the membrane so that we no longer have more than one aqueous phase, we would for the most part retain no signal that the catalytic event had occurred. Therefore the components of the catalytic system become difficult to recognize, and isolation has so far failed, with a very few possible exceptions.

Prior kinetic study is likely to tell us what we should expect and what we should not be likely to find, and to help guide isolative procedures. We learn first of all what kind of a reactive site to look for. In certain cases no cofactor or cosubstrate has been recognized, so that we are led to expect a site serving for the substrate alone. In other cases Na+ serves as a cosubstrate and modifies the binding of the substrate (1-7). That information implies a more complex arrangement, and should sharpen our ability to recognize the binding structure. To give a less favorable example, the inadvertent study of two transport systems in admixture led to the impression that two amino acid molecules might enter together in a complex with the transport receptor site (8). The support for that impression, namely the phenomenon of "competitive acceleration," disappeared, however, when the contributing systems were recognized (9) and studied in isolation (10). That case illustrates the importance of recognizing from the beginning of isolation whether we are observing the behavior of only one catalytic system or the sum of the behavior of two or more parallel transport systems.

To avoid confusion with other phenomena of related biological interest, I will define transport in a classical, kinetic manner, as the catalytic event that can be represented kinetically by the simplified formulation,

\[ S + E \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P \]

given that S and P are exactly the same chemical species and differentiated only by their position in two different aqueous phases. In this formulation E is not intended to specify an enzyme, but a molecule bearing any kind of catalytic site, as described by kinetic study. In the case of transport, that site mediates transport rather than the
conversion of the substrate into another molecular species. We encounter occasionally a tendency to define *enzyme* so broadly as to include any protein with a reactive site at which reversible binding occurs, without regard to the question of whether the substrate is destabilized by that binding. Such a usage is deplored.

The above definition of transport does not delight some biochemists whose interest falls on an interesting companion process falling outside the definition. I refer to *group translocations* (11), where P differs both chemically and physically from S, and where the process may be the sum of a transport and an enzymic event. We encounter in that area the occasional biochemical presumption that all transports will be found to have that nature, or that only those having such a character deserve our interest. We need only recall the enormous importance attached to the transport of inorganic ions and the hydrogen ion to reject such a limitation. One also notes an occasional tendency to regard passive transports as of inferior importance. A significant biological aspect is that these "facilitated diffusions" so often have a built-in capacity for operating uphill, and do so in other contexts. Accordingly, processes meeting the above kinetic definition will be included in the present discussion, whether they operate uphill or not.

B. TYPES OF INTERACTIONS BETWEEN ANALOGS

The following subsections list formally the types of interaction that may be found between two analogs for mediated transport.

1. *Complete and Homogeneous* (Case I)

Here I refer to the classical case of competition between two analogs, both of which may serve as substrates. Diagram 1 of Figure 1 shows the effect of the concentration of b on the rate of transport of a. It can also represent, presumably with different numbers on the coordinate scales, the effect of the concentration of a on the transport of b. The curve is drawn to represent precisely a rectangular hyperbola with an asymptote of zero velocity at infinite analog concentration.

2. *Homogeneous, but Complete in Only One Direction* (Case II)

In this case, the two curves describing the interaction between two analogs are still precise rectangular hyperbolas, but now one of them has the appearance of diagram 2 in Figure 1. Although one analog is able to suppress all transport of the other, for the converse aspect of the interaction a distinct component of migration escapes inhibition even at very high analog concentrations. This behavior may mean that the
Fig. 1. Diagrams to illustrate the effect on the uptake of a solute of adding increasing quantities of an analog. Diagram 1: The inhibition is complete and homogeneous, so that the curve is a precise rectangular hyperbola. Diagram 2: The inhibition does not concern all modes of the uptake, and is therefore incomplete. Diagram 3: The inhibition is inhomogeneous. The curve of diagram 3 is obviously not a rectangular hyperbola; instead it represents the sum of two rectangular hyperbolas.

transport of one of the substrates occurs by two agencies, one reactive and the other totally unreactive with the analog.

3. Homogeneous, but Complete in Neither Direction (Case III)

For this case diagram 2 (Fig. 1) describes both aspects of the interaction, although it is of course unlikely that the completeness of inhibition is the same in both directions.

4. Complete, but Homogeneous in Only One Direction (Case IV)

By describing one aspect of the interaction in case IV as heterogeneous I mean that the sum of two presumably nonidentical rectangular hyperbolas is necessary to represent it. Diagram 3 of Figure 1 illustrates the nature of the inhibitory action in one direction, Diagram 1 that in the other direction.

5. Complete, but Heterogeneous in Both Directions (Case V)

Kinetic curves of the type illustrated by diagram 3 of Figure 1 now describe both phases of the interaction. We cannot, of course, decide by simple inspection whether each analog has top reactivity with the same system, or whether analog a is more reactive with system A and analog b more reactive with system B.

Beyond these five categories we encounter (and very commonly) various combinations of incompleteness and heterogeneity, which will not require enumeration.

II. Problems in Discrimination

A. GENERAL

1. The Nonsaturable Component

Where do the problems in discriminating among these several cases actually lie? First of all, one must usually subtract or disregard a
small component of migration that appears not to be subject to saturation to secure correspondence to models I, IV, or V \((9,11,12)\). Since in the case of amino acid transport that component appears probably also mediated \((12)\), pure cases of these three types may not actually exist.

2. *Low Acuity of Simple Kinetic Study*

Let me remind you next that case I cannot reliably be discriminated from cases IV and V on the basis of an apparently hyperbolic character of the kinetics. When a rate curve is the sum of two rectangular hyperbolas, as in diagram 3 of Figure 1, the two \(K_i\) values specified by the curve must be far apart before we can recognize the duality, given the usual standards of accuracy. The \(K_i\) values selected for diagram 3 are separated by an order of magnitude; nevertheless, rather good accuracy must be obtained if the Lineweaver-Burk plot corresponding to it is to be recognized as deviating from linearity. The direct kinetic approach is very inefficient for recognizing whether or not the catalytic sites represent a single homogeneous type. This deficiency of the kinetic method is especially unfortunate for transport, because as I have mentioned, attempts to isolate mediating structures before kinetic study have not been productive.

3. *Consequences of Overlooked Heterogeneity*

When heterogeneity of catalysis is overlooked, the \(K_m\) and \(K_i\) values extracted from kinetic curves are of course arbitrary values that do not describe any single catalytic system. Such an oversight occurred for methionine uptake by the Ehrlich cell. Presumably as a consequence of that oversight, the respective \(K_m\) and \(K_i\) values describing the interaction between methionine and glycine for uptake were not consistent with each other. As a result it was concluded that methionine and glycine do not share the same transport agency, even though each inhibits the uptake of the other \((13,14)\). Subsequent study has established that about half the uptake of methionine and nearly all the uptake of glycine (each at 1 mM) actually occurs by the so-called \(A\) system \((9,15,16)\). This example shows that a wrong decision for homogeneity may lead to a wrong decision on the question whether a transport agency is shared or not. Figure 2 represents the situation just described. Merely to conclude that the total transport of two analogs is not identical may be superficial; to discover what modes of transport they have in common is what is needed.
Fig. 2. Illustrating a consequence of overlooked heterogeneity in mediation of transport. The small letters designate substrates, the boxes lettered with capitals, transport-catalyzing systems. A solid arrow passing through the box indicates that transport occurs; where the arrows cross there is competition for transport. The dotted lines mean that the inhibitory analog can occupy the site and thus produce inhibition but that no transport of the inhibitor takes place. To determine whether transport mediating agencies \( A \) and \( B \) (left-hand diagram) are in fact one and the same, the \( K_m \) of each substrate \( a \) and \( b \) is compared with its \( K_i \) value as an inhibitor of the transport of the other. The model shown here as model Ib represents two variants of model I in our list, these two being distinguished by the identity or non-identity of systems \( A \) and \( B \). In the case discussed in the text, because neither \( K_m \) resembled the corresponding \( K_i \) value, the conclusion was drawn that \( A \) and \( B \) are distinct agencies, and therefore that substrate \( a \) inhibits system \( B \) without being transported by it. The relation actually found among neutral amino acids for transport in the Ehrlich cell is shown instead by model V (right-hand diagram). Because the transport of the competing substrates is shared between two parallel systems the observed \( K_m \) and \( K_i \) values describe no single transport system; hence there is no reason that they should be identical.

4. Definition of \( K_i \) for Transport

In the transport field, incidentally, inhibitory analogs have usually proved to be competing substrates, although that situation is by no means universal (17). We frequently compare the inhibitory effect of the substrate with that of its analogs, an idea that I have observed occasionally to bewilder those who hear it for the first time. Such a comparison is readily made by curves of the form of those shown in Figure 1, but with the dimension \( v/[S] \) rather than \( v \) assigned to the ordinate scale. When this is done, we observe, as indeed we must, the very same inhibitory effect on adding more substrate as we do on adding an analog whose \( k_i \) equals the \( k_m \) of the substrate. In transport kinetics, we need further to reject the definition for \( K_i \) prescribed by some authorities, namely that it equals the dissociation constant for the EI complex. In the usual case the experimentally determined \( K_i \)
will instead equal \((k_{-1} + k_2)/k_1\); the value of \(k_{-1}/k_1\) will then remain unavailable to us. Graphing the rate coefficient, \(v/\lbrack S \rbrack\), as in Figure 1, rather than simply the rate, offers the advantage that one may show on the same plot the saturating effects of either the substrate or an analog, the essential similarity of these effects being emphasized by that plot.

Before we can conclude that approximately hyperbolic kinetic curves mean that two analogs compete homogeneously for transport, we must show that the simplicity of the kinetics persists as we modify the structure of either analog or modify the environment. In studying organic substrates, we have the advantage that their structures can be modified freely, so that catalytic heterogeneity can eventually be made conspicuous and described.

B. EXAMPLES OF THE DEMONSTRATION OF CASE V INTERACTIONS

1. The A and L Systems of the Ehrlich Cell

I will now proceed to describe how the interaction among neutral amino acids for uptake by the Ehrlich cell was shown to correspond in general to Case V.

Figure 3 will recall the situation presented. Almost every neutral amino acid examined was inhibitory to the uptake of any test neutral amino acid. But note (upper left) that their effectiveness in inhibiting glycine uptake was almost entirely uncorrelated with their effectiveness in inhibiting leucine uptake. In contrast, good correlation was observed between effectiveness on glycine and on alanine uptake, and an even better correlation between effectiveness in leucine and on phenylalanine uptake (9). The latter correlation has held up through tests of scores of additional analogs of leucine or phenylalanine. We interpreted Figure 3 to mean that at least two transport systems contribute to the uptake of most neutral amino acids, according to Model V. One system, designated L, had reactivity centering around such amino acids as leucine and phenylalanine; the other, designated A, around such amino acids as alanine and glycine.

The strategy followed to establish the applicability of model V was to modify one of the substrates to restrict its reactivity to only one of the two systems (15,16). Case V could thus be reduced to case II, as Figure 4 shows. We have discovered that \(N\)-methylating an amino acid is a useful procedure for restricting its reactivity. The graph at the left shows that \(\alpha\)-(methylamino)-isobutyric acid \(\text{(MeAIB*)}\) is able

* The abbreviations used are: MeAIB, the \(N\)-methyl derivative of \(\alpha\)-aminoisobutyric acid; BCH, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid.
Fig. 3. Correlation between the inhibitory activity of various neutral amino acids on the uptake of four amino acids by the Ehrlich cell. The test amino acids were at 1 mM, the inhibitors in general at 5 mM (10 mM for DL forms). The coefficients of correlation are as follows: Between glycine and leucine (upper left), 0.09; between glycine and alanine (lower left), 0.87; and between leucine and phenylalanine (lower right), 0.93.

...to inhibit only a very restricted portion of the uptake of phenylalanine. Presumably this is the portion of uptake occurring by the $A$ system, with which MeAIB is highly reactive, whereas it appears to be unreactive with other systems. If we now also $N$-methylate phenylalanine (lower right) its uptake is restricted (except for the usual small nonsaturable component) to routes accessible to MeAIB inhibition. Note that the ordinate scales on the graphs of Figure 4 are identical. In fact the two curves marked $I = \text{MeAIB}$ are superimposable. Hence we conclude that $N$-methylation has eliminated the system $L$ uptake shown by phenylalanine, to expose clearly the small component of uptake that occurs by the $A$ system. If we examine instead the uptake of MeAIB (graph at upper right), phenylalanine or $N$-methylphenylalanine are equivalent in their inhibitory action. Hence the model
Fig. 4. Analysis of the $A-L$ dichotomy illustrated by the transport of phenylalanine. The problem is, does each neutral amino acid enter the cell only by a single transport system, or does it enter by two systems in parallel? Do the inhibitory actions between neutral amino acids correspond to their sharing of transport systems? See text for discussion.

sketched at the extreme upper right, corresponding to case II in our list, is confirmed. By $N$-methylation of both analogs, paired kinetic curves are in fact obtained (graphs at right), so that the interaction was finally simplified to case I.

The parallel operation of these two transport systems has been confirmed in other ways also, particularly by taking advantage of the Na$^+$ independence of system $L$ and the Na$^+$-requiring nature of system $A$ (16). Figure 5 summarizes the general properties of the $L$ system, which appears to occur in many cells and tissues. Figure 6 summarizes in turn the properties of system $A$, which appears also to occur widely, although it is conspicuously absent in the various erythrocytes examined. A glycine-specific transport system (Fig. 7) may be found instead in these cells (18-21). Evidence can also be cited that such a system participates in renal transport (22).

An interesting feature of the $A$ system of the Ehrlich cell is the tendency of the $V_{\text{max}}$ values for various substrates to cluster in the region of 4–7 millimoles of amino acid per kg cell water per minute. Only rather extreme changes in structure succeed in changing $V_{\text{max}}$ beyond this range (17,23). This tendency is consistent with the applicability of the equilibrium assumption of Michaelis and Menten, and shows that the rate of the slowest step in the transport process is largely independent of amino acid structure.
THE L SYSTEM

Formally defined for: The Ehrlich cell


V\text{max}: Characteristically variable

Dependency on Na\textsuperscript{+}: None

pH sensitivity: Minimal

Exchanging properties: Very strong, so that net operation shown only with special care

Other apparent occurrences: Ubiquitous, including mature mammalian erythrocyte. Similar systems may lack exchanging property.

Fig. 5. Summary of properties of the L system.

THE A SYSTEM

Formally defined for: The Ehrlich cell

Substrates:

range: All neutral amino acids
Characterizing: AIB and MeAIB (glycine, proline, sarcosine, serine, methionine, norleucine)

least reactive: Valine; other branched-chain, apolar amino acids

V\text{max}: Rather constant for all but a few substrates.

Dependency on Na\textsuperscript{+}: First-order; causes one Na\textsuperscript{+} to migrate per amino acid molecule

pH sensitivity: Scarcely detectable at pH 5

Exchanging properties: Weak; weakly reversible

Other apparent occurrences: Hamster intestine; kidney, bony tissue; brain.

Conspicuously absent in erythrocytes.

Fig. 6. Summary of observations on the A system.

2. The A and ASC Systems of the Ehrlich Cell

At this stage a component of the uptake of alanine had been observed to be much more difficultly inhibited by a combination of MeAIB and phenylalanine (15) than it should be if systems A and L accounted for
THE GLYCINE SYSTEM

Formally defined for: Avian erythrocytes (Vidaver)

Substrates: Glycine, sarcosine.

Dependency on Na⁺: Non-hyperbolic (second-order?) More than one sodium migrates for each amino acid molecule. Evidence that Na⁺ gradient contributes energy.

Exchanging properties: Not demonstrated.

Other occurrences: Rabbit reticulocytes; possibly, the kidney, etc. Conspicuously absent from Ehrlich cell.

Fig. 7. Summary of properties of the glycine system.

Fig. 8. Analysis of the $A-ASC$ dichotomy, illustrated by the transport of alanine and its N-methyl derivative. See text for discussion.

all alanine uptake. Figure 8 shows a demonstration of that component. For this experiment, attention was restricted to Na⁺-requiring systems by deducting uptake retained in the absence of Na⁺. The new component is measured here by the difference in the effectiveness (left) of $N$-methylalanine and alanine in slowing the uptake of 1 mm alanine-$^{14}$C. If the uptake of alanine is observed in the presence of 25 mm $N$-methylalanine (lower right), the total rate of saturable uptake corresponds to the difference between the asymptotes of the curves on the left. If we observe instead the uptake of 1 mm $N$-methylalanine as the substrate, the two inhibitors are now equally complete in their action (upper right). We believe that we have in this way identified a third transport system, for which alanine, serine, and cysteine proved typical substrates—hence
the abbreviation, system ASC (17). Figure 9 summarizes the features observed for this system. Such amino acids as glycine, AIB, histidine, leucine, valine, and phenylalanine show little or no measureable Na+-dependent uptake beyond that inhibitable by MeAIB, i.e., no significant uptake by system ASC. Proline and the four-carbon aliphatic and hydroxyaliphatic amino acids are, however, also ASC substrates.

We were assisted in concluding that system ASC really functions independently of the A system by the discovery that a system very similar to it operates in the rabbit reticulocyte (18) and the pigeon erythrocyte (24). One can propose an alternative hypothesis, that the reactive sites for the A system occur in pairs or clusters, and that when one of these is filled by MeAIB, the adjoining site(s) may be prevented from receiving an amino acid unless it is one of those listed above as an ASC substrate. The unusual stereospecificity of the ASC component (17), as well as its separate existence in erythrocytes, argues against any such model.

3. Biological Support for Kinetic Discriminations

In several other cases decisions as to the separateness of two systems participating in the transport of a single substrate have had confirmation by the loss of one and not the other activity from the biological

THE ASC SYSTEM

Formally defined for: The Ehrlich cell

Substrates: Characteristic, 3- and 4-carbon aliphatic and hydroxyaliphatic amino acids; proline, cysteine.

No inhibition detected by MeAIB, MeAla (discrimination from A system)

$V_{\text{max}}$: Characteristically variable; several amino acids inhibit without migrating detectably by it.

Dependency on Na+: First order.

$\text{pH}$ sensitivity: Intermediate. Characteristically less than for A system.

Stereospecificity: Exceptional

Exchanging properties: Weak

Other occurrences: In rabbit reticulocyte, pigeon erythrocyte, but with strong exchanging action, and with several sodium ions migrating for each amino acid molecule. Ubiquitous?

Fig. 9. Summary of observations on the ASC system.
context. For example, several transport systems are lost from the rabbit reticulocyte as it matures (18); in fact these systems are lost on different time schedules (25). In *Salmonella typhimurium* a broad-scope transport system contributing to histidine transport was found defective in a mutant whereas a more specific high-affinity system was found undisturbed (26). This situation, incidentally, does not correspond to a generalization suggested by Koch's work (27) that the more specific high-affinity systems may depend for their operation on a broad-scope transport agency. On treatment of rats with actinomycin D, differences can be observed in the time required for intestinal absorptive activities to be lost, corresponding approximately to a greater sensitivity of an A-type than of an L-type system (28). On osmotic shock in the cold, *Escherichia coli* cells lose their transport activity for several amino acids corresponding to one of the transport systems discriminated kinetically. At the same time, however, the transport activity for certain other amino acids is scarcely affected. In addition, a protein can be detected in and isolated from the supernatant shock fluid, which forms complexes with just those amino acids whose transport has been lost. Furthermore, the dissociation constants of these complexes usually correspond well with the $K_m$ values of those amino acids for transport (29).

C. EXAMPLE OF PARTIAL ELUCIDATION OF A CASE III INTERACTION

We faced next a more serious problem in the kinetics of mixed systems in the interactions between neutral and cationic amino acids for transport. Here we were dealing with a type III interaction. Phenylalanine and lysine will be selected as typical substrates. Each of these two shows a component of uptake by the Ehrlich cell which escapes inhibition by the other even at extreme concentrations. In each case, another component of uptake is readily inhibited by the other member of the pair (23,31). Figure 10 shows three models, one of which might account for the behavior just described. Model IIIA proposes that no interaction at all occurs between the two transport systems typical of these two substrates, which we call the $L$ and $\text{Ly}^+$ systems, but instead that they compete for transport via a third system which does not discriminate between amino acids with neutral and positively charged side chains. Model IIIB proposes instead that the two characteristic reactive sites are close enough together so that filling one with phenylalanine slows but does not stop lysine transport; filling the other with
lysine slows but does not stop phenylalanine transport. (A simplified form of this model had already been eliminated on kinetic grounds for the type II interaction between methionine and MeAlB (17).) The current enthusiasm for the subject of biological regulation leads too freely, I think, toward explanations of type III interactions by models of the type IIIB, without sufficient regard for the possible relevance of model IIIA. A third model, IIIIC, suggests that phenylalanine and lysine slow each other’s transport by entering modifier sites operating on the L and Ly+ sites, respectively. The modifier sites are assumed not to serve for transport. The near identity of the $K_m$ values of lysine uptake in the presence and in the absence of phenylalanine, and the similarity of the $K_m$ of phenylalanine for uptake and its $K$, in inhibiting lysine uptake, make model IIIIC rather unlikely.

It soon became clear that another complication would have to be added to any of the models of Figure 10. The inhibition of lysine transport by neutral amino acids proved not to be of a single, homogeneous sort, but to fall into at least two categories (23,30). Only one of these clearly resembles the L system in its structural selectivity; the other category of inhibitory effect showed itself as an incremental inhibitory action produced by any of several short-chain amino acids over that produced by phenylalanine alone. Contrary to an early prediction (30), the intensity of this effect by each test amino acid proved to be uncorrelated with its reactivity with the A system; furthermore, neither inhibitory action required the presence of Na+. To represent this second mode of inhibition, we may for consistency propose a second $L^{n+}$ system for model IIIA (23). For model IIIB or C, we may instead add a modifier site operating on the $Ly^+$ transport system, as illustrated at the far right in Figure 10.
Our recent identification of a model amino acid, 2-aminobicyclo-
[2.2.1]heptane-2-carboxylic acid (BCH), whose transport appears to be
limited to the L system, has permitted a new examination of the alter-
natives of Figure 10 (31).

This amino acid has only a weak interaction for transport with lysine.
By comparing homoarginine with lysine we could show that this inter-
action must relate mainly if not entirely to the α-zwitterionic form of
lysine, and not to the cationic substrate. The circumstance that a
type-substrate of the L system could be shown to have little or no in-
fluence on the uptake of cationic amino acids appears to have excluded
model IIIB. The parallel observation that BCH was able to eliminate
the component of phenylalanine uptake subject to lysine or homoargin-
ine inhibition, whereas it was not able to eliminate the component of
the uptake of the basic amino acids subject to inhibition by neutral
amino acids, appeared to exclude model IIIA, under which these com-
ponents are assigned to the same agency. In this way both of the simpli-
fying identitities of transport-reactive sites suggested by models A and
B, Figure 10, appear to have been excluded. Hence we must think of
another way in which neutral and cationic amino acids can interact to
explain the type III interaction seen in this case.

D. DESCRIPTION OF HETEROGENEITY IN MODE OF
EXODUS FROM THE CELL

1. General

Up to this point in my discussion entry into isolated cells has been
used to measure the activity of transport-mediating structures which
according to all evidence appear to be situated in the plasma membrane.
The migration of solutes out of the cell may also be used to detect these
structures; an irreversible operation for entry only appears to be the
exceptional case, whether the transport is an uphill one or not (32).
Our tests with the Ehrlich cell fail to reveal clear-cut differences in the
characteristics for operation inward and outward, except in the value of
$K_m$, which is typically much higher on the inside than on the outside
(32), a circumstance noted earlier by Winkler and Wilson for $\beta$-galacto-
side transport by E. coli (33).

2. Countertransport Phenomena

Mediation of outward transport has advantages for studying the
phenomenon of accelerative exchange diffusion, a kinetic manifestation
of special importance in the study of transport (34–38). The phenom-
enon may be described as follows: As the concentration of a substrate is increased on one side of a membrane, so that its flux into the other phase is increased, one observes also an increase in its flux from the second to the first phase. Another name for the same phenomenon "the trans effect" has an obvious, descriptive meaning (38). In the first term, the words, exchange diffusion are interpretative in nature; they assume a mechanism under which a portion of the movement of the solute in the first direction occurs by exchange with solute already in the second phase. If energy is required for net transport, no corresponding energy requirements occurs for this transport by exchange.

An enzymologic analog of the "trans" effect can be proposed. Suppose one were to increase the glutamate concentration in a solution of a suitable transaminase. He would presumably note an accelerated amination of any quantity of α-ketoglutarate present, although the total amount of α-ketoglutarate present would not change. The analogy can be extended further; one could obtain the same acceleration by adding alanine, if that amino acid is a suitable substrate. Similarly, phenylalanine already accumulated in cells will accelerate the uptake of, for example, leucine. Or the exodus of the accumulated phenylalanine can be greatly accelerated by adding leucine (or, interestingly, lysine) to an amino-acid-free suspending medium. The two analogous phenomena under comparison produce neither net amination nor net transport. The physical requirements for the phenomena are in each case parallel: There should not be a free and rapid interconversion of pyridoxal phosphate and pyridoxamine phosphate, or of the transport receptor site in its orientation from one side to the other side of the membrane, except by the catalytic process under observation.

The external concentration of an amino acid sufficient to produce a half-maximal stimulation of exodus of an analog appears to be the same as the concentration required to produce a half-maximal velocity of uptake of the amino acid. Consequently, the occurrence of uptake by exchange has not in our experience given rise to kinetic evidence of heterogeneity; the only change appears to be an increase in $V_{\text{max}}$ (9,17).

Accelerative exchange diffusion must be discriminated from another phenomenon, inhibitory exchange diffusion or "the cis effect." In this case it is the parallel flux of an analog that is inhibited, while the opposed flux is unaffected or slowed only to a lesser degree. The key to the discrimination is the adequacy of the separate measurement of influx and efflux. For this purpose, measurement of the kinetics of exodus in response to an external analog has advantages over measurement of uptake in response to an internal analog. One can make the external
volume as large as he likes; hence the escaping substrate will be diluted so greatly with solvent as to make reentry negligible. Despite this opportunity, however, serious errors have been made by underestimating the degree of dilution required to minimize reentry. The volume required may be enormous where the $K_m$ for uptake is very low, perhaps as low as $10^{-6}$ M, and the $V_{max}$ relatively high. Conceivably, even drawing the external medium through a layer of cells may not protect the investigator from confusion between effects on exodus and effects on reentry.

3. Special Importance of Substrates Specific to a Single System for the Study of Exodus

The study of exodus is accordingly superior to the study of entry for the characterization of systems serving to mediate exodus by exchange, and for differentiating them from systems that fail to show accelerative countertransport. The kinetic study of exodus has been found to suffer, however, from the difficulty of obtaining clearly saturating concentrations of transport substrates and their analogs inside the cell. Therefore one may be able to obtain only approximate values for the kinetic parameters for exodus. He may be unable to establish the participation of a second system in the exodus of an amino acid by the method used in Figure 8 for the ACS system, that is, by the properties of an exodus system retained after one or more other systems have been saturated, because he is unable to saturate them. Therefore the characterization of exodus catalyzed by mixed systems depends to an unusual extent on the development of a substrate specific to each contributing system. With such substrates we can use more direct methods, as we did in Figure 4 to show that a component of the entry of phenylalanine occurs by the A system, in addition to the principal component by the L system. MeAIB has proved suitable for the identification of exodus as well as entry by the A system (32). The new analog mentioned earlier, 2-aminobicyclo[2.2.1]hexane-2-carboxylic acid or BCH, appears to be uniquely high in its specificity to the L system, and suitable for the demonstration of the role of that agency in exodus as is illustrated in Figure 11 (32). Taurine (39,40) serves in the same way for the $\beta$-system (see Fig. 12 for a description of this system), and sarcosine for the glycine system. The overlap in reactivity is such that an analog specific to the ASC system may prove unattainable. Just how suitable such model compounds will prove for the analysis of separate systems in other cells and tissues has not been fully determined.
Fig. 11. Concentration dependence of phenylalanine exodus from the Ehrlich cell; its near elimination by the presence of excess 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid in the cell. The cells had been loaded to an estimated level of 50 mM of the inhibitor in the cellular water, and to selected phenylalanine-\(^{14}\)C levels. The decline in the cellular phenylalanine-\(^{14}\)C content through exodus into 800 volumes of choline-containing Na\(^+\)-free Krebs-Ringer bicarbonate medium, pH 7.4, was then observed during 10 min at 37°C. The abscissa shows the apparent cellular phenylalanine level at 5 min. Reproduced from the Journal of Biological Chemistry (32), with permission.

THE \(\beta\) SYSTEM

Defined for: Ehrlich cell

Substrates: Taurine, \(\beta\)-alanine, N-Me derivatives and analogs. Sulfonic group acceptable

Reversibility: Scarcely detectible. Exchange not detected.

Na\(^+\) dependency: Not first-order in pigeon erythrocyte, although about one sodium ion migrates for each amino acid molecule.

Other occurrences: Kidney, rabbit reticulocytes, avian erythrocytes. Ubiquitous?

Fig. 12. Summary of observations for the \(\beta\)-system.
III. Summary

Although we have used amino acids to illustrate the problems of the kinetics of mixed systems in transport, the problem seems unlikely to be limited to this class of substrates. For many classes of metabolites, homogeneity in transport has often been taken for granted, as indeed it was for some years in the neutral amino acids. Mediated intestinal transport of monosaccharides is not limited to sugars that compete with α-glucose for absorption. The mediated migration of the alkali metals into yeast shows kinetic evidence of heterogeneity that has not yet been fully analyzed (41). Indeed, one component of Na⁺ migration from the Na⁺-rich yeast cell depends on the presence of amino acids (42), and in the pigeon red blood cell the complex of the amino acid with the ASC site appears to be an efficient exchange-diffuser for Na⁺ (7), especially in presence of threonine or cysteine. Hence we have emphasized in this discussion the general need to deal with heterogeneity in the study of transport. Amino acid transport by the Ehrlich cell serves merely as a model for discovering what kinetic definition is necessary and possible. The wealth of regulatory interactions among catalytic activities proposed for microorganisms emphasizes the need for methods for checking just how interdependent the transport systems are.

References

DYNAMIC THREE-DIMENSIONAL MODEL FOR ENZYMIC TRANSAMINATION

By V. I. IVANOV and M. YA. KARPEISKY, Moscow, U.S.S.R.

CONTENTS

I. Introduction .......................................................... 22
   A. Comparison between Enzymic and Model Systems .................. 22
   B. Hypothesis on the Principles of Enzyme Action .................. 24

II. State of the Active Site of Aspartate Aminotransferase Prior to Interaction with Substrates ........................................... 25
   A. Some Properties of AAT ............................................ 25
   B. Multipoint Attachment of the Coenzyme to the Protein ......... 26
      1. The Covalent Aldimine Bond of PLP with the \( \epsilon-NH_2 \) Group of a Lysyl Residue and Ionic Binding of the Phosphate Group .... 26
      2. Evidence for Binding of the Pyridine Nitrogen Atom to a Proton-Donating Group of Apo-AAT .................................. 27
      3. On the Interaction of the 2-Methyl Group of PLP with the Protein .................. 30
      4. Plausibility of Electrostatic Interactions Involving the Phenolic Group of PLP and the Apoenzyme ............................ 32
   C. On the Significance of Multipoint Binding ...................... 33

III. Detailed Mechanism of Enzymic Transamination .................. 34
   A. Electronic and Stereochemical Aspects of the Formation of Substrate Aldimines of AAT ............................................. 34
   B. Peculiarities of the Enzymic Aldimine–Ketimine Transition .... 39
   C. Remaining Stages of the Reaction .................................. 41
   D. Explanation of Some Properties of AAT Based on the Proposed Model .......................... 41

IV. Verification of Some Implications of the Model ................. 43
   A. Role of the Coenzyme Methyl Group ............................. 43
   B. Simulation of the Proton Accepting Stage by the Action of Light upon AAT ........................................ 44
   C. Change of Coenzyme Surroundings in the Course of the Enzymic Reaction ........................................... 46
      1. Identification of the Enzyme–Substrate Intermediates with the Aid of Circular Dichroism (CD) .......................... 46
      2. The CD Evidence for Changes in Mutual Orientation of the Coenzyme Ring and Protein in the Course of Substrate Aldimine Formation ........................................ 50
      3. Alternation in the Ionization States of the Proposed Tyrosyl Group at Different Stages of the AAT-Catalyzed Reaction .............. 50

V. Conclusion: Interpretation of the Hypothesis “Stabilization–Orientation–Change of Conditions by Positional Change” as Applied to Other Enzymes .......................... 51

References ............................................................... 52
I. Introduction

A. COMPARISON BETWEEN ENZYMIC AND MODEL SYSTEMS

To evaluate the degree of efficiency of enzymic catalysis one must compare the rate of an enzymic reaction to that of a corresponding reaction proceeding without an enzyme. Nonenzymic systems simulating an enzymic process are designated "model systems." A model system that realizes the reaction under consideration through the same intermediates as the enzyme is called a "congruent model system." It is not obvious a priori that such congruent systems should exist. In principle it may be supposed that enzymic reactions proceed by mechanisms radically different from ordinary chemical reactions. Such a supposition appears unlikely at the present, since congruent model systems for a number of enzymic reactions have been found and studied.

One such reaction is the transamination of α-amino acids discovered by Braunstein and Kritzman (2):

\[
R_1\text{--C--COOH} + R_2\text{--C--COOH} \rightleftharpoons R_1\text{--C--COOH} + R_2\text{--C--COOH} \quad (1)
\]

It is catalyzed by pyridoxal phosphate (PLP)-dependent enzymes—the aminotransferases. A general mechanism for reactions involving PLP has been proposed by Braunstein and Shemyakin (3). Metzler, Ikawa, and Snell carried out similar reactions in nonenzymic systems containing heavy metal ions in addition to PLP and substrates, and independently suggested an analogous mechanism (4). On the basis of this general mechanism the reaction of transamination can be formulated by the following scheme (Schlenk and Fisher (5), the "shuttle" mechanism) (equation 2).