Transporters for Cationic Amino Acids in Animal Cells: Discovery, Structure, and Function

R. DEVÉS AND C. A. R. BOYD

Programa de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Universidad de Chile, Santiago, Chile; and Department of Human Anatomy and Genetics, University of Oxford, Oxford, United Kingdom

I. Introduction

The transport of cationic amino acids across mammalian cell membranes was, for a long time, thought to occur through a single transporter referred to as system y⁺. Thus, for more than 20 years, evidence related to mechanistic or physiological questions was interpreted assuming that basic amino acids were transported largely by this system in a number of different cell types (reviewed in Refs. 44, 59, 90, 194, 237, 253). In the past few years, the use of new molecular and kinetic experimental approaches has unveiled a more complex picture, involving various clearly distinct transporters that show differences in structure, substrate specificity, mechanism, site of expression, and regulation (reviewed in Refs. 18, 52, 123, 125, 127, 129, 157, 158, 159, 208, 218). These findings have raised new questions, as...
well as the possibility for new interpretations of early observations.

Here we review information generated in different areas in which understanding of cationic amino acid transport is being developed, from the molecular to the kinetic and from the strictly mechanistic to the physiological.

We start by discussing from a historical perspective the functional description of several transport systems. Our aim is not to provide a historical record, but rather to revisit some of the classical papers in the light of the new observations. In general, emphasis has been placed on primary experimental evidence rather than on the original interpretations of the findings at the time when they were reported, and thus we have not held back from showing the data from relevant early studies.

We also present the new molecular evidence that has led to the description of two families of proteins that are involved in cationic amino acid transport (recently reviewed in Refs. 52, 123, 127, 158, 159, 208). This work has given us the primary amino acid sequences of a number of molecules about which nothing was known 7 years ago. In addition, it has suggested their putative membrane topologies, shown their sites of expression, and in some cases, shed light on novel transport mechanisms. We have sought to relate these proteins to the transport systems previously identified on the basis of functional studies, some of which have been carried out over the last three decades (reviewed in Refs. 44, 194), and others only much more recently (64, 222, 226). Then, with both the molecular and kinetic evidence at hand, we discuss the functional and, when possible, the mechanistic properties of the different transporters.

This analysis is preceded by a discussion of the kinetic methodology that can be used, first, to discriminate between transporters that operate in parallel and, second, to disclose the interactions between a transporter and its substrates. This approach should be useful in the design of future experiments, not only in relation to amino acid transport, but in other areas of transport physiology as well. It might be argued that molecular methods will obviate the problems of interpretation, which result from the presence of multiple transporters with overlapping specificities. However, this limitation also applies to transport studies in expression systems, and it is especially important in the design of successful cloning strategies.

Finally, we have reviewed selected areas of physiology in which the function of a given tissue is directly influenced by the activity of one or more cationic amino acid transporters. The biological importance of these is exemplified in relation to a variety of topics including nutrition, endocrinology, immunology, and genetic disease. The discovery that nitric oxide (NO) plays a central role as a mediator of both regulatory and cytotoxic functions and the fact that arginine is its immediate precursor place cationic amino acid transport in a new context (150).

In view of the current widespread availability of bibliographic data bases, we have not attempted a comprehensive review. Instead, we have discussed with detail those observations that appeared more relevant to us, with special emphasis in the experimental strategies that have been used in disclosing the new facts. That is why original experiments are amply shown.

More generally, this review of a tightly defined topic exemplifies the nature of scientific discovery as physiology encounters molecular biology. As the “postsequencing era” in biology and medicine emerges, the subject of this review provides a paradigm for the unpredicted different ways in which gene function has been discovered; we have tried to let the reader see the primary evidence for this.

II. IDENTIFICATION OF DISTINCT TRANSPORT ACTIVITIES FOR CATIONIC AMINO ACIDS

Traditionally, amino acid transport systems have been classified considering two main criteria: 1) the substrate specificity, i.e., which amino acid or group of amino acids is transported by the system, and 2) the Na\(^+\) dependency of the rate of transport, generally defined with reference to the rate measured in the presence of K\(^+\) or choline salts. Four transport systems for cationic amino acids have been defined according to these criteria, and they are listed in Table 1.

Our definition of a “cationic amino acid transport system” applies to transporters exhibiting affinities and translocation rates for cationic amino acids, which are higher than or equivalent to those for other types of amino acids. Thus transporters that interact only weakly with dibasic amino acids, such as systems ASC (254) and asc (74), will be excluded from any further analysis.

Only one of the four systems (\(y^+\)) listed in Table 1 is selective for cationic amino acids (although it does present weak interaction with neutral amino acids) (48, 51,

<p>| Table 1. First description of cationic amino acid transport systems |
|-------------------------|-------------------|----------------------|</p>
<table>
<thead>
<tr>
<th>System</th>
<th>(Na^+) Dependence</th>
<th>Characteristic Substrates</th>
<th>Described in</th>
</tr>
</thead>
<tbody>
<tr>
<td>(y^+)</td>
<td>–</td>
<td>Lys, Arg, Orn</td>
<td>Reticulocytes, fibroblasts</td>
</tr>
<tr>
<td>(b^{L, +})</td>
<td>–</td>
<td>Lys, Arg, Leu</td>
<td>Blastocysts</td>
</tr>
<tr>
<td>(y^+L)</td>
<td>+ (CAA)</td>
<td>Lys, Arg</td>
<td>Erythrocytes</td>
</tr>
<tr>
<td>(y^+)</td>
<td>+ (NAA)</td>
<td>Leu, Met, Gln</td>
<td></td>
</tr>
<tr>
<td>(B^3+)</td>
<td>+</td>
<td>Lys, Arg, Ala, Val, BCO</td>
<td>Blastocysts</td>
</tr>
</tbody>
</table>

CAAs, cationic amino acids; NAA, neutral amino acids; BCO, 3-amino-endo-bicycle[3.2.1]octane-3-carboxylic acid; +, positive; –, negative. References are as follows: system \(y^+\), Christensen and Antonioli (48); White et al. (239); system \(b^{L, +}\), Van Winkle et al. (222); system \(y^+L\), Devés et al. (64); system \(B^3+\), Van Winkle et al. (226).
The other three systems (B\textsuperscript{0, +}, b\textsuperscript{0, +}, and y\textsuperscript{-L}) accept a wider range of substrates, including cationic and neutral amino acids; they differ, however, in their interactions with inorganic monovalent ions. System B\textsuperscript{0, +} is Na\textsuperscript{+} dependent and will not operate at an appreciable rate, unless this cation is present (226). System b\textsuperscript{0, +} is Na\textsuperscript{+} independent, and it functions in the presence of Na\textsuperscript{+}, K\textsuperscript{+}, choline, or Li\textsuperscript{+} salts (222). Finally, system y\textsuperscript{-L} exhibits a more complex pattern in its cation interaction; whereas the transport of lysine through this system is unaffected by Na\textsuperscript{+} replacement, the affinity of system y\textsuperscript{-L} toward neutral amino acids is dramatically decreased if Na\textsuperscript{+} in the medium is substituted with K\textsuperscript{+} (64).

In this section, we examine the first evidence that led to the identification of these four transport systems.

**A. System y\textsuperscript{+}: the Na\textsuperscript{+}-Independent Cationic Amino Acid Transporter**

The concept of system y\textsuperscript{+} as a Na\textsuperscript{+}-independent transporter for cationic amino acids, which interacts less strongly with neutral amino acids (but only when Na\textsuperscript{+} is present), originated from the early work of Christensen and co-workers (48, 49) with Ehrlich cells and reticulocytes. Afterward, it became the paradigm for cationic amino acid transport and was extended to nearly all other cells and tissues analyzed (see Ref. 237 for a comprehensive review). The more recent evidence showing that some transport systems are able to recognize cationic and neutral amino acids with comparably high affinity, whereas others exhibit a marked preference for cationic amino acids (64, 222, 226), poses the question as to whether the earlier observations reflect the operation of a single transport entity (system y\textsuperscript{+}). It is possible that the properties originally attributed to system y\textsuperscript{+} represent the sum of at least two separate transport activities, differing in their affinities toward neutral amino acids.

The importance of this question calls for a reexamination of the early data, especially considering that 30 years ago the existence of multiple transport systems for cationic amino acids was also considered, although later rejected, by Christensen and co-workers (42, 50) in studies with Ehrlich cells.

In the original paper describing the entry of labeled lysine into Ehrlich cells, Christensen (42) noted that lysine influx was partially, but substantially (60−70%), inhibited by phenylalanine, whereas lysine was able to eliminate a small portion of phenylalanine uptake (20%) (Fig. 1). It was concluded that there was a “lysine accepting system” that could also recognize neutral amino acids with specificity resembling that of system L for neutral amino acids (leucine, methionine, and phenylalanine); thus the authors referred to this system as a “positive-charge tolerant variant of the L system” and tentatively named it “system L\textsuperscript{+}.”

In a subsequent paper (51), it was shown that at least two pathways for cationic amino acids could be distinguished: the former “lysine-accepting agency” (L\textsuperscript{+}) and a second route, which resisted inhibition by phenylalanine, referred to as the “lysine-prefering agency.” The latter system was by definition selective for cationic amino acids and was named “Ly\textsuperscript{+}.”

---

**FIG. 1.** Cationic and neutral amino acids compete for transport in Ehrlich cells. A: inhibition of L-[\textsuperscript{14}C]lysine (1 mM) influx by phenylalanine. B: inhibition of L-[\textsuperscript{14}C]phenylalanine (1 mM) influx by lysine. Arrow indicates additional inhibition produced by 10 mM aminoisobutyric acid (AIB) and L-phenylalanine. External medium was Krebs-Ringer bicarbonate solution (pH 7.4, 37°C). [Modified from Christensen (42).]
The investigation of lysine transport was then extended to rabbit reticulocytes (48). A saturable transport process for lysine, arginine, and ornithine could also be demonstrated in these cells, but the observations differed in two important respects from those in Ehrlich cells: 1) the strength of the interaction of this system with neutral amino acids such as leucine, phenylalanine, and methionine was much lower [the apparent inhibition constants \((K_i)\) for these amino acids in reticulocytes were \(\sim 50\) mM]; and 2) cationic amino acids did not affect the influx of neutral amino acids. The authors concluded, correctly, that there was no basis to propose the existence of a system whose receptor site did not differentiate between cationic and neutral side chains. However, instead of recognizing the differences between the transport phenotypes of Ehrlich cells and reticulocytes, an attempt to unify the observations in these two experimental systems was made. This led to the proposal that the movement of cationic amino acids across these membranes occurred via a single transport system (system \(L\)) that was able to bind cationic and neutral amino acids (50). Interestingly, in both types of cells, the association with neutral amino acids was reduced when \(\text{Na}^+\) in the medium was replaced by choline (48, 50), but as discussed in section IVB, they showed differences in their interactions with \(K^+\) (213).

We now reexamine the evidence that led Christensen and co-workers to reject their original hypothesis which stated 1) that two transport systems for cationic amino acids, differing in their specificities toward neutral amino acids, were necessary to account for the transport properties of Ehrlich cells; and 2) that one of these systems was able to transport with equivalent efficiency both neutral and cationic amino acids.

First, the inhibition of lysine influx by amino acids with nonpolar side chains of medium size (phenylalanine, leucine, and methionine) was initially associated with the operation of system \(L\) (51). In a logical next step, the interaction between cationic substrates and the recently discovered cyclic amino acid analog 2-amino-endo-bicyclo[2.2.1]heptane-2-carboxylic acid (BCH), a specific substrate for system \(L\), was investigated (50). It was found that the influx of BCH into Ehrlich cells was not affected by lysine. It was concluded (correctly) that “cationic amino acids have exceedingly little effect on uptake by the \(L\) system” (cited from Ref. 50). However, the experiment did not give evidence against a system that would transport lysine, methionine, leucine, and phenylalanine (but not BCH), and this possibility was not recognized.

Second, homoarginine was found to be a weak inhibitor of phenylalanine fluxes, affecting \(3\text{–}8\%\) of the flux, although most of this effect was already apparent at \(3\) mM homoarginine (50). Phenylalanine, on the other hand, inhibited a larger fraction of homoarginine flux (60%) (49). The lack of correspondence in the magnitude of these two effects was interpreted as reflecting the inability of neutral amino acids to interact with the cationic amino acid transporter. Again, although the experiment showed that cationic amino acids did not affect the major route for neutral amino acid entry (presumably system \(L\)), it did not rule out the existence of a broad-scope system (such as \(L^+\)) in Ehrlich cells.

Independent evidence for a transport system with marked selectivity toward cationic amino acids (as found for reticulocytes) is to be found in the work of White et al. (239) with cultured human fibroblasts. It was here that the term \(\text{system } y^+\) was introduced to indicate that the system did not serve for lysine exclusively, but also for arginine, ornithine, and other cationic analogs. Arginine influx into human fibroblasts was seen to be independent of \(\text{Na}^+\) and \(\text{pH}\) and was inhibited by neutral amino acids with low affinity (Fig. 2). Homoserine was significantly more effective than serine or glutamine, and these interactions were undetectable when \(\text{Na}^+\) was replaced by choline. This behavior resembles more closely the observations in reticulocytes than those in the Ehrlich cells. Further evidence suggesting that the main pathways for cationic amino acids in Ehrlich cells and rabbit reticulocytes do not represent the same activity comes from a study of the structural selectivity in the interaction of neutral amino acids and alkali metal ions with the cationic transporters in the two cell types (213). These observations are discussed in section IVB.

We believe, therefore, that the current idea that cationic amino acid transport in Ehrlich cells, reticulocytes, and fibroblasts occurs through a single transport system (239) is not consistent with the experimental data. We propose that the designation “system \(y^+\)” be reserved for the transport of cationic amino acids via a \(\text{Na}^+\)-independent route showing marked specificity for positively charged substrates. This system would account for most of the flux in the case of reticulocytes and fibroblasts, but only a smaller fraction of the flux in the case of Ehrlich cells.

As discussed in sections IVB and IVC, independent evidence, obtained more recently, supports the notion that distinct cationic amino acid transporters differing markedly in their interactions with neutral amino acids coexist in various cell types (64, 222, 226).

### B. Systems B\(^{0,+}\) and b\(^{0,+}\): the \(\text{Na}^+\)-Dependent and \(\text{Na}^+\)-Independent Broad-Scope Transporters

In the course of transport studies in mouse blastocysts, Van Winkle and co-workers (222, 226) identified two novel transport systems carrying both neutral and cationic amino acids, but differing in their requirements for \(\text{Na}^+\): system \(B^{0,+}\) is \(\text{Na}^+\) dependent, and system \(b^{0,+}\) is \(\text{Na}^+\) independent.

The existence of system \(B^{0,+}\) became apparent when
Lysine was found to be an effective competitive inhibitor of L-alanine uptake, and the kinetic parameters for these two amino acids, measured either directly or in transport-inhibition experiments, were consistent with the conclusion that they shared the same pathway (Table 2). Other cationic amino acids were also found to be good inhibitors of alanine influx, and the transporter was shown to interact with bicyclic amino acid analogs, such as 3-aminoendo-bicyclo[3.2.1]octane-3-carboxylic acid (BCO) and BCH. The difference of these interactions from those exhibited by any of the previously described systems (A, ASC, L, or y⁺) justified the designation of system B⁰⁺⁺ as a newly identified transporter.

During these studies, it was observed that a substantial portion of mediated lysine influx persisted in the absence of Na⁺, suggesting that a Na⁺-independent system may be operating in parallel to the Na⁺-dependent system B⁰⁺⁺. This possibility was explored by Van Winkle et al. (222) in a later paper. It was found that the lysine flux measured in a Li⁺-containing medium was substantially inhibited by 10 mM arginine and leucine. In a mirror experiment, leucine influx (in the presence of choline) was found to be inhibited by 10 mM lysine and arginine. Cyclic amino acid analogs (BCH, BCO) did not affect the rate under the same conditions. The analysis of the effect of varying concentrations of unlabeled amino acids on the flux of radioactive lysine or leucine in (Li⁺ medium) (Fig. 3) led to the identification of a Na⁺-independent and broad-scope transporter that showed high affinity for leucine and lysine. The transporter was designated system b⁰⁺⁺ and accounted for most of the leucine (88%) and lysine (98%) flux at low concentration (1 μM). A “cation-prefering” system was also identified that carried only a small fraction (2%) of the lysine flux (226).

C. System y⁺L: the Cation-Modulated Broad-Scope Transporter

More recently, a kinetic study of the partial inhibition of lysine influx by neutral amino acids led Devés et al.

| TABLE 2. Kinetic parameters for the transport of L-lysine, L-alanine, and BCO through system B⁰⁺⁺ in mouse blastocysts |
|-------------------------------------------------|-----------|-----------|-----------|-----------|
|                   | V\text{max} | K\text{m} | K\text{i} |
|                   | (fmol/embryo⁻¹/min⁻¹) | (μM) | (μM) | (μM) | |
| L-lysine            | 150        | 140       | 35        | 101      | 320      |
| L-alanine           | 190        | 35        | 110       | 101      | 110      |
| BCO                 | 48         | 430       | 29        | 110      |         |

V\text{max}, maximum velocity; K\text{m}, Michaelis constant; K\text{i}, inhibition constant. K\text{m} values were measured using 3 different concentrations of labeled substrate and 2 or 3 different concentrations of unlabeled analog. Data were analyzed on the basis of Dixon plots. External medium contained 140 mM Na⁺. [From Van Winkle et al. (226).]
(64) to identify a novel transport system for cationic amino acids in human erythrocytes. The transporter was designated system $\gamma^+$L. Neutral amino acids were found to inhibit lysine (1 $\mu$M) entry, with the effect reaching a maximum at $\sim$50% of the original flux (Fig. 4A). On the basis of a kinetic analysis of the effect of leucine on lysine
influx (at varying substrate concentrations) (Fig. 4B) (see sect. IV A), it was shown that lysine enters the erythrocytes through two transporters: 1) a high-affinity low-capacity transporter, which recognizes leucine and lysine with comparable affinities (system y$^+$L), and 2) a lower affinity high-capacity transporter, which is specific for cationic amino acids (system y$^-$) (64). This proposal subsequently received support from inactivation experiments with the sulfhydryl reagent N-ethylmaleimide (NEM), which selectively inhibited system y$^+$, thus permitting the study of system y$^-$L in isolation (63).

The substrate specificity of system y$^-$L, in the presence of Na$^+$, resembles that of system b$^{0,+}$ described in section II B, but the two systems differ in their interactions with monovalent inorganic cations. Whereas system b$^{0,+}$ is Na$^+$ independent, the specificity of system y$^-$L varies depending on the ionic composition of the medium. In Na$^+$ or Li$^+$ medium, system y$^-$L interacts strongly with both neutral and cationic amino acids, but if these ions are replaced by K$^+$, the affinity for neutral amino acids is dramatically decreased (9, 63). This behavior is illustrated in Figure 5. It is very important for the correct identification of system y$^-$L to keep in mind that, as shown in Figure 5, inset, at sufficiently high concentrations of neutral amino acids, inhibition will be observed in the absence of Na$^+$. Thus the concentrations of amino acids used when searching for this activity must be appropriate.

III. IDENTIFICATION OF COMPLEMENTARY DEOXYRIBONUCLEIC ACID SEQUENCES ENCODING PROTEINS INVOLVED IN CATIONIC AMINO ACID TRANSPORT

The experimental possibilities made available by recombinant DNA technology have led in recent years to the molecular identification of various proteins involved in the transport of cationic amino acids. Products of two types of genes have been identified: 1) the cationic amino acid transporters (CAT), encompassing four homologous proteins [CAT-1, CAT-2A, CAT-2(B), and CAT-3] that accept arginine, lysine, and ornithine as substrates (reviewed in Refs. 52, 103, 123, 125, 127) and 2) the broad-scope amino acid transport proteins (BAT), with two members (rBAT/D2 and 4F2hc), whose expression induces transport of both cationic and neutral amino acids in Xenopus laevis oocytes. The acronym rBAT was originally developed to represent “related to b$^{0,+}$ amino acid transporter.” It has been suggested that the proteins in this group are not transporters but are transport activators. For this reason, they are here referred to as “transport proteins” (reviewed in Refs. 18, 157-159).

In this section we discuss the structural properties of these proteins and briefly mention some of their distinctive functional characteristics. The kinetic properties of the transport activities induced by these proteins are examined with more detail in section IV.

A. Cationic Amino Acid-Specific Transporters: the CAT Family

1. mCAT-1: the constitutive and widely distributed cationic amino transporter

A) IDENTIFICATION. In 1991, Kim et al. (110) and Wang et al. (230) discovered that, when expressed in Xenopus oocytes, the membrane receptor for ecotropic murine leukemia viruses (ecoR) induced cationic amino acid transport. Albritton et al. (6) had previously cloned the cDNA...
FIG. 5. Interaction of system y′L with leucine, but not lysine, is affected by inorganic monovalent cations in human erythrocytes. L-[14C]lysine (1 μM) influx in presence of varying concentrations of unlabeled L-lysine (A) or L-leucine (B). External medium (pH 6.8, 37°C) contained either Na+/ (154 mM NaCl, 5 mM sodium phosphate), K+/ (154 mM KCl, 5 mM potassium phosphate), or Li+/ (154 mM LiCl, 5 mM potassium phosphate). Calculated inhibition constants are as follows (in mM): $K_i \text{Lys} = 9.5 \pm 0.67$ (Na+/), 8.65 ± 0.33 (K+), 9.83 ± 0.7 (Na+), 4.52 ± 0.26 (Li+). [Redrawn from Devés et al. (63).]

encoding ecoR by transfection of mouse DNA into human cells and selection for susceptibility to ecoR infection. It was noticed that the predicted transmembrane topology of the protein encoded by ecoR was homologous to that of various membrane transport proteins in Saccharomyces cerevisiae including the gene products Can1 (97), Ctr (153), and Hip1 (207), which are arginine, choline, and histidine transporters, respectively. As expected from this finding, injection of mRNA, prepared from in vitro transcription of ecoR cDNA into Xenopus oocytes, was able to induce amino acid transport activity (110, 230). Thus expression of a functional receptor resulted in the selective induction of arginine, lysine, and ornithine uptake. The overall transport properties and the expression pattern of ecoR were compatible with system y′+L. To reflect its cellular function, the virus receptor was renamed mCAT-1 (mouse cationic amino acid transporter) (53).

Alternative names have been used to describe the genes encoding the mouse and human CAT-1 proteins or the gene product itself (see Table 3). In this review, CAT-1 is used throughout.

B) STRUCTURE. The mCAT-1 transporter is a single polypeptide of 622 amino acids and a relative molecular mass of 67 kDa. On the basis of sequence analysis, the protein was initially predicted to contain 14 membrane-spanning domains (6). With the consideration that the putative NH$_2$ terminus lacked a leader sequence, a structural model was proposed that placed the NH$_2$ terminus on the cytoplasmic side and assumed that every hydrophobic domain was membrane spanning. As pointed out by Closs (52), this model is supported by the finding of similarities in the localization of the transmembrane segments 1 through 7 of mCAT-1 and the protein permeases for yeast and fungi (203).

An alternative model assuming 12 potentially membrane-spanning segments has been proposed (107, 179). This model considers the evolutionary relationship of mCAT-1 with members of the APC family of transporters (arginine, polyamines, choline) as well as the conserved nature of structural features to make the prediction. The permeases in this family contain 9–12 hydrophobic domains and have been shown to exhibit ~20% identity to mCAT-1 (179). The model is consistent with the proposal that the mCAT-1 sequence may have arisen from duplication of a primordial gene that encoded a six transmembrane sequence, since transmembrane region 1 exhibits some homology with transmembrane region 7 as do transmembrane regions 6 and 12. Similarities have also been observed between the extracellular loops 3 and 6 (107).

As discussed by Closs (52), evidence supporting the 14 transmembrane model has also come from immunostaining of unpermeabilized cells with antibodies raised against peptides present in the third and fourth putative extracellular loops in the 14 transmembrane model (244). This finding argues against the 12 transmembrane model, for in this case, the corresponding peptide is located intracellularly. Support for the 14 transmembrane model has also been sought in analyses of N-glycosylation (reviewed in Ref. 52). Two putative N-linked glycosylation sites (Asn-223 and Asn-229) conserved in all CAT proteins are present in the third extracellular loop. Another potential glycosylation site (Asn-373) is exposed extracellularly in the 12 transmembrane segment model but intracellularly in the 14 transmembrane segment model. Mutation of Asn-223 and Asn-229 to histidine results
<table>
<thead>
<tr>
<th>Alternative names</th>
<th>mCAT-1, ecoR, ERR, MLV-R, ATRC1a, H13a</th>
<th>mCAT-2, CAT2α</th>
<th>Tea, mCAT-2, CAT2β</th>
</tr>
</thead>
<tbody>
<tr>
<td>First described as</td>
<td>Receptor for ecotropic MuLV</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Chromosomal localization</td>
<td>13 (Human)</td>
<td>8 (Human)</td>
<td>8 (Human)</td>
</tr>
<tr>
<td>Number of amino acids</td>
<td>622</td>
<td>657</td>
<td>657 (Human)</td>
</tr>
<tr>
<td></td>
<td>629 (Human)</td>
<td>657</td>
<td>657 (Human)</td>
</tr>
<tr>
<td>Identity, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With mCAT-1</td>
<td></td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>With mCAT-2A</td>
<td></td>
<td>61</td>
<td>97</td>
</tr>
<tr>
<td>Predicted molecular mass, kDa</td>
<td>67</td>
<td>71.8</td>
<td></td>
</tr>
<tr>
<td>N-glycosylation</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Predicted TMs</td>
<td>12 or 14</td>
<td>12 or 14</td>
<td>12 or 14</td>
</tr>
<tr>
<td>First identified in</td>
<td>(Ubiquitous)</td>
<td>Liver</td>
<td>Activated T cells/macrophages</td>
</tr>
<tr>
<td>( K_m ) (L-Arg), mM</td>
<td>0.07–0.25</td>
<td>2.15–5.2</td>
<td>0.19–0.25</td>
</tr>
</tbody>
</table>

CAT, cationic amino acid transporter. The term mCAT-2 has been used by different groups to designate 2 distinct mCAT-2 isoforms (53, 102). In this review, we follow the convention recently used by Closs (52) according to which the isoform expressed in liver is designated mCAT-2A and the isoform expressed in lymphocytes and macrophages is mCAT-2(B). * Human gene. Depending on the algorithm used to analyze the structure, 12 or 14 transmembrane domains (TMs) are predicted (see text). A range of independent determinations is given for \( K_m \) values; individual determinations were listed recently by Closs (52).

in a protein that migrates with the same mobility as the N-glycosidase-treated wild-type mCAT-1. This suggests that Asn-373 is nonglycosylated (although not necessarily intracellular). In both models, the viral binding site is located in the third extracellular loop of mCAT-1 (5, 251).

The complete amino acid sequence and the putative organization of mCAT-1 in the membrane (according to the 12- and 14-transmembrane segment models) are shown in Figure 6.

**C) FUNCTION.** Injection of mCAT-1 cRNA into X. laevis oocytes was found to induce cationic amino acid uptake (110, 230). Transport activity was followed by measuring either uptake of radioactively labeled substrates (Fig. 7) or cationic amino acid-specific inward currents (Fig. 8). In both functional expression assays, transport of L-arginine, L- ornithine, and L-lysine was found to be Na⁺ independent and saturable [Michaelis constant (\( K_m \)) values 70–100 μM]. Radiolabeled arginine uptake (100 μM) was inhibited by unlabeled L-cationic amino acids, but not by L-neutral amino acids (glutamine, serine, homo serine, and 2-methylaminoisobutyric acid) or D-isomers (0–3 mM) (110). Consistently, neutral amino acids were seen to induce currents only at very high concentrations and provided Na⁺ was present. The \( K_m \) for L-cysteine was 24.7 mM and that for L-homoserine was ~10 mM (230).

Increase in the binding of gp70 (the murine ecotropic viral coat protein that binds to the receptor) was also observed in injected oocytes, showing that expression of a functional receptor protein coincides with the increase in L-arginine uptake (110, 230).

**D) TISSUE DISTRIBUTION.** Expression of mCAT-1 has been found in all tissues investigated with the exception of the liver. Two transporter transcripts (7 and 7.9 kb) that result from use of alternative polyadenylation sites have been reported. Greatest expression was found in the testis (which synthesizes large amounts of arginine- and lysine-rich proteins), but transcripts were also present in bone marrow, brain, stomach, spleen, kidney, lung, ovary, uterus, large and small intestine, thymus, heart, and in trace amounts in skeletal muscle and skin (102, 110). The complete amino acid sequence and the putative organization of mCAT-1 in the membrane (according to (102), and the smallest (\(< 6 \) kb) appears to be unique to cardiac muscle. The absence of mCAT-1 in liver is supported by the failure of murine ecotropic retroviruses to infect this tissue (56, 92). The tissue distribution of mCAT-1 is listed in Table 4.

**E) HUMAN CAT-1 AND OTHER SPECIES.** A human cDNA, homologous to the murine ecotropic retroviral receptor, was cloned by Yoshimoto et al. (249) from a T-cell line. The human cDNA (originally called H13) presents 87.6% amino acid identity to its murine counterpart, and its sequence predicts a protein with 629 amino acids (~68 kDa). The gene is ubiquitously expressed in human tissues except in liver. The genomic DNA was also isolated from a cosmid library derived from human lymphocytes, and its organization was elucidated; it was mapped to chromosome 13 (249). This gene was cloned independently by Albritton et al. (4), designated ATRC1 and mapped to 13q(12-14).

More recently, a rat homolog was also cloned (170, 245), and it exhibits 95% homology to the mouse gene.

2. mCAT-2: the tissue-specific and regulated cationic amino acid transporters

Two transporters that are structurally homologous to mCAT-1 but differ in either kinetic properties, tissue distribution, or regulated expression have been identified.
**FIG. 6.** CAT transporters. A: deduced amino acid sequence and alignment of mouse cationic amino acid transporters mCAT-1 and mCAT-2A. NH$_2$-terminal residues are numbered 1 [initiating methionine (M) in both sequences] and COOH-terminal amino acids as residues 622 (mCAT-1) or 657 (mCAT-2A). Homology is shown as follows: a vertical solid line shows identity; two dots show strong chemical similarity of residues; one dot shows similarity; no symbol shows residues that are dissimilar (or are not aligned in optimization procedure). Horizontal lines (designated TM for transmembrane) indicate sequences that (on basis of their hydrophobicity) are predicted to span the membrane, and they are numbered I-XIV. Dotted line for TM VII and TM X indicates particular uncertainty as to whether these 2 sequences do traverse membrane (see Fig. 6B and text). Symbol ($\phi$) shown above some asparagine residues (N) indicates potential N-linked glycosylation. Shown in bold (residue 107 or 109 of mCAT-1 or mCAT-2A) is conserved glutamic acid residue (E), which was shown to be essential for transport and sequence YGE (residues 235–237 of mCAT-1) known to be required for viral binding [ecoR].

**B:** 2 alternative models for orientation of mCAT-1 in membrane. Model A: 14 transmembrane segment model (numbered as shown on sequence). Model B: 12 transmembrane segment model, in this model sequences corresponding to TM VII and X are not membrane spanning. Third loop contains viral binding site that is represented by a pale dashed line. Glycosylated sites are represented by Y. Dark dashed line indicates domain (41 amino acids) that determines affinity for substrate; this sequence is extracellular in 14 TM model and intracellular in 12 TM model [Redrawn from Closs (52)].

**C:** a comparison of divergent sequence in 2 protein isoforms from alternately spliced mCAT-2 RNA with equivalent mCAT-1 sequence. Asterisks indicate residues that are identical in all 3 CAT proteins. [Redrawn from MacLeod et al. (125)].
These proteins have been designated mCAT-2 or mCAT-2A (first identified in hepatocytes) (53, 108) and mCAT-2 or mCAT-2(B) (first identified in macrophages and lymphocytes) (54, 102, 108, 124). Here we use the acronym mCAT-2A to refer to the hepatocyte isoform and mCAT-2(B) to the macrophage and lymphocyte isoforms, as recently proposed by Closs (52).

A) IDENTIFICATION AND STRUCTURE. In 1990, MacLeod et al. (126), using a subtraction-differential screening assay, were able to identify several novel cDNA clones from two closely related murine lymphoma cell lines with distinct degrees of phenotypic differentiation. One of these genes was named Tea (T-cell early activation receptor) because it was induced early in the response of normal T cells to mitogens; this gene was mapped to chromosome 8 (124). The gene was not expressed in quiescent splenic T cells but was induced 16-fold when the T cells were activated with the mitogen concanavalin A. The cDNA revealed significant homology with only one protein, the mouse ecotropic retroviral receptor (ecoR) (whose transport function was unknown at the time), but it was substantially shorter. In a subsequent report, the original cDNA sequence for Tea, which had been truncated at the NH₂ terminus, was corrected (179) and shown to encode a protein 658 amino acids long (i.e., containing 205 amino acids more than the original version). Independently, this protein, which showed 61% amino acid sequence identity to mCAT-1, was found to be expressed in lipopolysaccharide-stimulated murine macrophages (55). We refer to this other protein as mCAT-2(B).

Using a Tea probe to screen a mouse liver cDNA library (known not to contain mCAT-1) under stringent conditions, Closs et al. (53) were able to identify a clone encoding for a protein showing 97% identity with Tea, which was designated mCAT-2A. The deduced sequences of the two proteins differ only by 20 amino acids within a stretch of 41 amino acids (Fig. 6). Because a single genomic fragment contains both exons, it has been proposed that the two isoforms [mCAT-2A and mCAT-2(B)] result from mutually exclusive alternate splicing of the...
As discussed in a following section, chimeric proteins have been constructed, and it has been shown that the proteins in this family contain domains that can function independently to control specific aspects of transport and retroviral receptor function (55, 108). Table 3 summarizes the properties of the different transporters in the CAT family.

C) TISSUE DISTRIBUTION. Whereas mCAT-1 is expressed nearly ubiquitously (except in the liver), mCAT-2A and mCAT-2(B) expression is restricted to a more limited number of tissues and cell types. The low-affinity isoform mCAT-2A is found in liver, skeletal muscle (most abundantly post-trauma), skin, ovary, and stomach, and it is absent from brain, large and small intestine, and kidney and quiescent or activated splenocytes. The high-affinity isoform mCAT-2(B) is expressed in lung, brain, activated macrophages and

<table>
<thead>
<tr>
<th>Transporter</th>
<th>mCAT-1</th>
<th>mCAT-2(B)</th>
<th>mCAT-2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>++</td>
<td>++++</td>
<td>--</td>
</tr>
<tr>
<td>Astroglial cells</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steady state</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>LPS + IFN-γ</td>
<td>++++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Heart</td>
<td>+</td>
<td>+/--</td>
<td>+</td>
</tr>
<tr>
<td>Intestine</td>
<td>++</td>
<td>+/---</td>
<td>+</td>
</tr>
<tr>
<td>Large</td>
<td>+++</td>
<td>+/--</td>
<td>---</td>
</tr>
<tr>
<td>Small</td>
<td>+++</td>
<td>+/-</td>
<td>---</td>
</tr>
<tr>
<td>Kidney</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>-</td>
<td>++++</td>
</tr>
<tr>
<td>Steady state</td>
<td>+</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Regenerating</td>
<td>+</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Lung</td>
<td>++</td>
<td>++++++</td>
<td>++</td>
</tr>
<tr>
<td>Macrophages</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Resident</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Activated</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Ovary</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Splenocytes</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Quiescent</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Activated</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Testis</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Thymocytes</td>
<td>+</td>
<td>+++</td>
<td>+--</td>
</tr>
<tr>
<td>Uterus</td>
<td>++</td>
<td>+++</td>
<td>+/--</td>
</tr>
<tr>
<td>Vascular smooth muscle cells</td>
<td>+</td>
<td>++</td>
<td>ND</td>
</tr>
<tr>
<td>Activated with IL-1β + TNF-α</td>
<td>+</td>
<td>++</td>
<td>ND</td>
</tr>
<tr>
<td>SL 12.4 thymoma cells</td>
<td>+++</td>
<td>++++</td>
<td>+/--</td>
</tr>
<tr>
<td>SL 2.3 thymoma cells</td>
<td>+++</td>
<td>+++</td>
<td>--</td>
</tr>
</tbody>
</table>

As modified from Wang et al. (230).

B) FUNCTION. Injection of mRNA derived from these clones into Xenopus oocytes induced, as expected, Na+-dependent and stereoselective cationic amino acid transport (53, 55, 102). However, in spite of the very high structural homology, the mCAT-2A and mCAT-2(B) transporters have been found to differ not only in their tissue distribution, but also in their affinities for the substrate and possibly in other kinetic properties, such as their sensitivity toward trans-stimulation. Whereas in the case of mCAT-2(B) the $K_m$ values for arginine, lysine, and ornithine were in the same range as those measured for mCAT-1 (55, 102), the transporter isolated from liver cDNA library, mCAT-2A, exhibited a marked lower affinity ($K_m = 2$–5 mM) for its cationic substrates and appears to be less responsive to trans-stimulation (see sect. IV B1) (53). This finding coincides with the observations, in hepatocytes, in which saturable arginine transport could not be demonstrated, suggesting that arginine crosses the membrane through a low-affinity route (238).
CATIONIC AMINO ACID TRANSPORTERS

April 1998

The patterns of expression of the mCAT proteins are shown in Table 4.

Recent studies have shown that the expression of these different tissue-specific isoforms may be regulated physiologically. Thus, during liver regeneration after partial hepatectomy, it has been shown (12) that the CAT-1 isoform (rather than CAT-2A) is expressed and can be induced additionally in response to hormones (insulin, glucocorticoids). The pattern of the induction of this gene is that of a classical delayed early response gene. Thus induction is cycloheximide sensitive (i.e., itself requires protein synthesis). There is also posttranscriptional regulation of CAT-1 gene expression and rapid turnover of the induced mRNA, suggesting tight regulation of expression of the protein by a number of separate mechanisms, some of which are tissue specific.

D) HUMAN cDNA. Human sequences for the CAT-2 transporters have also been reported by Closs et al. (54). Analyses of the deduced amino acid sequences of hCAT-2A and hCAT-2(B) demonstrated 90.9% identity with the respective murine proteins. In their functional domains (42 amino acids), the human CAT-2 transporters differ only by one residue from the respective mouse proteins. As expected, the kinetic properties observed for the three isoforms of the transporter encoded in the murine CAT genes were also seen in the human. Hoshide et al. (99) showed that hCAT-2 mapped to chromosomal location 8p21.3. In keeping with data discussed in section V, there were not any mutations in this gene associated with the inherited metabolic disorder lysinuric protein intolerance (LPI).

3. rCAT-3: the brain-specific cationic amino acid transporter

Recently, homology screening of a rat brain cDNA library, with a mCAT-1 probe, led Hosokawa et al. (100) to the identification of a cDNA (rCAT-3) encoding a novel member of the murine CAT family whose expression is restricted to the brain. The gene product has 619 amino acids and a calculated molecular mass of ~67 kDa. The predicted amino acid sequence of rCAT-3 shows 53–58% identity with those of the other members of the CAT family. Transient expression of rCAT3 into COS-7 cells induced system y+/ activity. Arginine uptake (100 nM) was Na+ independent, not affected by neutral amino acids (1 mM), and inhibited by K+-induced depolarization. The $K_m$ for arginine was 103 μM.

B. Broad-Scope Amino Acid Transport Proteins: the BAT Family

1. rBAT and D2: the Na+ independent broad-scope amino acid transport proteins

A) EXPRESSION CLONING. In 1992, a novel class of amino acid transport-related proteins was identified in three separate laboratories by expression cloning from rabbit and rat kidney cDNA libraries. These proteins were designated "neutral amino acid transporter" (209) or D2 (rat kidney) (234) and rBAT (rabbit kidney) (20).

In the first of these studies, Tate et al. (209) failed to recognize the ability of the protein to induce cationic amino acid transport, and it was thought to represent a neutral amino acid transporter. It was noticed, however, that its specificity toward neutral amino acids was not identical to that of system L. Subsequently, Bertran et al. (20) and Wells and Hediger (234) reported the independent cloning of one identical and one homologous sequence and were able to identify this protein as responsible for the induction of cationic and neutral amino acid transport with a specificity analogous to that reported for system b0,+ in rat blastocysts (Fig. 9).

Interestingly, in addition to inducing the uptake of various cationic and neutral amino acids, injection of D2 or rBAT cRNA into Xenopus oocytes was found to induce cystine uptake 200- to 400-fold over control values. Cationic amino acids, in excess, inhibited the uptake of neu-

![FIG. 9. Uptake of L-[3H]arginine (A) and L-[3H]leucine (B) induced by rabbit broad-scope amino acid transporter protein (rBAT), in Xenopus oocytes, is inhibited by various neutral and cationic amino acids.](http://physrev.physiology.org/)

Downloaded from http://physrev.physiology.org/ by 10.220.33.3 on April 18, 2017
FIG. 10. BAT transport proteins. Comparison of amino acid sequence of rat D2 and 4F2hc. NH2-terminal methionine residues are numbered 1 and COOH-terminal amino acids as residues 683 (D2) and 527 (4F2hc). Similarity between 2 sequences is indicated by symbols between optimally aligned sequences (see Fig. 6). Two alternative membrane topologies have been proposed for D2, assuming either 1 TM domain or 4 TM domains. Four putative transmembrane sequences are indicated (TM I to TM IV) by horizontal lines; these are dotted for TMs II, III, and IV, indicating relative uncertainty as to whether these sequences do in fact cross membrane. Potential N-glycosylation sites are indicated (\(\text{Asn-Gly-Asp-Ser} \)).

B) STRUCTURE. D2 and rBAT are single polypeptides of 683 and 677 amino acids, respectively (79% identity). The transporter exists in glycosylated form (84 and 87 kDa), and the unglycosylated form has a molecular mass of 78 kDa. Surprisingly, in contrast to most transport pro-
teins, hydrophobicity analysis does not predict a large number of membrane-spanning segments (Fig. 10). Two alternative membrane arrangements have been proposed. One has a single transmembrane domain (20, 234) and the other four such segments (209).

The first proposal postulates that the rBAT/D2 cDNA encodes a type II membrane glycoprotein, containing a cytoplasmic NH$_2$ terminus, a single transmembrane segment [residues 87–107 (D2); 81–102 (rBAT)], and a N-glycosylated extracellular COOH terminus; there are seven putative N-glycosylation sites in rBAT and D2. Homology has been found between the COOH-terminal domain and a family of carbohydrate-metabolizing enzymes and related proteins that lack the membrane-spanning domain (20, 234). This family includes a maltase-like gene of the mosquito Aedes aegypti (34% identity, 55% similarity) (101) and an oligo-1,6-glucosidase of Bacillus cereus (30% identity, 49% similarity) (233). In the case of the rat isoform (D2), an aspartic acid, which is considered to be part of the catalytic site of amylases and $\alpha$-glucosidases, is conserved. However, catalytic activity could not be demonstrated (234).

In addition, rBAT/D2 shows amino acid sequence homology (~30% amino acid identity) with a type II membrane glycoprotein, the human and murine cell surface antigen 4F2 heavy chain (4F2hc). The two proteins share four amino acid sequence fragments (10–18 amino acid residues) that are highly conserved (67–80% identity) (30, 115, 162, 171, 211). The 4F2 surface antigen is composed of two chains (heavy and light) that are linked by a disulfide bond (see sect. IV). The alternative proposal (141, 209) postulates that the D2 protein has four sequences (each 23–28 amino acids), which are potential transmembrane domains. The second and fourth putative transmembrane domains (residues 390–413 and 588–610, respectively) include charged amino acid residues.

The two alternative models were tested by Moskovich et al. (141) using two different methods. In the first approach, the surface of COS-7 cells, expressing D2, was probed with antibodies directed against putative extracellular and cytoplasmic domains (see scheme in Fig. 10C). Antibodies were added in the presence or absence of Triton X-100 to permeabilize the cells and were then visualized with fluorescent labeling. In the second approach, right-side-out rat renal brush-border membrane vesicles were subjected to limited surface proteolysis by papain, and the fragments were subsequently probed with several site-specific antibodies to determine approximately where the cleavages occurred. Cleavage of D2 with papain produces several fragments that remain firmly embedded in the membrane, and thus both approximations yielded results consistent with the four membrane-spanning topological model (141, reviewed in Ref. 208). The unusual membrane topology that has been predicted for the broad-scope amino acid transport proteins has led to the proposal that rBAT and D2 are not the complete transporters. Three possibilities have been suggested: 1) the carrier could be a homodimer, 2) the cloned polypeptide could be part of a heterologenous carrier that can associate with silent transporters in the oocyte, or 3) it could be a specific activator of an endogenous carrier. Wells and Hediger (234) have suggested that these proteins may undergo dimerization mediated by leucine zippers. In addition, a cysteine residue that may play a role in the heterodimeric structure of the surface antigen 4F2 is conserved in the case of rBAT and D2. This residue is located just COOH terminal to the transmembrane region.

Recently, Wang and Tate (231) obtained evidence suggesting that in the rat kidney and jejunal brush-border membrane, D2 is found in association with a 50-kDa protein, and the association has been shown to involve one or more interprotein disulfide bonds. When brush-border membranes were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions, D2 was seen to migrate partly (15–50%) as a 135-kDa species; this fraction increased to 90% when NEM was included in the buffer. Because the D2 cDNA clone encodes a 85-kDa subunit, it follows that the formation of 135-kDa species must involve association of D2 proteins with ~50-kDa proteins of oocyte origin. Analogous results were obtained in rabbit kidney brush-border membranes.

As discussed by Wang and Tate (231), the proposed oligomeric organization may explain why the expression of D2 in COS-7 cells failed to induce amino acid transport activity (142). It is possible that the 50-kDa or similar proteins required for functionally competent heterodimers are absent in COS-7 cells. This is consistent with the Western analysis of membranes from D2 cDNA-transfected COS-7 cells, which under nonreducing conditions did not show D2 species in the 135- to 140-kDa range (although intense bands corresponding to the D2 monomers were visible). On the contrary, a small but significant amount of disulfide-linked oligomers (135–140 kDa) has been detected in oocytes injected with D2/cRNA, in addition to D2 monomers and higher aggregates (180–200 kDa), the latter presumably representing disulfide-linked homodimers of D2 subunits (142, 231, reviewed in Ref. 208). As pointed out by Tate (208), if the formation of such a heterodimer is a prerequisite for expression of transport activity, the upper limit of transport must then depend on the availability of 50-kDa protein in oocyte. This would explain why, in D2-injected oocytes, 10-fold increase in total D2 (24–48 h after injection) produces only ~1.5-fold increase in the rate of leucine uptake (142).

Further insight into the role of rBAT has come from an analysis of two mutants of the human gene (M467T and M467K) which, as discussed below, are responsible for the genetic defect cystinuria type I (32). Chillarón et
al. (41) showed that both mutants display an intracellular trafficking defect that impairs their transport to the oocyte surface and maintains them in the intracellular location, probably the endoplasmic reticulum. However, a clear lack of correlation between the amount of rBAT protein in the oocyte surface and the induced amino acid transport was observed. Despite the low amount of M467T protein reaching the plasma membrane, the transport activity eventually (at 7 days) became the same as in the wild-type injected oocytes. It was concluded that rBAT is necessary, but not sufficient, for amino acid transport activity.

Recently, to evaluate whether rBAT functions as a component of an amino acid transporter or as a transport activator, COOH-deletion mutants of the human gene were prepared and assayed for their ability to stimulate transport. Several deletions (Δ141–685, Δ261–685, and Δ511–685) were made by Miyamoto et al. (137). Mutant cRNA was synthesized and injected into Xenopus oocytes, and the uptake of cystine, arginine, and leucine was determined. The wild-type cRNA resulted in the induction of system b₀⁺⁺-like amino acid transport. Of the mutants, only Δ511–685 stimulated transport, but the activity in this case matched that of system y⁺⁺L (induced by 4F2hc); that is, mutant Δ511–685 stimulated the transport of arginine and leucine (not of cystine), and only the transport of leucine was Na⁺ dependent. Unfortunately, the authors refer to this activity as y⁺⁺-like. It is interesting to notice that the Δ511–685 deletion in rBAT renders a protein of a similar size to the single transmembrane-protein 4F2hc. This led to the proposal that both rBAT and 4F2hc are likely to have a single transmembrane segment and further that the COOH terminal of rBAT is important in determining its specificity as a transport activator.

In another recent study, Peter et al. (165) suggested that the D2-stimulated activities of both neutral and cationic amino acids appear to consist of, at least, two distinct pathways and that these activities bear functional similarities to transporters in native oocytes that might be activated by formation of complexes with the D2 protein.

C) TISSUE DISTRIBUTION. Clones of D2 and rBAT hybridized intensely to a species of 2.2 kb and weakly to another of 4.4 kb in mRNA from kidney and intestine (rat and rabbit, respectively) (20, 209, 234). A 2.2-kb species was also seen in mRNA from a kidney-derived cell line LLC-PK₁ (234). Because the relative intensities of the bands do not change with stringency, they appear to reflect the use of different polyadenylation sites. At low stringency and after long exposure, bands were seen in pancreas, liver (2.2 kb), heart, brain (2.4 kb), and lung. No hybridization was seen to mRNA from skeletal muscle (20, 209, 234).

The site of expression of D2 in the kidney was studied by Kanai et al. (104) by a combination of in situ hybridization and immunocytochemistry with antibodies that differentiate recognize specific segments of proximal tubule. The D2 antisense RNA hybridized in the same segments that were strongly positive for anti-ectoATPase, but negative both for carbonic anhydrase type IV and the glucose transporter GLUT-2. These findings show that, in the renal proximal tubule, D2 is strongly expressed more distally in the S3 segment, with only weak expression in segments S1 and S2. The signal is absent in all other parts. The S3 expression thus coincides with transport activity for cystine and other amino acids as characterized by microperfusion studies. Renal proximal tubular brush-border membrane localization of D2 was independently confirmed in two other laboratories (81, 167). A less intense labeling was seen in the brush-border membranes of jejunal epithelial cells (167).

As reported by Pickel et al. (167), an unexpected finding of these immunocytochemical localization studies was that, in the intestine, intense D2-specific immunolabeling was seen in a selected population of enteroendocrine cells and enteric neurons. The neuronal labeling was localized within dense-core vesicles in axon terminals opposed to the basal lamina near fenestrated blood vessels. In an extension of this study, Nirenberg et al. (154) reported highly granular immunolabeling for D2 in the chromaffin and ganglion cells of rat adrenal medulla. In addition, labeled varicose processes were detected in brain stem and spinal cord nuclei. Ultrastructural examination of the nuclei of the solitary tract of rats showed that D2 was localized predominantly in axon terminals. Further work is required to characterize the anti-D2 reactive protein in these cells.

D) HUMAN rBAT AND OTHER SPECIES. A human rBAT cDNA was isolated by screening a human kidney cortex cDNA library for expression of Na⁺⁻independent transport of L-arginine in Xenopus oocytes (19). The clone also induces L-cystine and L-leucine uptake, and it is found in kidney, small intestine, pancreas, and liver. The isolated clone (685 amino acids) encodes a 78-kDa protein 85 and 80% identical to rBAT and D2, respectively. In another report, the human rBAT clone was isolated by low-stringency screening of a human kidney cDNA library using the radiolabeled D2 insert as a probe (118). Southern blot analysis of genomic DNA from a panel of mouse-human somatic cell hybrids showed that the gene for human rBAT resides on chromosome 2 (118). Since the discovery that mutations in human rBAT are responsible for one form of cystinuria, there has been an intense study of this human gene (reviewed in Ref. 159); this work is reviewed in section V.

In a recent report, Mora et al. (140) have shown that OK cells (a "proximal tubular-like" cell line, derived from opossum kidney) express a rBAT transcript. Poly(A)+ RNA from OK cells induced system b₀⁺⁺-like transport activity in oocytes, and the induction could be suppressed by hybrid depletion with human rBAT antisense oligonu-
cletides. A polymerase chain reaction-amplified cDNA fragment (~700 bp) from OK cell RNA was shown to correspond to an rBAT protein fragment 65–69% identical to those from human, rabbit, and rat kidneys. To show that the rBAT protein is functionally related to this transport activity, OK cells were transfected with human rBAT antisense and sense sequences. Transfection with rBAT antisense, but not with rBAT sense, resulted in the specific reduction of rBAT mRNA expression and b+c-like transport activity. The activity was further localized to the apical pole of confluent OK cells (140). The properties of the rBAT and D2 proteins are summarized in Table 5.

2. 4F2hc: the cation-modulated and broad-scope amino acid transport protein

The homology (~30% identity) detected between the broad-scope amino acid transport inducing proteins, rBAT and D2, and the heavy chain of the cell surface antigen 4F2hc (Fig. 10) prompted the investigation of the possible involvement of this protein in transport (17, 235). The 4F2 cell surface antigen is induced during the process of cellular activation and remains at constant level in exponentially growing cells (162). It was originally identified by the production of a mouse monoclonal antibody (mAb4F2) against human T-cell lines (93, 95) and mapped to human chromosome 11 (166). The cDNA clones for the human, mouse, and rat heavy chain antigens (4F2hc) have been isolated and are 75% identical at the amino acid level (30, 115, 162, 171, 211). The 4F2hc gene is a member of the family of growth-related genes characterized by a low level of constitutive expression in resting cells and a high level of expression after cell activation or neoplastic transformation (246, 247). It has been recently proposed that 4F2hc is identical to the fusion protein-1 (FRP-1) that regulates virus-mediated cell fusion of monocytes. The FRP-1 is principally detected in concanavalin A- or interleukin-2-stimulated lymphocytes, although it is scanty in resting lymphocytes (155). The pattern of expression of 4F2 in malignant cells (squamous cell carcinomas) has been correlated with the tumor-spreading pattern, differentiation, and metastatic behavior (183).

A) STRUCTURE. The 4F2 surface antigen is constituted by a 85-kDa glycosylated heavy chain covalently linked to a 41-kDa highly hydrophobic light chain. The heavy chain is a type II membrane glycoprotein with cytoplasmic NH2 terminus, a single transmembrane segment, and glycosylated extracellular COOH terminus (115, 162, 171, 211). Northern analysis has demonstrated that its expression is widely distributed in mouse tissues (162).

The rBAT/D2 and 4F2hc show a very similar localization of the single putative transmembrane domain within their sequences (Fig. 10). Neither rBAT nor 4F2hc has the conserved aspartic (or glutamic) acid residues, which are part of the catalytic site for the homologous amylases and a-glucosidases and which are conserved in D2.

B) FUNCTION. As expected, Bertran et al. (17) and Wells et al. (235) found that the injection of 4F2hc mRNA (human) induced a broad-scope amino acid transport activity. However, the expression of 4F2hc produces a smaller increase in basal amino acid uptake (3- to 4-fold) than the expression of rBAT. The uptake of arginine, lysine,

---

**TABLE 5. Properties of the BAT transport proteins**

<table>
<thead>
<tr>
<th>Properties</th>
<th>rBAT</th>
<th>4F2hc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternative names</td>
<td>D2 or NBAT (rat) SLC3A1 (human), D2H (human)</td>
<td>FRP1, CD98</td>
</tr>
<tr>
<td>First described as</td>
<td>Transporter</td>
<td>Surface antigen</td>
</tr>
<tr>
<td>Chromosomal localization</td>
<td>2p 16.3 (SLC3A1)</td>
<td></td>
</tr>
<tr>
<td>Number of amino acids</td>
<td>677</td>
<td>529</td>
</tr>
<tr>
<td>Predicted molecular mass, kDa</td>
<td>683 (D2)</td>
<td>529 (Mouse)</td>
</tr>
<tr>
<td>Glycosylated, kDa</td>
<td>663 (SLC3A1)</td>
<td>527 (Rat)</td>
</tr>
<tr>
<td>Predicted TMs</td>
<td>70 (D2)</td>
<td>26</td>
</tr>
<tr>
<td>N-glycosylation</td>
<td>86 (SLC3A1)</td>
<td></td>
</tr>
<tr>
<td>Half-saturation constants, µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_m ) (L-Arg)</td>
<td>85 ± 7</td>
<td>43</td>
</tr>
<tr>
<td>( K_m ) (L-Leu)</td>
<td>90 ± 12</td>
<td>ND</td>
</tr>
<tr>
<td>( K_m ) (L-Cystine)</td>
<td>41 ± 7</td>
<td>=</td>
</tr>
<tr>
<td>Characteristic tissue or cell*</td>
<td>Kidney, intestine</td>
<td>Lymphocyte, placenta</td>
</tr>
</tbody>
</table>

BAT, broad-scope amino acid transporter protein. For rBAT column, unless indicated, properties listed refer to isoform found in a rabbit kidney cortex cDNA library (rBAT). For 4F2hc column, unless indicated, properties listed refer to human 4F2hc. For predicted TMs, 2 different membrane arrangements have been proposed (see text). \( K_m \) values were measured in rBAT- and 4F2hc-injected *Xenopus laevis* oocytes. Values for rBAT are from Chillarón et al. (40) and for 4F2hc from Wells and Hediger (235). * Cell/tissue in which transport function has been demonstrated (see text). All other references are given in text.
sine, ornithine, leucine, but not cystine was stimulated \([K_m\) for L-arginine was 43 \(\mu M\) (235)]. Interestingly, the Na\(^+\) dependence of cationic and neutral amino acid uptake was found to differ. Whereas lysine and arginine were transported at the same rate in the presence or absence of Na\(^+\), the interaction with neutral amino acids was markedly Na\(^+\) dependent. Thus uptake of L-arginine (15 \(\mu M\), in 4F2hc-injected oocytes, was strongly inhibited by 2 mM L-leucine, L-methionine, or L-homoserine in the presence of Na\(^+\), but not in its absence (N-methyl-D-glucosamine) (Fig. 11). The substrate specificity induced by 4F2hc corresponds to that of system y\(^{+}\)L (9, 64, 72, 73).

C) TISSUE DISTRIBUTION. Northern blot analyses have demonstrated that 4F2hc gene is expressed at relatively high levels in adult testis, lung, brain, kidney, and spleen and at significantly lower levels in liver and cardiac and skeletal muscle (162).

Fei et al. (73) showed that mRNA transcripts for 4F2hc (and not for D2) are expressed at high levels in placenta and JAR cells. Consistently, injection of mRNA isolated from human placental choriocarcinoma cells (JAR) into \(X.\) \(læ\)evis oocytes induced the transport of L-leucine and arginine, but only leucine transport was Na\(^+\) dependent as expected for system y\(^{+}\)L. Moreover, hybrid depletion of JAR cell mRNA using antisense oligomer specific for the 4F2hc RNA completely abolished the induction of y\(^{+}\)L activity in oocytes (73).

A recent report by Broer et al. (30) has proposed that the antisense cRNA transcribed from 4F2hc, when coinjected with poly(A)\(^+\) RNA from C6-BU rat glioma cells, completely suppresses a system “L-like” amino acid transport activity, otherwise induced by poly(A)\(^+\) RNA injection in these cells. The possibility, therefore, that 4F2hc regulates the expression of more than one protein, as has been suggested for D2 by Peter et al. (165), should be considered in future experiments. The properties of the activity induced by 4F2hc are given in Table 5. Figure 12 compares the substrate specificity of mCAT-1, D2, and 4F2hc.

### IV. SPECIFICITY AND MECHANISM

#### A. Kinetic Theory and Methodology

Technical accounts of experimental procedures for measurements of amino acid fluxes have been thoroughly reviewed elsewhere (e.g., Refs. 90, 109, 111). For this reason, the various techniques available for the study of amino acid transport, as well as the biological preparations commonly employed, are not discussed here. This section focuses on experimental strategies (based on assays of function) that have been developed and/or used recently to identify and characterize cationic amino acid transport systems. Two problems are addressed: 1) the determination of kinetic parameters of transporters acting in parallel and 2) the analysis of transport specificity.

1. Determination of transport parameters for substrates interacting with parallel pathways

One of the distinctive features of amino acid transport is the existence of multiple systems with overlapping specificities in a single membrane. Thus the first step in the characterization of amino acid transport, in an intact biological system, requires dissecting the activity of interest.

As has been clearly noted by Christensen (45, 46), finding out whether there is transport heterogeneity is not trivial, and the analysis will be misleading if it rests solely on the examination of substrate saturation curves. He points out that a complex system will give curves corresponding (within reasonable standards of accuracy) to a rectangular hyperbola unless the \(K_m\) values of the parallel systems lie very far apart. For example, two transporters having the same maximum velocity \((V_{max})\) but \(K_m\) values differing by fourfold give a saturation curve that is indistinguishable from that expected for an homogeneous system. Even a 10-fold difference in \(K_m\) may not be clearly

![Figure 11](https://via.placeholder.com/150)

**FIG. 11.** Uptake of neutral amino acids, but not cationic amino acids, in 4F2hc-injected \(Xenopus\) \(læ\)evis oocytes is Na\(^+\) dependent. A: uptake of various \(^{14}\)C-labeled amino acids (15 \(\mu M\)) was measured in presence of 100 mM Na\(^+\) or 100 mM N-methyl-D-glucosamine (open bars). B: effect of 2 mM homoserine (HS), leucine (Leu), and methionine (Met) on L-[\(^{14}\)C]arginine (15 \(\mu M\)) influx. Experiments were performed in presence of either 100 mM Na\(^+\) (solid bars) or 100 mM N-methyl-D-glucosamine (open bars). [Modified from Wells et al. (235).]
pathways that do not differ in their ionic dependence, or that of two substrates that share one transport route, but can move independently through other transporters.

A second approach that has been used exploits the difference in the specificities of the parallel systems. Usually, a substrate analog is used to abolish the entry of labeled substrate by one of the routes. The analog, which may or not be transported, acts as a partial competitive inhibitor. From the comparison of the substrate saturation curves, in the presence or absence of the analog, information regarding the interaction of the substrate with the two pathways has been obtained. It is uncommon, however, to find an inhibitor, especially a substrate analog, that will interact with one of the systems exclusively. The most typical finding is an inhibitor that interacts with different affinities with the two systems. If this is the case, the determination of kinetic parameters by simply subtracting one component of the flux from the total activity, to subsequently analyze the inhibitor-sensitive and inhibitor-insensitive components separately, would not be accurate.

A new method for the kinetic analysis of dual-transport systems, which overcomes these difficulties, has been developed by Devés et al. (64) and applied recently to the study of the two cationic amino acid transporters in human erythrocytes and placenta (64, 72). The method is based on the determination of the “permeability ratio,” a constant that groups together the substrate transport parameters, $V_{\text{max}}$ and $K_i$, for the two transporters (see below). This constant determines the relative contribution that each pathway makes to the flux at low substrate concentration. Two variants of the method have been developed, which differ in the agent used to separate the contribution of the two systems. These are as follows: 1) inhibition with an unlabeled competing analog or reversible inhibitor (64) and 2) inactivation with an irreversible inhibitor (63).

A) DETERMINATION OF TRANSPORT PARAMETERS FOR TWO SYSTEMS ACTING IN PARALLEL USING A REVERSIBLE INHIBITOR OR UNLABELED COMPETING SUBSTRATE. The method relies on the use of an inhibitor or substrate analog that interacts preferentially, although not necessarily exclusively, with one of the transport systems.

1) Kinetic equations: rate equation for a substrate transported through two parallel systems in the presence of a competitive ligand. The rate equation for the transport of a substrate (S) through two transporters (A and B) in the presence of a competitive ligand (I) (located on the cis-side) is given by

$$v = \frac{V_{\text{maxA}} [S]}{K_{mA}(1 + [I]/K_{iA}) + [S]} + \frac{V_{\text{maxB}} [S]}{K_{mB}(1 + [I]/K_{iB}) + [S]}$$

(1)

The total rate of transport ($v$) is given by the sum of the rates through each pathway (the corresponding parame-
ters are identified by subscripts \(A\) and \(B\). Rate and concentration are assumed to be related through a Michaelis-Menten type of function. \(V_{\text{max}}\) represents the maximum rate of transport, \(K_m\) represents the half-saturation constant for the substrate, and \(K_i\) represents the apparent inhibition constant. The same equation applies if \(I\) is either a nontransported inhibitor or a transported substrate analog.

The relative transport rate at varying inhibitor concentrations (\(II\)) can be written as

\[
\frac{v}{v_0} = \frac{V_{\text{maxA}}/V_{\text{maxB}}}{K_{\text{mA}}(1 + [I]/K_{\text{mA}}) + [S]} + \frac{1}{K_{\text{mB}}(1 + [I]/K_{\text{mB}}) + [S]}
\]

where \(v_0\) is the rate of transport in the absence of inhibitor. This equation can also be written as

\[
\frac{v}{v_0} = \frac{F}{1 + [I]/K_{\text{clap}}} + \frac{1}{F + 1}
\]

When expressed in this form, the dependence of the relative transport rate on the inhibitor concentration (at constant substrate concentration) is seen to be determined by three parameters: the apparent inhibition constants for each system (\(K_{\text{clap}}\) and \(K_{\text{clap}}\)) and constant \((F)\), which is the ratio of the rates of transport through the more sensitive pathway \((v_A)\) and the less sensitive pathway \((v_B)\)

\[
K_{\text{clap}} = K_{\text{clap}}(1 + [S]/K_{\text{mclap}})
\]

\[
K_{\text{clap}} = K_{\text{clap}}(1 + [S]/K_{\text{mclap}})
\]

\[
F = \frac{v_A}{v_B} = \frac{V_{\text{maxA}}(K_{\text{mB}} + [S])}{V_{\text{maxB}}(K_{\text{mA}} + [S])}
\]

At very low substrate concentration \([S] \ll K_m\), these parameters are simplified and give directly the two inhibition constants and the substrate permeability ratio \((F_{S\rightarrow A})\)

\[
K_{\text{clap}}(S\rightarrow 0) = K_{\text{clap}}
\]

\[
K_{\text{clap}}(S\rightarrow 0) = K_{\text{clap}}
\]

\[
F_{S\rightarrow 0} = \left(\frac{v_A}{v_B}\right)_{S\rightarrow 0} = \frac{V_{\text{maxA}} \cdot K_{\text{mB}}}{K_{\text{mA}} \cdot V_{\text{maxB}}}
\]

\(F_{S\rightarrow 0}\) represents the ratio of the rates of transport through system \(A\) and system \(B\) at very low substrate concentration. If \(F_{S\rightarrow 0}\) equals one, both systems contribute in the same proportion to the total flux.

At saturating substrate concentration, \(F_{S\rightarrow \infty}\) is directly related to the maximum velocities for the two systems

\[
F_{S\rightarrow \infty} = \left(\frac{v_A}{v_B}\right)_{S\rightarrow \infty} = \frac{V_{\text{maxA}}}{V_{\text{maxB}}}
\]

According to this description, the effect of a competitive analog on the rate of entry of a substrate through two transporters (\(Eq. 1\)) is determined by six kinetic parameters: two substrate half-saturation constants \((K_{mA}\) and \(K_{mB}\)), two maximum rates of transport \((V_{\text{maxA}}\) and \(V_{\text{maxB}}\)), and two inhibition constants \((K_A\) and \(K_B\)). Even if the effect of the analog followed \(Equation\ 1\) strictly, it would be difficult to justify attempting to fit data to an equation with so many unknowns. The problem can be simplified considerably if an appropriate sequence of steps is followed experimentally.

\(II\) Experimental approach. Step 1 is the determination of the permeability ratio for the substrate \((F_{S\rightarrow 0} = V_{\text{maxA}} \times K_{mB}/V_{\text{maxB}} \times K_{mA})\).

In a first step, the effect of varying concentrations of the inhibitor on the influx of labeled substrate is studied under initial rate conditions. The substrate concentration is kept fixed at a very low value. Relative transport rates, in the presence or absence of inhibitor, are plotted against the inhibitor concentration and analyzed on the basis of \(Equation\ 3\). The principal objective of this experiment is to measure the permeability ratio for the substrate \((F_{S\rightarrow 0})\); this constant groups the kinetic parameters \((V_{\text{max}}\) and \(K_m\)) of the two systems involved (\(Eq. 9\)).

As shown in \(Equation\ 3\) and \(Equations\ 7\)–\(9\), the effect of an inhibitor on the flux of a substrate that crosses the membrane through two transport routes \(A\) and \(B\) (and is present at very low concentration) is a function of the permeability ratio \((F_{S\rightarrow 0})\) and of the inhibition constants \((K_A\) and \(K_B\)). In the case of a selective inhibitor, the permeability ratio determines the fractional inhibition, whereas the inhibition constant for the sensitive component determines the steepness of the function.

This behavior is illustrated in Figure 13, which shows theoretical plots corresponding to three different situations: 1) the two systems have equal “permeabilities” \((V_{\text{maxA}}/K_{mA} = V_{\text{maxB}}/K_{mB})\) and thus \(F_{S\rightarrow 0}\) equals one, 2) the inhibitor sensitive system \((A)\) has a higher permeability \((F_{S\rightarrow 0} > 1)\), and 3) the inhibitor-sensitive system \((A)\) has a lower permeability \((F_{S\rightarrow 0} < 1)\). In all three cases, the affinities of the two systems for the inhibitor differ by a factor of 1,000 \((K_A/K_B = 1,000)\). Figure 4A shows the result obtained in this type of experiment in a study of lysine influx in human erythrocytes (64). L-Leucine is seen to affect the two transporters for cationic amino acids in these cells with markedly different affinities. The \(K_i\) values for systems \(y^+\) and \(y^-\)L were 0.022 ± 0.003 and 30.36 ±
and thus is not expected to produce pronounced biphasic behavior, is necessary because the substrate, unlike the inhibitor, ability ratio ($F_{n}$) analysis is done, however, using the value for the permeance of a very low concentration of labeled substrate. The concentrations of unlabeled substrate ($S$) are used to inhibit the substrate. The experimental design is identical to the one described in the first step, except that unlabeled substrate replaces the inhibitor; that is, varying concentrations of unlabeled substrate ($S$) have a higher permeability ($F_{A}$), and $S$ inhibitor-sensitive system ($A$) has a lower permeability ($F_{B}$). In all 3 cases, $K_{m}/K_{A} = 1,000$.

Step 2 is the determination of the substrate half-saturation constants ($K_{mA}$ and $K_{mB}$) and relative maximum velocities ($V_{maxA}/V_{maxB}$) for the two systems.

Once the permeability ratio has been measured, the systems can be directly probed for their interactions with the substrate. The experimental design is identical to the one described in the first step, except that unlabeled substrate replaces the inhibitor; that is, varying concentrations of unlabeled substrate ($S$) are used to inhibit the flux of a very low concentration of labeled substrate. The analysis is done, however, using the value for the permeability ratio ($F_{S-o}$) determined in the previous step. This is necessary because the substrate, unlike the inhibitor, is not expected to produce pronounced biphasic behavior, and thus $F_{S-o}$ cannot be accurately estimated in this experiment. Substrate self-inhibition curves, corresponding to the three situations described above (Fig. 13), are shown in Figure 14. The $K_{m}$ values for the substrate for systems $A$ and $B$ are assumed to differ by 10-fold ($K_{mA} = 10$ and $K_{mB} = 100$). For the sake of comparison, we also show the theoretical curves that would be obtained if a single system were assumed to be responsible for the flux. It is evident that this experiment by itself would be insufficient to resolve whether one or two systems are involved. The $K_{m}$ values obtained with the one model fit are seen to vary depending on the relative contribution of the high-affinity ($A$) and low-affinity ($B$) systems to the overall transport rate ($F_{S-o}$). Thus, as indicated in Figure 14, the behavior of two transporters with $K_{m}$ values of 10 and 100 could be fitted reasonably well by a curve assuming one transporter with $K_{m}$ 70, 38, or 18, depending on the relative $V_{max}$ of the two pathways.

Once the half-saturation constants for the substrate are calculated, the relative maximum rates can be obtained by substituting into the permeability ratio (Eq. 9).

Step 3 is relating the inhibitory effects to the implicated systems. When the permeabilities ($V_{max}/K_{m}$) for the two transport systems are unequal ($F_{S-o}$ not equal to 1), it is straightforward to deduce which of the two systems is affected by the inhibitor. However, if the $V_{max}/K_{m}$ values are similar ($F_{S-o} \sim 1$), the question cannot be resolved on the basis of this evidence alone. A third step is thus required that involves measuring the effect of the inhibitor at a higher substrate concentration. This experiment should clearly reveal which system is affected by the inhibitor because, as shown by Equation 6, if the two systems

![Image](http://physrev.physiology.org/)

**FIG. 13.** Effect of an external unlabeled competing analog on influx of a labeled substrate that moves through 2 transport routes $A$ and $B$ (and is present at very low concentration) is a function of “permeability ratio” ($F_{S-o}$) and of apparent inhibition constants for two systems ($K_{A}$ and $K_{o}$). Theoretical plots (generated on basis of Eq. 3) are shown for 3 different situations: 1) 2 systems have equal “permeabilities,” or rates of transport at low substrate concentration ($F_{S-o} = 1$), 2) inhibitor-sensitive system ($A$) has a higher permeability ($F_{S-o} = 4$), and 3) inhibitor-sensitive system ($A$) has a lower permeability ($F_{S-o} = 0.25$). In all 3 cases, $K_{m}/K_{A} = 1,000$.

![Graph](http://physrev.physiology.org/)

**FIG. 14.** Substrate self-inhibition for 2 parallel systems may not exhibit a pronounced biphasic behavior. Plots for 3 situations described in Fig. 13 ($F_{S-o} = 1, F_{S-o} = 4$, and $F_{S-o} = 0.25$) are shown. Curves were generated with Equation 3. Substrate $K_{m}$ values for 2 systems are assumed to differ by 10-fold ($K_{mA} = 10$ and $K_{mB} = 100$). For comparison, theoretical curves assuming a single system are also shown, and corresponding $K_{m}$ values are given in figure.
tems have different $K_m$ values, their relative contribution to the total flux will vary as a function of the substrate concentration. Thus, whereas at very low substrate concentration $F_{S\rightarrow 0} = (V_{maxA}/K_{ma})/(V_{maxA}/K_{ma})$, when the substrate concentration is saturating $F_{S\rightarrow 0} = V_{maxA}/V_{maxB}$ (Eq. 10). If a high-affinity and low-capacity system is the target, the inhibited fraction decreases at higher substrate concentrations. The opposite is true if the analog affects a low-affinity and high-capacity system. Examples for these two cases are given in Figure 15. The application of this experimental strategy to the case of lysine transport in human erythrocytes is shown in Figure 4B.

It is noteworthy that, even if there is no ambiguity regarding which system is affected by the inhibitor, consistency among the parameters determined in steps 1 and 2 can be tested by comparing the effect of the inhibitor at higher substrate concentrations (step 3) with the predicted values. Relative transport rates can be estimated at any substrate concentration by introducing the calculated parameters into Equation 2 (see Fig. 4B) (64).

Absolute values for the maximum velocities can be obtained multiplying the permeability constant ($V_{max}/K_m$), estimated from the transport rate measured at very low concentration, by the $K_m$ (72). Alternatively, $V_{max}$ can be calculated by fractionating the total flux at high substrate concentration.

B) DETERMINATION OF TRANSPORT PARAMETERS FOR TWO SYSTEMS ACTING IN PARALLEL USING SELECTIVE IRREVERSIBLE INHIBITORS. This approach requires finding a protein reagent which, by forming a covalent derivative with a relevant amino acid residue, inactivates one of the transporters selectively. Whereas reversible inhibition is usually produced without delay and disappears when the free inhibitor is removed, irreversible inhibition would ordinarily develop gradually and would persist after the free inhibitor has been eliminated.

*Step 1* is selective inactivation of one transporter and

---

**FIG. 15.** Fractional inhibition caused by a selective competing analog interacting with 2 parallel pathways varies as a function of substrate concentration if the 2 systems have different affinities for the substrate. Examples for 2 cases are given. A: inhibitor-sensitive system has higher affinity and lower $V_{max}$. B: inhibitor-sensitive system has lower affinity and higher $V_{max}$. Curves were generated on basis of Equation 2, substituted with parameters indicated on figure.

**FIG. 16.** N-ethylmaleimide (NEM) is a partial irreversible inhibitor of L-[14C]lysine influx into human erythrocytes. Cells were treated with NEM (0.2 mM, 25°C) for varying times, and extent of inactivation was estimated by measuring residual flux with 1 mM L-lysine. Inset: replot of these results after subtracting NEM-resistant flux (value after 15 min of incubation). Inactivation rate constant was calculated from equation $\ln v/v_o = -kt$, where $v/v_o$ is relative transport rate in presence and absence of NEM and $t$ is time. Rate constant of inactivation ($k$) was 0.53 ± 0.027 min⁻¹. [From Devés et al. (63).]
determination of the permeability ratio. To find out the conditions that are necessary to block one of the transporters selectively, the cells are treated with a fixed concentration of irreversible inhibitor for varying lengths of time, and residual transport rates are estimated after removing the free inhibitor. It should be noted that the variable to be controlled is time of reaction and not concentration, as was the case with reversible inhibition. If a single system is affected by the inhibitor, the rate decreases monexponentially with time until it reaches a constant value that represents the flux through the resistant pathway.

When low substrate concentrations ([S] << [Km]) are used to estimate the residual flux, the fractional inhibition obtained, after the sensitive system has been fully inhibited, is directly related to the permeability ratio for the substrate (Fss). Figure 16 shows the effect of the sulfhydryl reagent NEM on the influx of lysine (1 μM) into human erythrocytes. The total lysine flux was reduced by ~60% after the reaction reached completion. The sensitive and insensitive pathways should be tested for homogeneity using substrate analogs whenever possible (63).

**Step 2** is determination of the substrate half-saturation constants (Km,s and Kmax) and relative maximum velocities (Vmax/L) for the two systems. Both Km,s and Kmax can be estimated from the effect of varying concentrations of unlabeled substrate on the flux of a low concentration of labeled substrate in untreated cells. This is identical to step 1 above but uses the Fss value determined in that step. The analysis is performed on the basis of Equation 3 (assuming Km,/[S] < 1). An independent measurement of the Km for the inhibitor-resistant pathway can be obtained by performing the same experiment in treated cells. In this case, inhibition should conform to Equation 13 (see below). This approach may be used to investigate the interaction of the transporters with its substrates or with any ligand that interacts with the systems reversibly. Figure 17 shows the application of this method to resolve substrate (see below). This approach may be used to investigate the interaction of the transporters with its substrates or with any ligand that interacts with the systems reversibly.

If the properties of the labeled substrate are being investigated, relative Vmax values can be obtained, as explained before, by replacing the half-saturation constants into the permeability ratio (Fss) measured in step 1.

**Step 3** is testing for consistency between the transport parameters. Consistency between the transport parameters for the substrate determined in steps 1 and 2 can be tested by monitoring the effect of the irreversible inhibitor with varying concentrations of labeled substrate, as previously explained. In the presence of a very low substrate concentration, the fractional inhibition is directly related to the permeability ratio, that is, to the Vmax/Km for the two systems. However, in the presence of a saturating concentration of substrate, the fractional inhibition depends solely on the ratio of the maximum velocities for the two systems (Eq. 10). Thus, when the transport parameters are known, it is possible to predict the fractional inhibition that should be obtained at any given substrate concentration (63).

2. **Analysis of transport specificity**

One of the basic assumptions of carrier-mediated models of transport is that the interaction of the substrate with the transporter involves two consecutive, and conceptually different, events: a binding step, in which substrate is recognized by a specialized site in the transporter, and a translocation step, representing the actual transference of the substrate from one compartment to the other. These two processes may exhibit different specificities, with an extreme case being that of a molecule which binds to the transporter but cannot undergo transport, for example, due to steric constraints. Thus studies of transport specificity should aim to disclose the interactions between the carrier and the substrate in these two steps.

It may seem that the most direct way to approach this problem is to determine the kinetic parameters for a
number of labeled substrate analogs. The reciprocal value of the half-saturation constant ($K_m$) would be expected to give an indication of the apparent affinity for the substrate, whereas the maximum velocity would reflect its translocation rate. However, this approach has both practical and theoretical weaknesses. From the experimental point of view, it would be impractical in the case of amino acid transport, because, as emphasized in this review, the molecules of interest are expected to cross the membrane through more than one transporter. From a theoretical point of view, the experimentally determined parameters, $K_m$ and $V_{max}$, do not directly reflect the dissociation constant or the rate of translocation of the substrate-carrier complex. Experimental considerations that should be taken into account in this type of studies are discussed below.

A) SPECIFICITY OF THE BINDING STEP. The study of the specificity of the binding step can be more directly addressed by following the effect of various unlabeled analogs on the flux of a single labeled substrate with both molecules initially present in the same compartment (cis-inhibition). Although this strategy is frequently applied in transport studies, it is important to emphasize some aspects that are not always considered.

1) Kinetics of cis-inhibition. If two amino acids bind to the same site, the flux of labeled substrate (S) in the absence or presence of unlabeled competing analog (I) is given by

$$\frac{v}{v_0} = \frac{K_m + [S]}{K_m(1 + [I]/K_i) + [S]} \quad (11)$$

Equation 11 assumes that the test substrate (labeled) moves through a single transport pathway (strategies that may be used to functionally isolate a transport system have been discussed above). The concentration of competing substrate producing 50% inhibition ([I]$_{0.5}$) is

$$[I]_{0.5} = K(1 + [S]/K_m) \quad (12)$$

and if the substrate concentration is experimentally set very low ([S] $<< K_m$), Equation 12 simplifies to

$$\frac{v}{v_0} = \frac{1}{(1 + [I]/K_i)} \quad (13)$$

In this case, [I]$_{0.5}$ = $K_i$.

Thus, if a large number of analogs are to be tested, as is usually the case in specificity studies, the experiments should be performed keeping the concentration of the labeled substrate at a very low level (at least one-tenth of the $K_m$). The demonstration of competitive inhibition requires verifying that the inhibitor can be displaced by the substrate at higher concentrations.

However, even if these considerations are observed, a comparison of the half-saturation constants, or inhibition constants, for a number of analogs does not directly reflect their relative equilibrium dissociation constants.

According to the carrier model, the transport of a substrate is represented by the following scheme

where $C_o$ and $C_i$ are the outward and inward looking forms of the carrier, respectively, and $C_{So}$ and $C_{Si}$ are the equivalent conformations for the carrier substrate complex. $K_{So}$ and $K_{Si}$ are equilibrium dissociation constants, and $f_1$, $f_{-1}$, $f_2$, and $f_{-2}$ are rate constants for the translocation steps indicated.

On the basis of this model, the half-saturation constant of a transported analog, measured either in a cis-inhibition experiment ($K_i$) or by following its transport directly ($K_m$) and assuming rapid equilibrium, is given by

$$K_m \text{ or } K_i = \frac{K_{So}(f_2 + f_{-1})}{f_1 f_{-1}} \quad (14)$$

Equation 14 shows that the half-saturation constant for the analog is not only a function of the substrate dissociation constant at the external face ($K_{So}$), but also of the microscopic rate constants for translocation. As shown in the kinetic scheme, $f_1$ and $f_{-1}$ represent the rate constants for the free carrier reorientation inward and outward, respectively, and $f_2$ represents the rate of inward movement of the carrier substrate complex. It follows that the relative values of the half-saturation constants will give an indication of the relative true dissociation constants, only if the rate of translocation of the substrate carrier complex for the analogs tested are similar. Otherwise, differences in the rate of translocation of the analogs must be considered in the analysis.

B) SPECIFICITY OF THE TRANSLLOCATION STEP. Once the specificity of binding has been defined, the question arises as to the relative facility with which these analogs are translocated by the carrier. As before, direct assessment of entry rates is not helpful, because it would require precise knowledge of the various routes involved in the transport of each solute. In addition, the maximum velo-
ity is not a direct measurement of the rate of translocation of the carrier-substrate complex at a given carrier concentration (C), as shown by the following equation for the $V_{\text{max}}$ for entry

$$V_{\text{max}} = \frac{(f_2 \cdot f_{-1})C}{f_2 + f_{-1}} \quad (15)$$

According to this expression, the $V_{\text{max}}$ depends not only on the rate of translocation of the carrier-substrate complex inward ($f_2$), but also on the rate of reorientation of the free carrier outward ($f_{-1}$). Any of these two steps could be rate limiting. A more informative experimental strategy is to determine the effect of an analog, located on the opposite compartment ($\text{trans}$), on the unidirectional flux of labeled substrate. The effect of the analog on the unidirectional flux of labeled substrate provides information regarding the transport properties of the carrier-antagonist complex (65).

If labeled substrate efflux is measured in the absence or presence of varying concentrations of analog, trans-acceleration will occur if the complex reorients to the inward-looking conformation faster than the free carrier; in contrast, trans-inhibition results if the complex reorients more slowly than the free carrier.

The determination of transport parameters by this method is particularly useful when systems with overlapping specificities are present, since the results depend exclusively on interactions with the system under study. The only requirement is that the labeled substrate moves through a single transporter.

1) Analysis of trans-acceleration experiments. On the basis of a general treatment of the carrier model (8, 66), the rate of exit of a substrate $S$ into a medium that contains an unlabeled analog $T$ ($v^T$) can be written as

$$v^T = \frac{v_o + v_{\text{max}}T_o/K_T}{1 + [T_o]/K_T} \quad (16)$$

where $v_o$ is the rate of exit measured in the absence of analog, $v_{\text{max}}$ is the rate in the presence of a saturating concentration of external analog $T$, and $K_T$ is the apparent half-saturation constant for the analog. This equation can be rearranged to

$$v^T - v_o = \frac{(v_{\text{max}} - v_o)[T_o]}{K_T + [T_o]} \quad (17)$$

If $v^T_{\text{max}}$ differs from $v_o$, then the analog will accelerate or retard the rate according to a Michaelis-Menten-type function. The $K_T$ value reflects the interaction between the carrier and the substrate at the external site.

The $K_T$ is a function of the concentration of labeled substrate. If the concentration of labeled substrate is very low, $K_T$ is, in theory, equal to the half-saturation constant determined in zero-trans-entry experiments (cis-inhibition) ($\text{Eq. 14}$). However, if the concentration inside is very high (saturating), the expression equals

$$(K_T)_{S_{\infty}} = \frac{K_T(f_3 + f_{-2})}{(f_1 + f_{-2})} \quad (18)$$

In this equation, $f_2$ represents the rate of inward movement of the analog-carrier complex (CoT), and $K_T$ corresponds to the half-saturation constant measured in an infinite-trans-experiment. Other constants are as in Equation 14.

The ratio between the rate in the presence or absence of the analog ($v^T/v_o$) can also adopt two extreme forms depending on the concentration of the substrate. At very low substrate concentration

$$\left(\frac{v^T_{\text{max}}}{v_o}\right)_{s=0} = \frac{1 + f_{-2}f_1}{1 + f_{-2}f_3} \quad (19)$$

Trans-acceleration will occur if $f_3 < f_1$. In a symmetrical system, where $f_1$ equals $f_{-1}$, the ratio cannot be larger than two, no matter how large the $f_3/f_1$. At very high substrate concentration

$$\left(\frac{v^T_{\text{max}}}{v_o}\right)_{s=\infty} = \frac{1 + f_{-2}f_1}{1 + f_{-2}f_3} \quad (20)$$

As before, trans-acceleration occurs only if $f_3 > f_1$, but the flux ratio can be larger than two depending on the relative values of $f_1$, $f_{-2}$, and $f_3$. In homoexchange when $f_3$ ($f_2$ in this case) might be equal to $f_{-2}$, the expression becomes

$$v^T_{\text{max}}/v_o = 0.5(1 + f_{-2}f_1) \quad (21)$$

Thus, although any of these conditions may be used in the analysis, it must be kept in mind that the concentration of substrate must be carefully controlled.

Figure 18 shows the trans-acceleration induced by increasing concentrations of lysine on the rate of labeled lysine efflux in human erythrocytes treated with NEM (system y$^+$L). Addition of L-lysine to the external medium was found to induce marked acceleration of L-$^{[14]}$lysine efflux. Data obtained in three independent experiments, using different cell samples, follow closely the behavior predicted by Equation 16, that is, L-lysine produces a dose-dependent acceleration that reaches saturation (Fig. 18B). The half-saturation constant for external L-lysine, calculated by fitting Equation 16 to these data, was found to be $10.32 \pm 0.49$ $\mu$M ($n = 3$), and this value agrees closely with the half-saturation constant measured in cis-
inhibition experiments. On average, excess L-lysine accelerated the rate of exit by 6.2 ± 0.54-fold (n = 20). The application of the concepts outlined above to a study of the specificity and cation dependence of the translocation step in system y+L is presented in section IV.

B. Functional Properties of Cationic Amino Acid Transporters

To avoid misinterpretations that may originate from the study of poorly dissected activities, the present analysis is based, predominantly, on information obtained following expression of defined gene products or in studies with intact systems, in which the contribution of parallel pathways has been separated.

1. System y+L, the cationic amino acid-specific transporters

A) SUBSTRATE SPECIFICITY. I) Interaction with cationic amino acids. The most widely distributed isoform of the cationic amino acid-specific transporters, mCAT-1, has properties that match those described for many cells (e.g., Refs. 48, 63, 72, 110, 230, 239) and are consistent with system y+L as defined in section II. This transporter has been shown to transport lysine, arginine, and ornithine with half-saturation constants (Km) in the range 0.040–0.25 mM; the maximum rates of transport for these analogs do not differ significantly in a given experimental system. The strength of interaction of cationic substrates with system y+L increases with increasing number of methylene groups between the α-carbon and the terminal cationic group (239). Although α-N-methylation reduces or eliminates the interaction, the system can recognize (and transport) arginine analogs that are methylated at the guanido group such as Nε-monomethyl-L-arginine, an inhibitor of the enzyme NO synthase (14, 76, 189, 190). The interaction is stereospecific (76, 110, 230, 239). Half-saturation constants (Km or Ki) for cationic amino acid analogs measured in a number of cell systems are listed in Table 6.

In erythrocytes and placenta, system y+L can be distinguished, on the basis of its affinity toward cationic amino acids, from the higher affinity (and lower capacity) transporter, system y+L (64, 72, 76). As shown in Figure 17, the inhibition of lysine influx by increasing concentrations of unlabeled amino acid is plotted against concentration of unlabeled amino acid (vT and v0 are rates in presence or absence of amino acid in trans-compartment, respectively) (see Eq. 17). Symbols denote 3 different experiments. Calculated half-saturation constant (Ks) was 10.3 ± 0.48 μM. Inset: results obtained for a wider concentration range. Saline solution contained 150 mM NaCl and 5 mM sodium phosphate (pH 6.8, 37°C). Maximum acceleration was on average 6.2-fold. [Modified from Angelo and Devés (8).]
TABLE 6. Interaction of system \( y^+ \) with cationic amino acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Retic</th>
<th>Fibr</th>
<th>HTC</th>
<th>Erythr</th>
<th>mCAT-1</th>
<th>mCAT-2 (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>70</td>
<td>41</td>
<td>200</td>
<td>56</td>
<td>70–250</td>
<td>187–380</td>
</tr>
<tr>
<td>L-2-Amino-3-guanidobutyric acid</td>
<td>1,600</td>
<td>5,300</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-2-Amino-4-guanidobutyric acid</td>
<td>120</td>
<td>390</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Homoarginine</td>
<td>19</td>
<td>89</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>180</td>
<td>90</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-CH(_3)-L-arginine</td>
<td>&gt;20,000</td>
<td>30,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-CH(_3)-L-lysine</td>
<td>&gt;20,000</td>
<td>20,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-mono-CH(_3)-L-arginine</td>
<td>590</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-mono-CH(_3)-D-arginine</td>
<td>151</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-CH(_3)-L-lysine</td>
<td>2,660</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-mono-CH(_3)-L-lysine</td>
<td>151</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-mono-CH(_3)-D-lysine</td>
<td>2,660</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-Arginine</td>
<td>700</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N^+ )-Lysine</td>
<td>14,800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ret, rabbit reticulocytes; Fibr, cultured human fibroblasts; HTC, hepatoma cell line McA-RH7777, Erythr, human erythrocytes. Reference are as follows: reticulocytes, Ref. 48; fibroblasts, Ref. 239; HTC, Ref. 238; erythrocytes, Ref. 76 and DeveÂs and Angelo, unpublished data; mCAT-1, Refs. 53, 102, 110, 230; mCAT-2(B), Refs. 55, 102.

expressed in stimulated lymphocytes and macrophages, mCAT-2(B) (see Tables 3 and 4). As mentioned in section III, both mCAT-2 proteins originate from a common gene \((mCAT-2)\) and only differ in a stretch of 41 amino acids (see Fig. 6); it follows that this segment must, directly or indirectly, account for their different affinities.

In a very elegant set of experiments, Closs et al. (55) tested this hypothesis by constructing chimeric cDNA in which 81 amino acids containing the divergent region of mCAT-2A and mCAT-2(B) were inserted into mCAT-1 \([mCAT-1/2A, mCAT-1/2(B)]\) and the equivalent portion of mCAT-1 was inserted into mCAT-2A \((mCAT-2A/1)\). The behavior of the chimeric proteins was then assessed in Xenopus oocytes. The transport parameters obtained for the chimeric constructs and the wild-type clones are shown in Table 7. There is clearly a good correlation between the characteristics of the wild-type proteins and the chimera containing the corresponding donor sequence. Similar results were obtained by Kavanaugh et al. (108), using a different chimeric construct in which the 5'-portion of mCAT-1 was ligated at nucleotide 1091 to the 3'-region of mCAT-2A and mCAT-2(B). The site of recombination occurs at amino acid 275 of mCAT-1.

II) Interaction with neutral amino acids. The interaction of system \( y^+ \) with neutral amino acids is one of the aspects that must be reconsidered in view of the identification of broad-scope systems that are able to recognize neutral and positively charged amino acids with comparable affinity (20, 64, 222, 226, 234). Transport studies with the cloned transporters show that system \( y^+ \) interacts weakly with neutral amino acids. In mCAT-1-injected oocytes, homoserine at 10 mM was found to produce a small inward current that was approximately one-half of that induced by 1 mM lysine (Fig. 8) (230), and at 3 mM, it did not affect the influx of L-[\(^{3}\)H]arginine (110). Similarly, neutral amino acids were shown to interact weakly with the mCAT-2(B) isoform and, in agreement with early observations in reticulocytes, homoserine, cysteine, and leucine were preferred over other neutral amino acids (102).

For \( mCAT-1 \), inhibition constants for these amino acids as competitors of labeled lysine influx were 20, 30, and 48 mM, respectively (48). Glutamine did not induce an inward current in oocytes expressing mCAT-2(B). The inability of glutamine to interact with system \( y^+ \) was also seen in placenta (72); in this case, glutamine \((10 \text{ mM})\) did not affect lysine influx, whereas leucine and methionine inhibited system \( y^+ \) by ~22% at this concentration. Finally, the relative potencies of homoserine and glutamine in fibroblasts (Fig. 2B) are also consistent with the behavior described above (230).

B) CATION DEPENDENCE. The cation dependence of
amino acid transporters has been one of the key criteria considered in their classification, and moreover, such information can offer valuable insight into the carrier mechanism. The transport of cationic amino acids through system y− is not significantly affected by changes in the ionic composition of the medium. Sodium replacement with choline decreases the rate of arginine and lysine influx (1 mM) through mCAT-2(B) by ~15% (102). The same was observed with mCAT-1 when the substitution was made with equimolar tris(hydroxymethyl)aminomethane (Tris) (110).

The cation dependence for the interaction of system y− with neutral amino acids has been largely deduced from the early experiments performed by Christensen and co-workers (48, 49) in Ehrlich cells and reticulocytes. A notion that appears to be widely accepted is that in these two cell types “neutral amino acid inhibition of cationic amino acid influx depends strictly on the presence of an alkali metal ion, where Na+ is better than Li+ or K+” (cited from White, Ref. 237). The implication is that Ehrlich ascites cells and reticulocytes possess the same carrier (system y−).

However, a reexamination of the results shows that the cation dependence of these transporters differs markedly and suggests that distinct carriers participate in the transport of cationic amino acids across their membranes. This can be illustrated by the following observations. The influx of arginine (50 μM) into reticulocytes was inhibited weakly by neutral amino acids, and the inhibition did not vary significantly in the presence of various inorganic cations. For example, hydroxyornorvaline (added at a concentration of 50 mM) reduced arginine influx by 35, 43, and 52% in the presence of K+, Na+, and Li+, respectively. Inhibition was substantially lower (5%) in the presence of choline (213). A different behavior was observed, in the same study, with Ehrlich ascites cells. In this case, the interaction with neutral amino acids was optimal in the presence of Na+ or Li+ and was undetectable in K+ or choline. In addition, in Ehrlich cells, the overall affinities were higher than in the case of reticulocytes. The cation dependence of neutral amino acid inhibition of lysine transport seen in Ehrlich cells corresponds to that observed for system y−L (see sect. ivB4).

The cation dependence has not been systematically studied in recent reports involving either cloned proteins or well-dissected transporters. Nevertheless, it has been shown that the weak interaction of the transporter [mCAT-2(B) or mCAT-1] with homoserine becomes undetectable when Na+ in the external medium is replaced by choline or Tris, respectively (102, 230).

Young et al. (252) reported that NaCl replacement with sucrose stimulated lysine influx in human erythrocytes. This effect was attributed to a removal of a competitive inhibition exerted by Na+ on system y−. It was pointed out that this was consistent with the proposal that the Na+ dependent interaction of neutral analogs with system y− involves Na+ binding at the position otherwise taken by the distal cationic group of the basic amino acid (50).

A recent report reexamined the effect of low ionic strength on lysine influx, separating the contribution of systems y− and y−L (62). The results show that NaCl replacement with sucrose increases influx through system y−L, but, in contrast to the original proposal, decreases influx through system y−. It was concluded that the inhibition of system y− is caused by the membrane depolarization that results from chloride removal, whereas the stimulation of system y−L is due to the enhancement of the negative surface potential. The effect of surface potential on lysine influx via system y−L is discussed in section ivB4.

C) TRANS-ACCELERATION. Although the experiments have not been performed under conditions allowing a detailed analysis, it has been clearly shown that system y− is subjected to trans-acceleration. In fibroblasts, unleaded amino acids present in the external medium (10 mM) increased the rate of exit of L-arginine (1 mM), so that after 2 min, the arginine retained by the cells was 50% compared with the control (in the absence of external amino acid) (239). Sodium replacement with choline did not alter the effect of cationic amino acids but prevented acceleration by L-homoserine and L-glutamine. L-Phenylalanine did not accelerate efflux. (This may be due to its bulkiness or to weak interaction with the binding site.) The same phenomenon was observed in hepatoma cells preloaded with unlabeled amino acids (incubation with 10 mM amino acid for 1 h). The Vmax for entry was seen to increase by approximately fourfold when the cells were loaded with arginine, lysine, or homoarginine (238).

The observation that arginine uptake was trans-stimulated by intracellular cationic amino acids in fibroblasts and hepatoma cells, but not in hepatocytes, suggested that this property might be exhibited by the mCAT-1 isoform, but not by mCAT-2A. To examine this hypothesis Closs et al. (53) developed an assay for trans-stimulation in mCAT-1- and mCAT-2a-injected oocytes. Oocytes containing either mCAT-1 or mCAT-2A were injected with water or unlabeled arginine to give intracellular arginine concentrations of 0, 0.5, or 5 mM. Subsequently, they were placed in media containing different concentrations of labeled arginine (0.1–12.5 mM), and uptake was followed (Fig. 19). Uptake mediated by both proteins increased after injection of arginine, but the relative increase in mCAT-1 activity exceeded that of mCAT-2A. At a calculated internal concentration of 5 mM, and with 12.5 mM of external labeled arginine, the relative increase in the presence or absence of arginine was 3-fold for mCAT-1 and 1.6-fold for mCAT-2A. Because the two transporters apparently reach similar rates at higher concentrations, and the differences in trans-stimulation are due to differences in the basal zero-trans-rates, it was proposed that the free carrier is more restricted in its movement in the case of mCAT-1 than in mCAT-2A (53).
However, the two transporters were not compared under equivalent conditions, since the maximum concentration inside is saturating for mCAT-1, but not for mCAT-2A, and it is possible that this might explain the observations in part. Similar results were obtained in another study with the three isoforms, but again, transport was measured under different conditions of saturation: the [S]/Km values for the substrate on the trans-side were 1.3 (mCAT-1), 0.8 (mCAT-2(B)), and 0.07 (mCAT-2-A), and thus it is not surprising that there was less stimulation in the case of mCAT-2A (55). Experiments with chimeric proteins gave similar results (55, 108). However, it is not absolutely clear to what extent these results reflect differences in the transport parameters rather than intrinsic mechanistic differences affecting the mobility of unloaded or loaded carrier forms.

A moderate trans-acceleration of the efflux of lysine (4 µM) of ~30% was observed in erythrocytes when saturating concentrations of arginine, lysine, and N\(^{\text{G}}\)-monomethyl-\(\text{L}\)-arginine were added to the external medium (76). The relatively smaller trans-acceleration observed in these cells may be due to their less negative membrane potential (~10 mV) (77).

D) STRUCTURAL DETERMINANTS OF TRANSPORT ACTIVITY.
Because the most conserved sequence in the yeast transporters for arginine, choline, and histidine (Ser-Leu-Gly-Glu-Leu) has a closely related sequence in the corresponding position (third putative transmembrane region) in the CAT transporters (Thr-Val-Gly-Glu-Leu), Wang et al. (229) hypothesized that the conserved glutamic acid residue might be critical for function. To test this hypothesis, mCAT-1 was mutated by substituting Asp for Glu at position 107. It was found that CCL-64 mink cells (which do not express mCAT-1 protein), when transfected with the wild-type or mutant mCAT-1 protein, acquired the ability to bind ecotropic retroviral gp70 and also became highly susceptible to infection by murine retroviruses. However, this highly conservative mutation was able to abolish transport activity totally, estimated either by two-electrode voltage-clamp analysis of arginine-induced currents (1 mM) or [\(^{3}\text{H}\)]arginine uptake (0–1 mM range). Only the wild-type transporter was able to confer transport activity above the endogenous background. It is concluded that Glu-107, which occurs in a conserved transmembrane region, is critical for cation transport. It is not known whether this substitution affects binding or translocation.

E) EFFECT OF MEMBRANE POTENTIAL. The analysis of arginine transport using voltage-clamp methods with overexpressed proteins demonstrated that transport mediated by the three isoforms of mCAT is electrogenic (105, 230). In voltage-clamped oocytes expressing mCAT-1, addition of arginine caused inward currents that were both concentration dependent and voltage dependent (105). At a given concentration, the inward arginine current increased in an exponential fashion with membrane hyperpolarization. The current induced by 10 µM arginine increased ~10-fold per 58.1 mV, whereas that induced by 1 mM arginine increased ~10-fold per 59.4 mV. The K_m values decreased, and V_max values increased with hyperpolarization. Cells loaded with arginine showed outward currents when placed in a medium devoid of arginine, and the steady-state current increased exponentially with membrane depolarization (~10-fold per 52 mV) (Fig. 20).

Kavanaugh (105) has proposed that among the molecular mechanisms that could underlie the observed effects of membrane potential, on the kinetic parameters, are actions arising from “ion well” effects on binding and unbinding of the charged substrate to a site on the protein that senses a fraction of the membrane electric field; this would explain the increase in K_m as the membrane potential is made positive. However, he also noted that the lack of effect of the concentration of arginine on the voltage dependence of influx must reflect an additional effect of membrane potential on the rate constants.

In this study, the data were analyzed on the basis of...
FIG. 20. Half-saturation constant ($K_m$) and maximum current ($I_{max}$) of arginine-induced inward currents are voltage dependent. A: concentration dependence of influx currents was studied at various membrane potentials by voltage clamp. B: effect of membrane potential on $K_m$. C: effect of membrane potential on $I_{max}$, normalized to value measured at $-120$ mV. [Modified from Kavanaugh (105).]

A model that assumes three carrier states, two forms of the free carrier (inward and outward looking) and one intermediate for the carrier substrate complex. As pointed out by Kavanaugh (105), the voltage dependence of system $y^+$ differs from that of the Na+-dependent glucose (217) and γ-aminobutyric acid (106) transporters, both of which approximate a maximum rate at hyperpolarized potentials; saturation occurs at membrane potentials where the rate-limiting step becomes independent of voltage. In this case, there is no evidence of saturation of arginine flux with voltage that continues to increase exponentially even at $-180$ mV. Such a model requires movement of a negative charge, from inside to outside, during free carrier reorientation (105).

A similar membrane potential dependence was observed, using a different experimental approach, for system $y^+$ in placental brush-border membranes (72) (Fig. 21). Mediated influx of l-lysine into human placental brush-border membrane vesicles occurs through two systems with properties corresponding to system $y^+$ and $y^-$ L. The influx of lysine through system $y^+$ changed markedly in response to alterations of membrane potential. In the presence of an inwardly directed thiocyanate gradient, the influx of lysine through this route was accelerated, whereas with an inwardly directed positive K$^+$ diffusion potential, lysine influx through system $y^+$ was reduced. A kinetic analysis showed that changing the membrane potential from 0 to negative (approximately $-60$ mV) almost doubled the $V_{max}$ and halved the $K_m$, leading to a fourfold increase in permeability ($V_{max}/K_m$). The experiment depicted in Figure 21 shows that the magnitude of the potential determines the proportion of lysine transport that occurs through each pathway in placental membranes. Because system $y^+$ exhibits accelerated exchange, it can be inferred that zero-trans-influx is limited by the rate of return of the free carrier to the external face of the membrane. Thus the increase of $V_{max}$ in response to hyperpolarization suggests that the free carrier has a negative charge that moves in the electric field. The effect of membrane potential on $K_m$ argues in favor of a separate effect at the level of substrate binding.

Electrogenic lysine transport through system $y^+$ has also been observed in human erythrocytes (see Fig. 28 (62). Hyperpolarization induced by the addition of valinomycin in the presence of an outwardly directed K$^+$ gradient, and depolarization by external chloride replacement with glucuronate, increased (5.8-fold) and decreased (6.7-fold) lysine influx, respectively. A reduction of influx
Inhibition of system y⁻ is selective in human erythrocytes (63). The other transporter for cationic amino acids (system y⁺L) is unaffected by the reagent. Pretreatment of the cells with NEM was found to inhibit the rate of entry of labeled lysine (1 μM) by ≈50% (maximum effect); the relative rate after NEM treatment (0.2 mM, 15 min) was 0.51 ± 0.086 (n = 13) (Fig. 16). N-ethylmaleimide inhibited this component of the flux (Kₘ for Lys, 109 μM) with monoeXponential kinetics, and the inactivation rate constant (k) was 0.53 min⁻¹ at a concentration of 0.2 mM (25 °C). With 0.1 mM NEM, the rate constant was 0.23 min⁻¹. The substrate (at equilibrium on both faces of the membrane) did not protect against inactivation. Following this report, NEM has been used in other studies as a selective inhibitor of system y⁺, thus allowing the properties of other parallel pathways to be examined independently (e.g., Refs. 71, 80, 161, 216).

2. System B₀⁺, the Na⁺-dependent broad-scope amino acid transporter

System B₀⁺ was first identified in mouse blastocysts, and activities with similar properties have subsequently been described, with varying degrees of rigor, in other tissues. No molecular evidence has yet been obtained for system B₀⁺.

A) SPECIFICITY AND IONIC DEPENDENCE. In mouse blastocysts, system B₀⁺ has been shown to transport L-alanine, L-valine, L-lysine, and BCO with relatively high affinity (Table 2) (226). L-Arginine, L-homoarginine, and L-α-alanine were seen to act as efficient competitors of L-alanine influx. Thus, at a concentration of 1 mM, they inhibited the influx of L-alanine (1.4 μM) by 79, 80, and 89%, respectively. L-Tryptophan and L-leucine were better inhibitors than L-cysteine and L-serine; these two groups of amino acids (200 μM) inhibited alanine (0.85 μM) influx by 65 and 91%, respectively. Sodium replacement with choline, or Li⁺, abolished the uptake of L-alanine almost completely (99%), and it also reduced total lysine uptake (~25%) (222, 226). For optimal activity, the system appears to require Cl⁻, since inhibition of B₀⁺-mediated glycine influx is observed when Cl⁻ is replaced by acetate (227).

It has been proposed that this activity is also responsible for the Na⁺-dependent transport of β-alanine, alanine, and lysine in rabbit distal ileum, which formerly was referred to as the β-alanine carrier (143, 144). Consistently, the transport of these three amino acids in rabbit distal ileum has also been shown to be Na⁺ dependent and Cl⁻ dependent (145, 146), and the affinity of β-alanine is similar in the two cases (1–2 mM) (143).

Studies on the endogenous B₀⁺ transporter of Xenopus oocytes by Taylor et al. (210) have suggested that system B₀⁺ exhibits “adaptive regulation”: upregulation during amino acid deprivation and downregulation during amino acid supplementation. Dietary effects on a system B₀⁺-like activity have also been reported in rat intestinal brush-border membranes (243).

B) DEVELOPMENT. The functional changes of system B₀⁺ during the development of preimplantation mouse and rat conceptuses have been studied (221). In the rat, system B₀⁺ appears to be present throughout this process,
but it has not been detected until the two-cell stage in the mouse. By the time they form blastocysts, system \(B^0_\text{Na}^+\) becomes more evident in mouse than in rat conceptuses. In addition, it has been proposed that system \(B^0_\text{Na}^+\) activity may be controlled, in part, by the uterus near the time of implantation. Incubation of early, late, and implanting blastocyst in Brinster's medium for 1 h before measuring their transport activity had no effect in early blastocysts (26 h before implantation) but produced a threefold increase in late blastocysts (6 h before implantation) and an eightfold increase in implanting blastocysts (221).

3. System \(b^{0,+}\), the Na\(^+-\)independent broad-scope amino acid transporter

We use the generic name system \(b^{0,+}\) to encompass the activity first identified in mouse blastocysts and the transport systems that are induced in \(Xenopus\) oocytes, after expression of the mRNA derived from the cDNA clones D2 and rBAT. System \(b^{0,+}\) has been shown to mediate the Na\(^+-\)independent transport of neutral and cationic amino acids.

A) SUBSTRATE SPECIFICITY. The relative affinities of system \(b^{0,+}\) for various amino acids can be deduced from the inhibitory effect of various neutral amino acids (1 mM) on arginine and leucine influx, in rBAT-injected oocytes (Fig. 9) (20). A very similar pattern of interaction was seen for system \(b^{0,+}\) in rat blastocysts, although the blastocyst-associated transporter does not seem to transport cystine (Van Winkle, personal communication, cited in Ref. 159). The specificity of system \(b^{0,+}\) is more limited than that of system \(B^0_\text{Na}^+\). It exhibits low affinity for amino acids that branch at the \(\beta\)-carbon (the \(K_i\) for valine is \(\sim 5\) mM, i.e., \(\sim 40\)-fold greater than the \(K_m\) for leucine; Ref. 222), and amino acids that branch at the \(\beta\)-carbon (BCH, BCO) do not affect the flux up to 5 mM. Both D2- and rBAT-injected oocytes have been shown to transport cystine with relatively high affinity (\(K_m \sim 60\) \(\mu\)M), and defects in the human cDNA have been shown to be associated with some forms of cystinuria, suggesting that cystine transport is mediated by a system \(b^{0,+}\)-like transporter in vivo (see sect. vi) (32).

B) ION DEPENDENCE. Sodium is not required for the interaction of system \(b^{0,+}\) with neutral or cationic amino acids. Sodium replacement with Li\(^+\), choline, or N-methyl-D-glucamine did not affect the rate of transport of either neutral or cationic substrates (20, 222, 234). Unlike system \(B^0_\text{Na}^+\), this system is \(\text{Cl}^-\) independent (223).

In rat blastocysts, Na\(^+\) replacement with uncharged osmolytes such as sucrose and mannitol was shown to induce an approximately threefold increase in lysine influx (0.42 \(\mu\)M) that was accompanied by a shift in apparent \(K_m\) from 61 to 5 \(\mu\)M (223). No effect on leucine influx was observed. Consistently, increasing concentrations of Na\(^+\) and other cations (K\(^+\), Li\(^+\), choline, Mg\(^{2+}\)) progressively reduced lysine influx in sucrose medium. The half-maximal inhibition concentration for monovalent cations was \(\sim 25\) mM, and inhibition levelled off at \(\sim 25\)–30% of the original flux. A similar effect was described for lysine influx into human erythrocytes (252) and for choline influx into mouse blastocysts (225).

In all these cases, the phenomenon was explained assuming that Na\(^+\) could compete with the cationic substrates by directly binding to the substrate site. However, their transport activity had no effect in early blastocysts (26 h before implantation) but produced a threefold increase in late blastocysts (6 h before implantation) and an eightfold increase in implanting blastocysts (221).

A similar effect was described for lysine influx into human erythrocytes (252) and for choline influx into mouse blastocysts (225).

In all these cases, the phenomenon was explained assuming that Na\(^+\) could compete with the cationic substrates by directly binding to the substrate site. However, their transport activity had no effect in early blastocysts (26 h before implantation) but produced a threefold increase in late blastocysts (6 h before implantation) and an eightfold increase in implanting blastocysts (221).
valent ions was nonspecific and partial, leveling off at \( \sim 30\% \) of the uninhibited flux; 2) inhibition exhibited a competitive component; 3) divalent cations were also inhibitory, but their potency was found to be higher; and 4) the flux of the neutral substrate leucine was unaffected by sucrose substitution (223).

C) TRANS-ACCELERATION AND EXCHANGE PROPERTIES. I) ELECTROGENICITY. The rBAT- and D2-injected oocytes have been shown to carry out electrogenic exchange of neutral and cationic amino acids. Using electrophysiological techniques, Coady et al. (58) were able to associate outward currents elicited by the addition of alanine to the external medium to the expression of rBAT. Thus rBAT-injected oocytes did not show appreciably different currents than noninjected oocytes when immersed in a solution devoid of amino acids. However, when alanine was added to the medium, outward currents of 60–100 nA were seen at \(-50 \text{ mV} \) in rBAT-expressing oocytes. Similar currents were seen after exposure to leucine, isoleucine, serine, and glutamine. In contrast, addition of 1 mM arginine to the external medium caused an inward current of 35 nA.

No amino acid currents were seen upon application of proline, methylaminoisobutyric acid, aspartic acid, or glutamic acid. Preincubation of the oocytes with alanine (5 mM) diminished the alanine-induced currents by 66\% and increased the arginine-induced current by 131\% (Fig. 22). The phenomenon was further investigated using the cut-open oocyte technique. In the absence of intracellular amino acids, addition of alanine to the external bathing solution did not appear to induce any currents in rBAT-expressing oocytes. However, when 0.5 mM arginine was present intracellularly, addition of alanine to the external bathing solution led to an outward current similar to that seen with the two-microelectrode system. It was concluded that amino acid exchange is the major mode of action of this protein.

Independent voltage- and current-clamp studies performed by Busch et al. (31) on rBAT-injected *Xenopus* oocytes led to the same conclusions. In the current-clamp mode, superfusion with L-leucine caused hyperpolarization, whereas superfusion with L-arginine depolarized the oocyte. As before, in voltage-clamp experiments, dibasic amino acids and neutral amino acids induced inward and outward currents, respectively. In an elegant experiment, changes in pH, from 6.25 to 8.75, did not affect the currents induced by saturating concentrations of arginine or leucine, but reversed the direction of L-histidine-induced currents from inward to outward (Fig. 23).

Sodium-independent currents of opposite polarity were also demonstrated by Ahmed et al. (1) in oocytes injected with the homologous cRNA D2 when exposed to either neutral or cationic amino acids, but in this study, the authors concluded that neutral/cationic amino acid exchange was not sufficient to account for the evoked currents.

II) SPECIFICITY OF EXCHANGE. In a recent report, Chilaro et al. (40) have shown that the substrate specificity of the *trans*-acceleration of arginine efflux in rBAT-injected oocytes is consistent with the substrate specificity observed in influx studies. This is expected for an exchange process according to the carrier mechanism. The efflux of L-[3H]arginine and L-[3H]leucine in injected oocytes increased eight- and sevenfold, respectively, when arginine (1 mM) was added to the external medium, whereas the efflux in uninjected oocytes was unaffected by the same additions (Fig. 24A). The \( K_m \) values for leucine and arginine, as stimulators of L-[3H]arginine efflux, were 67 ± 26 and 65 ± 5 \( \mu \text{M} \), respectively (i.e., similar to the \( K_m \) values for influx, 85 ± 7 and 90 ± 12 \( \mu \text{M} \), respectively), but the maximum acceleration was twofold higher for L-arginine. In influx experiments, L-leucine is also transported more slowly \([V_{\text{max}}(\text{Arg}), 211 ± 11 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{oocyte}^{-1}; V_{\text{max}}(\text{Leu}), 59 ± 4 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{oocyte}^{-1}]\).

The authors proposed that rBAT expression induces an obligatory amino acid exchange system. However, there is some ambiguity as to the exact magnitude of the acceleration of rBAT-associated efflux, that is, with regard
The difference between a net and an exchange mode of operation is quantitative and depends on the relative rates of translocation of the free carrier and the loaded carrier. In a true obligatory exchange system, such as the Cl-/HCO₃⁻ anion exchanger, the difference between these two rates is as large as 40,000-fold (78, 112). Thus accurate measurements of the net transport rates are required to distinguish unambiguously between these two possibilities. Nevertheless, it is evident that, as concluded in several studies (31, 40, 58), this rBAT-associated transport activity can operate as an efficient amino acid exchanger.

In a recent report, Mora et al. (140) studied the transport of L-cystine in a “renal proximal tubular” cell line (OK cells) and showed that this activity has similar characteristics to those induced by rBAT cRNA in oocytes. The uptake of cystine was Na⁺ independent and shared by arginine and leucine. Both cationic and neutral amino acids, added to the external solution at 1 mM, were seen to stimulate the rate of efflux of L-[³H]arginine, but the stimulation was larger for cationic amino acids (arginine, 17-fold; lysine, 12-fold; leucine and phenylalanine, 6-fold). The difference could result from

1) a divergence in the rates of translocation of the carrier-substrate complex with neutral and cationic amino acids,
2) differences in the electrogenicity of the homoexchange of cationic substrates versus their heteroexchange for neutral amino acids, and
3) the participation of parallel pathways.

III) Energetics. In their study of the exchange properties of the rBAT-induced activity, Chillarón et al. (40) found that the steady-state levels of accumulation for 50 μM L-[³H]arginine, L-[³H]leucine, and L-[³⁵S]cystine, after 3–6 h of incubation, were found to be higher in oocytes expressing rBAT than in uninjected controls; in addition, the accumulation of arginine in rBAT-injected oocytes was higher than in mCAT-1-injected oocytes (Fig. 25). The same results were obtained for rBAT-injected oocytes in the presence or absence of Na⁺. It was proposed that the accumulation is the consequence of the obligatory exchange mode of operation of rBAT. However, countertransport is found not only for obligatory exchange mechanisms, but is an inherent property of carrier-mediated transport, whether obligatory or not. The difference in the magnitude of the accumulation observed in mCAT-1-injected oocytes and rBAT-injected oocytes is consistent with the properties of these two transporters.

IV) Stoichiometry. Chillarón et al. (40) examined the stoichiometry of the homo- and heteroexchange of L-leucine and L-arginine via rBAT by comparing the unidirectional influx and efflux of labeled amino acids under identical substrate distribution conditions (Fig. 24B). Oocytes were loaded with leucine or arginine (labeled or unlabeled) by incubating for 4 h with 1 mM amino acid. The loaded oocytes were then placed in solution containing 250 μM arginine or leucine (labeled or unlabeled) as shown in Figure 24B. For each experimental situation, the

to the tightness of the coupling. This arises from the fact that the basal arginine efflux in injected oocytes was lower than that of uninjected controls, and therefore, there is uncertainty regarding the magnitude of the rBAT-induced net efflux under zero-trans-conditions.
rate of entry equalled the rate of exit, in agreement with an \( n:n \) stoichiometry. The heteroexchange of internal leucine for external arginine was seen to be favored over the exchange in the reverse direction. The fastest reaction was arginine homoexchange. The experiment was performed at a single substrate concentration; in a true obligatory exchange system, influx and efflux should be identical at all substrate concentrations.

The proposal that rBAT expression induces a classical obligatory exchange system, in which the flux of a given substrate is coupled to the flux of another, has been recently challenged by Coady et al. (57). They have observed that alanine and aminoisobutyric acid (AIB) are equally effective in the stimulation of arginine efflux in rBAT-injected oocytes but that only the first of these amino acids is transported into the cell at a rate similar to neutral amino acids; that is, the specificity of system \( y^{+}/L \) depends on the ionic composition of the medium. A transporter with these characteristics has been described in human erythrocytes (64), brush-border membranes from human placenta (72, 80), human lymphocytes (28), and oocytes injected with 4F2hc mRNA (17, 235). It has also been induced in oocytes after injection of mRNA obtained from choriocarcinoma cell line (JAR cells) (73). It does not tolerate \( \alpha-\text{NH}_{2} \)-group substitution, as

change to occur but would be slow to debind. However, this predicts that the affinity for AIB should be substantially greater than that of alanine and arginine, which is not the case. A deeper understanding of this interesting phenomenon requires that studies be extended to include more substrate analogs and that the model be subjected to quantitative tests.

4. System \( y^{+}/L \), the broad-scope and cation-modulated amino acid transporter

System \( y^{+}/L \) is a broad-scope transporter that binds and translocates cationic and neutral amino acids. Sodium ion replacement with \( K^+ \) does not affect lysine transport but markedly decreases the affinity of the transporter for neutral amino acids; that is, the specificity of system \( y^{+}/L \) depends on the ionic composition of the medium. A transporter with these characteristics has been described in human erythrocytes (64), brush-border membranes from human placenta (72, 80), human lymphocytes (28), and oocytes injected with 4F2hc mRNA (17, 235). It has also been induced in oocytes after injection of mRNA obtained from choriocarcinoma cell line (JAR cells) (73).

A) SPECIFICITY OF THE BINDING STEP. In \( Na^+ \) medium, the binding specificity of system \( y^{+}/L \) resembles that of system \( b^{	ext{H}^+} \), although the substrate affinities are somewhat higher. The half-saturation constants of system \( y^{+}/L \) for lysine and leucine are comparable (\( K_m \) for L-lysine, 9.5 ± 0.67 \( \mu \)M; \( K_m \) for L-leucine, 10.7 ± 0.72 \( \mu \)M); the system also interacts strongly with L-methionine, L-glutamine, and with considerably less affinity with smaller amino acids such as L-serine, L-alanine, and L-glycine (Table 8) (8, 9, 63). It does not tolerate \( \alpha-\text{NH}_{2} \)-group substitution, as
shown by the lack of effect of N-methyl-L-leucine on lysine influx, and does not interact with BCH (5 mM) or proline (2 mM) (63).

Although, in general, the apparent affinity increases with overall size (Fig. 26, Table 8), the carrier site can accurately discriminate between side chains of comparable hydrophobicity. The different apparent affinities for leucine and isoleucine (11 and 250 μM, respectively) suggest that a methyl group substituent attached to the β-carbon interferes with binding; this is consistent with the observation that the addition of an extra carbon atom (which in general increases binding) is detrimental when added as a methyl group at this position (see alanine-aminobutyric acid transition in Fig. 26).

System y’L has been shown to bind and transport various cationic substrates including L-lysine, L-arginine, L-ornithine, and the methylated analog of L-lysine N0-monomethyl-L-arginine (76). The affinity is highest for L-arginine. The system is stereospecific, but the preference for the L-isomer is considerably more marked with the neutral amino acid leucine (900-fold) than with the cationic substrates lysine or arginine (15- and 56-fold, respectively) (63, 76).

B) CATION DEPENDENCE OF THE BINDING STEP. As shown in Table 8, binding of cationic amino acids to system y’L occurs equally well in Na+ or K+ medium, but its association with neutral amino acids is strictly dependent on the cation present (9). In K+, the carrier behaves as a cationic amino acid specific carrier, interacting weakly with neutral amino acids. Replacement of Na+ with K+ reduced the affinity of the carrier for all neutral amino acids tested, but the reduction was more important for those amino acids that are the best substrates in Na+ (leucine, methionine, glutamine). Thus, in K+ medium, the transporter not only binds neutral amino acids with lower affinity, but it is also less able to discriminate among them. In contrast, Li+ potentiates neutral amino acid binding, and in general, the apparent affinities are higher than in Na+. In the presence of Li+, elongation of the nonpolar side chain makes a more important contribution to binding (Fig. 26), and the carrier is considerably more tolerant toward β-carbon substitution. Therefore, in Li+ medium, the binding strength is higher, but again, the selectivity is less marked than in the presence of Na+. The guanidinium ion (30 mM) has also been shown to potentiate neutral amino acid binding to system y’L, and the magnitude of the effect depends on the length of the side chain (Fig. 26). Thus, with respect to glycine, guanidinium exhibits a “K+ like behavior” and with respect to norleucine a “Na+ like behavior” (9).

A remarkably similar cation dependence was described by Thomas et al. (213) in Ehrlich cells 25 years ago. Most notably, apparent affinities for the same series of substrates (alanine-norleucine) were found to increase in the presence of Li+ or Na+, whereas the corresponding affinities in K+ or choline were reported to be considerably lower. Moreover, the relative effect of the addition of successive methylenes in Li+ and Na+ medium is qualitatively similar in the two types of cells. This is in agreement with the proposal that an important fraction of the cationic amino acid uptake in Ehrlich cells occurs through a transporter analogous to system y’L.

In the framework of these pioneering studies, Christensen et al. (50) proposed an explanation for the difference...
by binding inside or outside the substrate site cannot be Both in human erythrocytes and in 4F2hc-injected oo-

dium were seen to stimulate eflux of [3H]arginine in

either the carrier, and not by direct interaction with the amino acid. Large stimulation by Na⁺ and Li⁺ was seen for amino acids with nonpolar chains, such as isoleucine and leucine, where direct interaction between the cation and amino acid side chain cannot be conceived (9).

Whether the cations affect the carrier conformation by binding inside or outside the substrate site cannot be decided on the basis of the observations made so far. The induction of distinct conformational changes in an enzyme, dialkyglycine decarboxylase, by K⁺ or Na⁺ has been reported (215).

C) SPECIFICITY AND CATION DEPENDENCE OF THE TRANSLOCATON STEP. A study of the specificity of the translocation step showed that system γ'L in human erythrocytes can accept a wide range of amino acids as substrates. Relative rates of inward translocation of the carrier-analog complex for various amino acids were estimated from their trans-effects on the unidirectional efflux of L-[14C]lysine (9). As shown in Figure 18, unlabeled L-lysine was able to markedly trans-accelerate lysine efflux. On average, the magnitude of the trans-acceleration was 6.1 ± 0.46 (n = 15). With the exception of L-tryptophan, all neutral and cationic amino acids tested were found to induce the a marked trans-acceleration of labeled lysine efflux. Some observations are shown in Figure 27. The half-saturation constants calculated from this effect agreed with those previously measured in cis-inhibition experiments (8, 76). This observation indicates that substrates exhibiting widely different affinities, when bound, are able to translocate at comparable rates.

The only structural feature that appears to impair translocation is bulkiness. Most notably, L-tryptophan, the largest amino acid tested, was not able to accelerate the rate of transport at concentrations that completely inhibit entry. This suggests that the tryptophan-loaded carrier and the free carrier reorient inward with a similar rate. Small analogs such as serine and alanine (which are weakly bound) were found to induce comparable trans-stimulation to the best substrates (8).

Interestingly, the degree of trans-acceleration caused by neutral amino acids (leucine and glutamine) did not differ significantly in Na⁺, Li⁺, or K⁺ medium, although, as expected, the concentrations required to produce the effect varied. The Kᵦᵣ values for activation matched those measured in cis-inhibition experiments (9). Thus the effect of ions on the interaction of neutral amino acids with system γ'L is exerted at the level of binding exclusively.

In agreement with the above findings, L-arginine and L-leucine, but not L-tryptophan, added to the external medium were seen to stimulate efflux of [3H]arginine in 4F2hc-injected oocytes, and moreover, the concentration of leucine required to produce stimulation was higher in the absence of Na⁺ than in its presence (40).

It can be concluded that, in the case of system γ'L, the binding step is more sensitive to modifications in amino acid structure than translocation. The observation that specificity is principally expressed in substrate binding indicates that the carrier reorientation step is largely independent of the forces of interaction between the substrate and the transport site (113).
FIG. 27. Effect of various amino acids on lysine efflux via system y\(^-\)L in human erythrocytes. NEM-treated erythrocytes were preloaded with L-[\(^{14}\)C]lysine, and efflux was measured in presence or absence of unlabeled amino acid outside. Rates were normalized using reference rates measured in presence of 1 mM lysine. Dotted line indicates average value for relative rate measured in absence of amino acid. Average trans-stimulation by lysine was 6.1 ± 0.46-fold (n = 15). Internal concentration of L-[\(^{14}\)C]lysine at beginning of experiment was 4–5 mM. Half-saturation constants (\(K_T\)) calculated on basis of Equation 16 are shown. In case of tryptophan, constant given corresponds to \(K_i\) for L-lysine entry. [Modified from Angelo et al. (8).]

cytes, system y\(^-\)L-mediated efflux has been shown to be highly dependent on the presence of substrates on the trans-side (Figs. 18 and 27; Refs. 8, 40). In 4F2-injected oocytes, the efflux measured in the absence of external amino acids was markedly stimulated by addition of 1 mM arginine or of 1 mM leucine to the external medium. This shows that in both cases the carrier-substrate complex translocates faster than the free carrier. Surprisingly, L-[\(^{3}\)H]leucine efflux in 4F2hc-injected was not stimulated by either leucine or lysine (40). The reason for this apparent asymmetry remains unknown.

Further evidence for amino acid heteroexchange comes from the observation that oocytes expressing 4F2hc, but not uninjected oocytes, showed a small, but reproducible, positive outward current when 10 mM L-leucine was present in the external medium in the absence of Na\(^+\) (1.2 ± 0.1 and −0.1 ± 0.1 nA for 4F2hc-injected and uninjected oocytes, respectively) (40). In the presence of Na\(^+\), the current was not detectable in agreement with the electrically silent influx observed in human erythrocytes (62).

As observed for rBAT, 4F2hc-injected oocytes were found to accumulate substrates to a level higher than mCAT-1-injected oocytes (Fig. 25A) (40). Consistently, system y\(^-\)L has also been found to sustain lysine accumulation in human erythrocytes (Fig. 25B; Devés and Angelo unpublished data). L-[\(^{14}\)C]lysine transport was seen to reach higher intracellular levels in NEM-treated cells (sys-

FIG. 28. Lysine influx via system y\(^-\)L in human erythrocytes is insensitive to changes in membrane potential. Hyperpolarization was induced by addition of 2 \(\mu\)M valinomycin in presence of a K\(^+\) gradient. Uptake of 1 \(\mu\)M L-[\(^{14}\)C]lysine in presence or absence of valinomycin (Val) was measured in intact (A) and NEM-treated cells (B). Calculated entry rates via system y\(^-\)L (NEM sensitive) and system y\(^+\)L (NEM insensitive) are shown in inset. External medium contained MOPS/Na\(^+\) (pH 6.8), 140 mM NaCl, and 4 mM KCl. [Modified from Devés and Angelo (62).]
tem y⁺ inhibited) than in untreated cells (systems y⁺ and y⁻L intact). Thus, although the initial (or unidirectional) flux is higher when both systems are functional, the magnitude of the accumulation is larger when only system y⁻L is active. The observation implies that, under these experimental conditions, system y⁺ functions as an exit route for lysine. The higher accumulation that is observed in the presence of system y⁻L alone must be due, as suggested by Chillarón et al. (40), to countertransport with endogenous amino acids. Countertransport is not expected with system y⁺, because its specificity is restricted to cationic amino acids (which are present at low intracellular concentrations); on the contrary, the concentration of neutral amino acids, which are substrates of system y⁻L, is significant (unpublished observations).

E) EFFECT OF MEMBRANE POTENTIAL. The influx of lysine through system y⁻L in human erythrocytes has been shown to be electroneutral, indicating that, under the experimental conditions used, the rate-limiting step does not involve charge movement (62). Neither hyperpolarization, induced by the addition of the K⁺ ionophore valinomycin in the presence of an outwardly directed K⁺ gradient (180), nor depolarization, induced by replacing external Cl⁻ with the impermeant anion glucuronate (16, 77), affected influx significantly. This finding is in agreement with the proposal that system y⁻L participates in the exchange of lysine for endogenous neutral amino acids plus Na⁺ (40, 62). The effect of hyperpolarization of lysine influx through system y⁺ and system y⁻L into human erythrocytes is shown in Figure 28.

F) EFFECT OF SURFACE POTENTIAL. Sodium chloride replacement with sucrose was seen to stimulate total lysine (5 μM) influx into human erythrocytes and to reduce the $Kₜₐₜ$ for lysine from 59 to 5 μM. This effect was initially attributed to the removal of a competitive inhibition exerted by Na⁺ on system y⁺ (254). A reexamination of this phenomenon has showed that the sucrose-induced stimulation can be explained by a selective activation of system y⁻L-mediated influx (as discussed above, the activity of system y⁺ was reduced in the presence of sucrose) (62). It has been proposed that the sucrose-induced stimulation of system y⁻L is consistent with a surface potential effect.

The rationale was as follows. A negative surface potential develops when erythrocytes are suspended in low ionic media (15, 94). The potential will produce an increase of the concentration of cations near the membrane interface, relative to that in the bulk face, and as a result influx will increase (212). Counterions that exert a "screening" effect, reducing the surface potential, should therefore act as inhibitors when added to sucrose media, and multivalent counterions should be more potent than univalent ones (133). The effect of the potential is expected to be more marked at low substrate concentrations, diminishing as the substrate approaches saturation. It follows that mobile cations are expected to inhibit with an apparent competitive behavior. In addition, inhibition is expected to be partial because the effect of mobile ions should lessen as the surface potential approaches zero. The observed effect of cations on the sucrose-induced stimulation of system y⁻L was entirely consistent with the theory exposed above. I) Increasing Na⁺, K⁺, Li⁺, and choline caused a partial reduction of the flux [mean

![Figure 29](http://physrev.physiology.org/fig/29)
TABLE 9. Properties of the four transporters for cationic amino acids

<table>
<thead>
<tr>
<th>Transport System</th>
<th>y⁺</th>
<th>B⁺⁺</th>
<th>b⁺⁺</th>
<th>y⁻L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Related cDNA</td>
<td>mCAT-1</td>
<td>Unknown</td>
<td>rBAT</td>
<td>4F2hc</td>
</tr>
<tr>
<td>Binding specificity, (K_a) or (K_i), (\mu M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arginine</td>
<td>89</td>
<td>+</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>99</td>
<td>140</td>
<td>48–79</td>
<td>10</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>48,000</td>
<td>+</td>
<td>135</td>
<td>11</td>
</tr>
<tr>
<td>L-Valine</td>
<td>ND</td>
<td>110</td>
<td>4,850</td>
<td>2,500</td>
</tr>
<tr>
<td>Cation dependence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Na⁺/K⁺)</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>(Li⁺/K⁺)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Na⁺/choline</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Chloride stimulation</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Membrane potential dependence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero-trans flux</td>
<td>Yes</td>
<td>Yes</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Exchange CAA/NA</td>
<td>ND</td>
<td>ND</td>
<td>Yes</td>
<td>No ((Na⁺))</td>
</tr>
<tr>
<td>Surface potential effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Lysine influx</td>
<td>ND</td>
<td>ND</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>L-Leucine influx</td>
<td>ND</td>
<td>ND</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>Inhibition NEM</td>
<td>Yes</td>
<td>ND</td>
<td>ND</td>
<td>No</td>
</tr>
<tr>
<td>Functional expression</td>
<td>Ubiquitous (not in liver)</td>
<td>Blastocysts, small intestine</td>
<td>Blastocysts, kidney (BBM), small intestine</td>
<td>Erythrocytes, placentas, lymphocytes, small intestine (BBM)</td>
</tr>
</tbody>
</table>

NAA, neutral amino acids; CAA, cationic amino acids; +, interaction, but constant not determined; ND, not determined; BM, basal membrane; BBM, brush-border membrane. Binding specificity, \(K_a\) (or \(K_i\)) values: system y⁺, constants for Arg and Lys are average values from measurements in rabbit reticulocytes, HTC (hepatoma cell line), human fibroblasts, human erythrocytes, or mCAT-1-injected oocytes (references are given in Table 6); constant for Leu was measured in reticulocytes (48); system B⁺⁺, constants for Lys and Val were measured in mouse blastocysts, Arg (79% inhibition at 1 mM), Leu (91% inhibition at 200 \(\mu M\)) (226); system b⁺⁺, constant for Arg (40) was measured in rBAT-injected oocytes, for Val, Lys, Leu measured in blastocysts (222); system y⁻L, constants for Arg (76), Leu, and Lys (65) were measured in human erythrocytes. References to other properties are given in text. Cation dependence indicates whether binding of amino acid is potentiated in \(Na⁺\) vs. \(K⁺\) medium, \(Na⁺\) vs. \(Li⁺\) medium, or \(Na⁺\) vs. choline medium.

affinity constant \((K_{0.5} = 25–34 \text{ mM})\), the effect reaching a maximum at 35–40% of the original flux. 2) Divalent cations (\(Mg²⁺, \text{Ca}²⁺\)) were also inhibitory, but lower concentrations were required \((K_{0.5} = 1.1–1.8 \text{ mM})\). 3) Sucrose stimulation was reduced at higher lysine concentrations. The effect of monovalent and divalent cations on lysine influx through system y⁻L is shown in Figure 29.

5. Summary

The properties (structural and functional) of these four systems are compared in Table 9. This summarizes the evidence reviewed above, which led to the conclusion that these four transporters are distinct and, moreover, are sufficient to account for experimental findings reported to date. Discrimination criteria that can be used to establish adequate experimental strategies to distinguish and characterize these systems are given in Table 10.

V. PHYSIOLOGY OF CATIONIC AMINO ACID TRANSPORT

If we consider just one of the naturally occurring cationic amino acids, arginine, we find that it has a wide variety of functions (reviewed in Ref. 13). These include a major role in nutrition, where arginine is especially important in catabolic states (starvation, injury, sepsis). Arginine is also central to nitrogen balance, because in the liver it is the immediate precursor for synthesis of urea; additionally, it is a precursor for the synthesis of creatine and of the polyamines (spermine, spermidine, and putrescine). Arginine is also the substrate for NO synthase and, thus, central to NO biology. Finally, it is also required for host immunity, possibly through a thymotrophic action that may relate to the ability of the amino acid to stimulate peptide hormone release from a wide variety of endocrine tissues (including insulin and glucagon from the pancreatic islets and growth hor-
TABLE 10. Discrimination criteria: characteristics that distinguish the transport system highlighted in bold (top) from the transport systems listed in first column

<table>
<thead>
<tr>
<th>y⁺</th>
<th>B⁺⁻</th>
<th>b⁺⁻</th>
<th>y⁻L</th>
</tr>
</thead>
<tbody>
<tr>
<td>y⁺</td>
<td>High affinity for NAA</td>
<td>High affinity for NAA</td>
<td>Very high affinity for NAA</td>
</tr>
<tr>
<td></td>
<td>Na⁺ dependent for CAA</td>
<td>Na⁺ independent for NAA</td>
<td>High exchange/net transport rate</td>
</tr>
<tr>
<td>B⁺⁻</td>
<td>Weak affinity for NAA</td>
<td>BCH not a substrate</td>
<td>BCH not a substrate</td>
</tr>
<tr>
<td></td>
<td>Na⁺ independent for CAA</td>
<td>Low affinity for valine</td>
<td>Low affinity for valine</td>
</tr>
<tr>
<td></td>
<td>Cl⁻ independent</td>
<td>Na⁺ independent</td>
<td>Na⁺ independent</td>
</tr>
<tr>
<td>b⁺⁻</td>
<td>Weak affinity for NAA</td>
<td>BCH transport</td>
<td>Cystine not a substrate</td>
</tr>
<tr>
<td></td>
<td>Na⁺ dependent for NAA</td>
<td>High affinity for valine</td>
<td>Na⁺ dependent for NAA</td>
</tr>
<tr>
<td></td>
<td>Cl⁻ dependent</td>
<td>Cl⁻ independent</td>
<td>Cl⁻ independent</td>
</tr>
<tr>
<td>y⁻L</td>
<td>Weak affinity for NAA</td>
<td>BCH transport</td>
<td>Cystine transport</td>
</tr>
<tr>
<td></td>
<td>NEM sensitive</td>
<td>High affinity for valine</td>
<td>Electrogenic exchange (NAA/CAA)</td>
</tr>
<tr>
<td></td>
<td>Na⁺ dependent for CAA</td>
<td>Na⁺ independent</td>
<td>NEM sensitive</td>
</tr>
<tr>
<td></td>
<td>Cl⁻ dependent</td>
<td>Electrogenic exchange</td>
<td>NEM sensitive</td>
</tr>
</tbody>
</table>

CAA, cationic amino acids; NAA, neutral amino acids; NEM, N-ethylmaleimide; BCH, 2-amino-endo-bicyclo[2.2.1]heptane-2-carboxylic acid.

mone and prolactin from the adenohypophysis of the pituitary gland). All of these effects require that the amino acid is transported into the cells of the responsive tissues.

In this section of the review, we attempt to bring together a variety of published work that emphasizes the relevance of cationic amino acid transport to many disparate areas of physiology.

A. Nutrition

1. Protein synthesis and growth

Nutritionally (119), the cationic amino acids are categorized into those that are essential (lysine, histidine, and arginine) and those that are nonessential (hydroxylysine and ornithine), although the distribution of these amino acids between the two groups is known to vary with age and species. Thus, in the human newborn, both lysine and histidine are essential with respective dietary requirements of ~3.0 and 1.5 mmol/day (152); however, in the adult, only lysine remains an essential amino acid with a daily dietary requirement of ~6 mmol. There are nutritional interactions between cationic amino acids, particularly, an “antagonism” between lysine and arginine. Thus Anderson and Dobson (7) showed, in chicks, that the rate of growth in control animals fed similar (and sufficient) quantities of arginine and lysine was greater (26%) than in animals fed an identical diet but supplemented with additional lysine (approximately doubling their intake of this amino acid). This inhibition of growth was reversed completely by further supplementation of the diet with arginine. Explanations for such an interaction include competition for shared transport systems (at the intestinal, renal, or cell/tissue level), although there are alternative (and/or additional) possibilities such as effects on appetite, amino acid metabolism, or protein synthesis. Dietary arginine is required for optimal growth of the young of many, if not all, species (136), and although citrulline (in excess) can substitute for arginine deficiency, ornithine is unable to do so. Renal de novo arginine synthesis from citrulline seems to be sufficient to meet the growth requirements for this amino acid. Citrulline itself is synthesized from glutamine in the small intestinal mucosa, and ~80% of the citrulline produced in this way is ultimately converted to arginine in the kidney (242). Complementary and prolactin from the adenohypophysis of the pituitary gland). All of these effects require that the amino acid is transported into the cells of the responsive tissues.

In this section of the review, we attempt to bring together a variety of published work that emphasizes the relevance of cationic amino acid transport to many disparate areas of physiology.

A. Nutrition

1. Protein synthesis and growth

Nutritionally (119), the cationic amino acids are categorized into those that are essential (lysine, histidine, and arginine) and those that are nonessential (hydroxylysine and ornithine), although the distribution of these amino acids between the two groups is known to vary with age and species. Thus, in the human newborn, both lysine and histidine are essential with respective dietary requirements of ~3.0 and 1.5 mmol/day (152); however, in the adult, only lysine remains an essential amino acid with a daily dietary requirement of ~6 mmol. There are nutritional interactions between cationic amino acids, particularly, an “antagonism” between lysine and arginine. Thus Anderson and Dobson (7) showed, in chicks, that the rate of growth in control animals fed similar (and sufficient) quantities of arginine and lysine was greater (26%) than in animals fed an identical diet but supplemented with additional lysine (approximately doubling their intake of this amino acid). This inhibition of growth was reversed completely by further supplementation of the diet with arginine. Explanations for such an interaction include competition for shared transport systems (at the intestinal, renal, or cell/tissue level), although there are alternative (and/or additional) possibilities such as effects on appetite, amino acid metabolism, or protein synthesis. Dietary arginine is required for optimal growth of the young of many, if not all, species (136), and although citrulline (in excess) can substitute for arginine deficiency, ornithine is unable to do so. Renal de novo arginine synthesis from citrulline seems to be sufficient to meet the growth requirements for this amino acid. Citrulline itself is synthesized from glutamine in the small intestinal mucosa, and ~80% of the citrulline produced in this way is ultimately converted to arginine in the kidney (242).
such hyperammoniemia; citrulline is less effective than either arginine or ornithine (70). This may reflect the relative affinities and translocation rates for the transport of these substrates in the hepatocyte. Additionally, depolarization of the hepatocyte membrane (associated with Na\(^+\)-coupled nutrient uptake into the liver after dietary protein intake) would favor net loss from the cell of the key intermedrate ornithine (required for ammonia incorporation into the urea cycle) through CAT-2A.

3. Other roles

Although in the human the essential cationic amino acids may enter the epithelium of the small intestinal mucosa in the form of lysine- or histidine-containing oligopeptides (rather than as the free amino acids), after their absorption from the intestinal tract, they will be used as essential nutrients by the very wide range of different cell types, which are synthesizing proteins at appreciable rates. In all such cells, the entry of lysine across the plasma membrane will be a condition necessary for cell survival. However, there are other processes that do not involve protein synthesis and that will also require the cell to have the relevant transport systems expressed. Some examples are given below.

A) INSULIN SECRETION. The release of insulin, clearly of major nutritional significance, is stimulated by many secretagogues in addition to the classical stimulant, raised plasma glucose. One of these is a raised concentration of arginine in venous blood. L-Arginine transport into pancreatic \(\beta\)-cells has been shown to produce membrane depolarization, opening of voltage-gated Ca\(^{2+}\) channels, increase of intracellular Ca\(^{2+}\) and, as a result, insulin release. It was proposed that this effect may be mediated by mCAT2-A, which has been found to be expressed in an insulinoma cell line (MIN6) (198). The effect of arginine would be most important during glucose-stimulated insulin secretion. In this instance, glucose metabolism will raise the membrane resistance by closure of \(K_{ATP}^{+}\) channels and thus potentiate the depolarization caused by the inward current generated by influx of cationic amino acid (11).

B) NITRIC OXIDE SYNTHESIS. Arginine delivery (mediated by its transport across the membrane) may be important for intracellular arginine-requiring enzymes. It has been observed that, in many cell types (cardiac muscle, lymphocytes, macrophages), the concentration of extracellular L-arginine is rate limiting to the production of NO by the enzyme NO synthase (reviewed by Simmons et al. in Ref. 197). However, in the whole animal, individual tissues appear to respond differently to arginine supplementation. Thus endothelial NO production has been observed to depend on extracellular arginine, mainly, when there is concomitant pathophysiology as in hyperlipidaemia or atherosclerosis (151); in macrophages and smooth muscle, such dependence is a more consistent finding (see sect. VD).

Mechanisms causing the transport of a substrate to be rate limiting (when due to synthesis within the cell, this might not have been predicted) include 1) the rate of utilization exceeding synthetic capacity, 2) intracellular compartmentalization, or 3) kinetic coupling of substrate entry to specific cytoplasmic enzymes. Whatever the mechanism(s) responsible, it suggests that cationic amino acid transporters may be targets for intervention in the pathway leading to NO biosynthesis. It is also pertinent to point out that tissue-specific expression of such transporters will itself determine the delivery of certain NO synthase inhibitors to target cells (see Refs. 14, 26, 76, 190).

C) SYNTHESIS AND TRANSPORT OF POLYAMINES. Recent work (reviewed in Ref. 2) has shown that inward rectification of K\(^+\) channels in nerve and muscle is due to interaction with cytoplasmic polyamines. Because ornithine is the precursor needed for polyamine synthesis, the transport of this amino acid may be relevant to nerve and muscle excitability. Polyamines themselves may cross cell membranes using some of the transport systems for cationic amino acids (134), as suggested by the inhibition exerted by the polyamine putrescine on ornithine transport in Ehrlich cells. Although the pathways for ornithine flux were not identified, they appear to involve two Na\(^+\)-independent routes, both inhibited (although with differing affinities) by the polyamine.

B. Epithelial Transport of Cationic Amino Acids

If an epithelium is to generate polarized transport of any solute, in one particular direction (whether absorptive or secretory), the transport properties of the input (apical) and output (basal) membranes must differ. Furthermore, if the luminal concentration of solute is to fall below the concentration in the relevant capillary bed, then its flux must be coupled to an external energy source. This requires the presence, in the epithelium, of an active transport system either for input or output (or both). In the case of cationic amino acids, the negative membrane potential will contribute to the driving force for amino acid influx at the apical surface and equivalent, but opposite, effects will apply to the efflux of cationic amino acids at the basolateral membrane.

It has been recognized for nearly 40 years that there are similarities between the transport of cationic amino acids in renal proximal tubule and the small intestine (135). This became clear from the observation that, in patients with the renal abnormality cystinuria (see sect. vB4a), the transport of lysine and ornithine was also defective in the intestinal tract. For this reason, and with considerable prescience, Milne et al. (135) postulated that the genetic defect underlying cystinuria was shared by
the small intestine. This notion was compatible with early studies (reviewed in Ref. 241) that indicated that in the in vitro hamster intestine there was net transport against a concentration gradient for the three basic amino acids and that these amino acids "shared the same transport system as cystine."

1. Intestine

A) BRUSH-BORDER MEMBRANE. Both Na\(^+\)-dependent and Na\(^+\)-independent transport systems for cationic amino acids have been described in the brush border of the small intestine, although the presence of both systems in all parts of the small intestine, and in different species, is not a consistent finding.

In rabbit jejunal brush-border membrane vesicles, Stevens and co-workers (204, 205) described a single Na\(^+\)-independent transport system for lysine that was thought to represent system y\(^{+}\). Similar conclusions were reached for guinea pig vesicles of small intestine (188). Cassano et al. (34) describes, in rat brush-border membrane vesicles, a Na\(^+\)-independent transporter for cationic amino acids that also interacts with leucine and phenylalanine; this activity is compatible with system b\(^0,+\).

A different pattern of expression has been found in the distal ileum of some species, including the rabbit. In this case, in addition to a Na\(^+\)-independent pathway, a Na\(^+\)-dependent transport system for lysine has been described (143, 163, 243). Recently, Munck and Munck (146) have suggested that this Na\(^+\)-dependent component of lysine transport shows functional properties characteristic of system B\(^0,+\) (see sect. IVB).

Finally, a recent kinetic analysis of lysine transport in chicken jejunal vesicles by Torras-Llort et al. (216) has shown the presence of two Na\(^+\)-independent transport systems that have half-saturation constants (zero-trans-lysine influx) of 2 and 160 \(\mu\)M. The high-affinity system is inhibited (in a Na\(^+\)-independent way) by neutral amino acids with high affinity (e.g., \(K_i\) for methionine is 20 \(\mu\)M) and, thus, has similar properties to system b\(^0,+\) (although the affinity for lysine appears to be significantly higher than in blastocysts) (see Table 9). The lower affinity system (also inhibited by neutral amino acids, but only in the millimolar range) is completely abolished after NEM preincubation, as found for system y\(^{+}\) in other cell types. This study made use of the procedure outlined in section IV A to distinguish between parallel transport systems. Similar conclusions have been reached in studies with Caco-2 cells (161). These cells are derived from a human colonic adenocarcinoma and undergo enterocytic differentiation in culture. The uptake of arginine (5 \(\mu\)M) was Na\(^+\) independent and could be dissected into two fractions. One pathway was found to be selective for cationic amino acids and inhibited by NEM and was associated with system y\(^{+}\). The other pathway recognized cationic and neutral amino acids and was insensitive to NEM; these characteristics are consistent with system b\(^0,+\), system y\(^{+}\)L, or a combination of the two (cation replacement experiments were not carried out). The location of these systems, either at the apical or basal membranes, was not addressed. Interaction between cationic and neutral amino acids at the apical membrane of Caco-2 cells was observed by Thwaites et al. (214). Although, in general, the experimental strategies used in this study do not allow a clear discrimination of the routes involved, at least a fraction of the lysine uptake (at 10 \(\mu\)M) appears to occur via system b\(^0,+\), since it is partially inhibited by cystine (200 \(\mu\)M).

B) BASOLATERAL MEMBRANE. There is evidence that efflux across the basolateral membrane constitutes the rate-limiting step of transepithelial cationic amino acid transport (reviewed in Ref. 36). Thus, during lysine transport across the intact epithelium, the intracellular amino acid concentration may exceed by fivefold that in the lumen, whereas for neutral amino acids, the accumulation of solute in the tissue is much less (1.3-fold for leucine under comparable conditions).

This fact must be considered in relation to the discovery of Robinson and Felber (181) that a number of neutral amino acids, added at the luminal side, stimulated (rather than inhibited) absorption of cationic amino acids from the distal ileum of some species, including the rabbit. In this case, in addition to a Na\(^+\)-independent pathway, a Na\(^+\)-dependent transport system for lysine has been described (143, 163, 243). Recently, Munck and Munck (146) have suggested that this Na\(^+\)-dependent component of lysine transport shows functional properties characteristic of system B\(^0,+\) (see sect. IVB).

Finally, a recent kinetic analysis of lysine transport in chicken jejunal vesicles by Torras-Llort et al. (216) has shown the presence of two Na\(^+\)-independent transport systems that have half-saturation constants (zero-trans-lysine influx) of 2 and 160 \(\mu\)M. The high-affinity system is inhibited (in a Na\(^+\)-independent way) by neutral amino acids with high affinity (e.g., \(K_i\) for methionine is 20 \(\mu\)M) and, thus, has similar properties to system b\(^0,+\) (although the affinity for lysine appears to be significantly higher than in blastocysts) (see Table 9). The lower affinity system (also inhibited by neutral amino acids, but only in the millimolar range) is completely abolished after NEM preincubation, as found for system y\(^{+}\) in other cell types. This study made use of the procedure outlined in section IV A to distinguish between parallel transport systems. Similar conclusions have been reached in studies with Caco-2 cells (161). These cells are derived from a human colonic adenocarcinoma and undergo enterocytic differentiation in culture. The uptake of arginine (5 \(\mu\)M) was Na\(^+\) independent and could be dissected into two fractions. One pathway was found to be selective for cationic amino acids and inhibited by NEM and was associated with system y\(^{+}\). The other pathway recognized cationic and neutral amino acids and was insensitive to NEM; these characteristics are consistent with system b\(^0,+\), system y\(^{+}\)L, or a combination of the two (cation replacement experiments were not carried out). The location of these systems, either at the apical or basal membranes, was not addressed. Interaction between cationic and neutral amino acids at the apical membrane of Caco-2 cells was observed by Thwaites et al. (214). Although, in general, the experimental strategies used in this study do not allow a clear discrimination of the routes involved, at least a fraction of the lysine uptake (at 10 \(\mu\)M) appears to occur via system b\(^0,+\), since it is partially inhibited by cystine (200 \(\mu\)M).

B) BASOLATERAL MEMBRANE. There is evidence that efflux across the basolateral membrane constitutes the rate-limiting step of transepithelial cationic amino acid transport (reviewed in Ref. 36). Thus, during lysine transport across the intact epithelium, the intracellular amino acid concentration may exceed by fivefold that in the lumen, whereas for neutral amino acids, the accumulation of solute in the tissue is much less (1.3-fold for leucine under comparable conditions).

This fact must be considered in relation to the discovery of Robinson and Felber (181) that a number of neutral amino acids, added at the luminal side, stimulated (rather than inhibited) absorption of cationic amino acids from the distal ileum of some species, including the rabbit. In this case, in addition to a Na\(^+\)-independent pathway, a Na\(^+\)-dependent transport system for lysine has been described (143, 163, 243). Recently, Munck and Munck (146) have suggested that this Na\(^+\)-dependent component of lysine transport shows functional properties characteristic of system B\(^0,+\) (see sect. IVB).

Finally, a recent kinetic analysis of lysine transport in chicken jejunal vesicles by Torras-Llort et al. (216) has shown the presence of two Na\(^+\)-independent transport systems that have half-saturation constants (zero-trans-lysine influx) of 2 and 160 \(\mu\)M. The high-affinity system is inhibited (in a Na\(^+\)-independent way) by neutral amino acids with high affinity (e.g., \(K_i\) for methionine is 20 \(\mu\)M) and, thus, has similar properties to system b\(^0,+\) (although the affinity for lysine appears to be significantly higher than in blastocysts) (see Table 9). The lower affinity system (also inhibited by neutral amino acids, but only in the millimolar range) is completely abolished after NEM preincubation, as found for system y\(^{+}\) in other cell types. This study made use of the procedure outlined in section IV A to distinguish between parallel transport systems. Similar conclusions have been reached in studies with Caco-2 cells (161). These cells are derived from a human colonic adenocarcinoma and undergo enterocytic differentiation in culture. The uptake of arginine (5 \(\mu\)M) was Na\(^+\) independent and could be dissected into two fractions. One pathway was found to be selective for cationic amino acids and inhibited by NEM and was associated with system y\(^{+}\). The other pathway recognized cationic and neutral amino acids and was insensitive to NEM; these characteristics are consistent with system b\(^0,+\), system y\(^{+}\)L, or a combination of the two (cation replacement experiments were not carried out). The location of these systems, either at the apical or basal membranes, was not addressed. Interaction between cationic and neutral amino acids at the apical membrane of Caco-2 cells was observed by Thwaites et al. (214). Although, in general, the experimental strategies used in this study do not allow a clear discrimination of the routes involved, at least a fraction of the lysine uptake (at 10 \(\mu\)M) appears to occur via system b\(^0,+\), since it is partially inhibited by cystine (200 \(\mu\)M).
in rabbit ileum, Munck and Schultz (147, 148) were able to show that neutral amino acids exerted their effect by accelerating the rate of exit of cationic amino acids across the basolateral membrane. In later studies, this effect has been found to be widespread, occurring in a number of species, and in colon as well as small intestine (35, 144).

The most detailed analysis of this phenomenon is to be found in the study carried out by Cheeseman (35) in vascularly perfused frog small intestine, which led to the conclusion that neutral amino acids stimulated lysine absorption from the basolateral side. He showed that lysine drained from the preloaded epithelium in an asymmetric way, the rate constant for efflux across the basolateral membrane (into the vascular bed) being approximately fivefold greater than that for efflux back into the lumen. He noted, in addition, that the backflux of lysine (tissue concentration ~3.5 mM) into the lumen was stimulated approximately fourfold by the addition, to the lumen, of ornithine or arginine (2 mM) and less importantly by cysteine (2-fold). In contrast, neither alanine, leucine, nor proline accelerated lysine backflux into the lumen.

Interestingly, the events at the basolateral side were found to differ. Lysine efflux across the basal membrane was inhibited fourfold by addition of arginine or ornithine (10 mM) to the lumen. As expected, when added to the vascular side (10 mM), the same amino acids trans-stimulated lysine efflux markedly (>7-fold); histidine produced a fourfold stimulation. However, remarkably, neutral amino acids (alanine, leucine) exerted a stimulatory effect whether added to the lumen or the vascular perfusate. It was further shown that very low concentrations of leucine in the vascular perfusate were sufficient to stimulate lysine efflux across the basolateral membrane; thus, even at a concentration of 10 µM, leucine was able to trans-stimulate by 2.4-fold (Fig. 30). Trans-stimulation at the basolateral membrane was found to be asymmetric, in that leucine in the tissue (~1 mM) did not stimulate lysine (1 mM) influx from the vascular compartment. This asymmetry was not found for the cationic amino acids, ornithine being able to trans-stimulate influx as well as efflux of lysine across the basal membrane.

Taken together, these results seem to provide strong evidence for the presence of system y$^+$L in the basolateral membrane, as suggested in several recent reports (8, 72, 159). System y$^+$L shows all the properties that are required to account for the leucine-induced stimulation of lysine absorption. It is broad scope, transporting leucine and lysine at equivalent rates and with high affinity ($K_m$ ~10 µM); it is subject to strong trans-acceleration and is able to sustain leucine-lysine exchange (Figs. 18 and 27 and Table 9). The heteroexchange of neutral cationic amino acid is electroneutral (and thus it circumvents the membrane potential effect against lysine exit) (Fig. 28). The binding of leucine is greatly reduced if Na$^+$ in the medium is replaced by K$^+$, but the binding of cationic amino acids is not affected (Figs. 5 and 11). This would explain why intracellular leucine does not inhibit lysine efflux across the basal membrane (the Na$^+$ concentration of the cytoplasm is low) but does stimulate from the external surface (in the presence of physiological, i.e., high, Na$^+$ concentrations) (Fig. 30). It also explains why ornithine is effective from both the intra- and extracellular compartments. An early description of countertransport between neutral and cationic amino acids in intestine can be found in the work of Reiser and Christiansen (176–178) in rat isolated enterocytes. The proposal that, in the basolateral membrane, system y$^+$L is responsible for cationic transport, is compatible with recent models, discussed below, of how cationic amino acids may be handled at this face of the cell in other epithelia (40, 72).

C) MULTIPLE TRANSPORTERS INVOLVED IN VECTORIAL TRANSEPIHELIAL TRANSPORT. Although the studies on cationic amino acid transport across in the frog small intestine, discussed above (35), do not provide definitive data on the nature of the cationic amino acid transporters at the brush-border membrane, they show that the transport system(s) present in this membrane is distinct from those at the basal surface. Thus, at the brush border, both the specificity and the magnitude of the trans-stimulation are more compatible with system y$^+$, although the weak interaction with cystine may indicate the additional participation of system B$^{0,+}$. The lack of effect of alanine in cis-inhibition experiments, at the luminal side, appears to rule out the participation of system B$^{0,+}$ in the frog small intestine. However, whatever the relative contributions of systems y$^+$ and B$^{0,+}$ at this surface, it is clear that the vectorial nature of epithelial absorption is compatible with the electrogenic properties of both systems which, in conjunction with an inside-negative membrane potential (29), will allow polarized release of cationic amino acids through a basally located (electroneutral) system y$^+$L. The notion that system B$^{0,+}$ may not play a major role at the apical pole of intestinal epithelial cells (at least in some species) is supported by the very low level of D2 protein detected in rat intestinal brush-border membranes. This is in contrast to findings in renal proximal tubule (167).

In agreement with the proposal that there are multiple transporters involved in lysine absorption across the small intestine, injection of poly(A)$^+$ mRNA isolated from rat small intestine into Xenopus oocytes was reported to induce at least three distinct activities (91). The systems differed in their Na$^+$ dependence and their interactions with neutral amino acids. A significant fraction (38%) of lysine influx (200 µM) was Na$^+$ dependent. L-Leucine was able to inhibit lysine influx in the presence of Na$^+$ with a $K_{0.5}$ of 311 µM, and nearly complete inhibition (~95%) was achieved at 5 mM. In addition, the Na$^+$-dependent fraction was inhibited by alanine with high affinity. Leucine, in the absence of Na$^+$, acted as a partial inhibitor (60% maximum inhibition). The $K_{0.5}$ for this effect was <1
nM (the authors calculate a $K_i$ of 825 $\mu$M, but inspection of the data shows that the value may be substantially lower). Thus this fraction of the flux must represent a transporter that recognizes lysine and leucine in the absence of Na$^+$. The third component is Na$^+$ independent with respect to lysine but Na$^+$ dependent with respect to leucine. Thus three different activities appear to be present, and all of them represent broad-scope transporters. The authors concluded that all three transporters represented previously undescribed routes, although the likelihood that one of these may represent system $y^*$ was acknowledged (91). The analysis in this review suggests that these transporters may correspond to systems $B_0^+$, $b^0^+$, and $y^*$, respectively. The Na$^+$-dependent system shows many properties of system $B_0^+$ including strong interaction with alanine (see Tables 2, 9, and 10), the Na$^+$-independent system is compatible with system $b^0^+$, and the cation modulated pathway is analogous to system $y^*$. Both system $y^*$ and system $B_0^+$ interact weakly with alanine (Fig. 3B and Table 8) as found for the Na$^+$-independent fraction of the flux.

Finally, Wolffram et al. (243) showed, in the rat, that an increased dietary protein load stimulates the transport through a Na$^+$-dependent lysine transporter, presumably, system $B_0^+$. Thus differential dietary dependence of transporter expression must be considered together with species differences when attempting to reconcile apparent contradictions in the published literature.

2. Kidney

The principles of transepithelial cationic amino acid transport exemplified in intestine are also applicable to the kidney. This topic (up until 1988) has been very well reviewed by Silbernagl (194).

In the kidney, there is controversy concerning both Na$^+$ dependence and electrogenicity of the brush-border transport of cationic amino acids. Thus, in studies using membrane vesicles, it has been variously concluded that there is Na$^+$-dependent secondary active transport (96), that there is electrogenic but Na$^+$-independent transport (89), and that there is electroneutral transport in the absence of Na$^+$ (206).

Apparently, contradictory findings have also been reported in electrophysiological studies. The ability of cationic amino acids, added to the lumen, to depolarize the apical membrane of the proximal tubule in situ has been shown to be totally Na$^+$ dependent in rat, whereas in newt, this Na$^+$ dependence is only partial (a substantial depolarization by 10 nM lysine remaining in Na$^+$-free media) (186, 98). As Silbernagl (194) points out, this implies that, if in the rat, systems other than electrogenic Na$^+$-dependent transport are involved in cationic amino acid reabsorption at the brush border, then this additional system(s) must either operate in an electroneutral fashion or have such a low $V_{max}$ so as to be undetectable by electrophysiological assay.

In conjunction with the intestinal data reviewed above, this conclusion is compatible with the notion that there may be variable contributions, possibly dependent on anatomical location, diet, and species, from systems such as $B_0^+$, $y^*$, and $b^0^+$ at this aspect of the small intestine and proximal tubule. As described below, the contribution of $b^0^+$ may be particularly important in the straight segment (S3) of the proximal tubule (where this system contributes to the high-affinity transport system for the handling of cystine and hence plays a role in the abnormalities of cystine reabsorption in cystinuria) (104).

At the basal membrane of proximal tubule, only a very small depolarization is seen in response to addition of cationic amino acids to the peritubular capillary perfusate (186). The observation that this depolarization has a slow onset may indicate that it results from (paracellular) backflux of the amino acid into the lumen of the tubule and uptake from that side. With the consideration of the substantial physiological net flux of cationic amino acids across the epithelium, and the absence of appreciable electric responses to transport across the basal membrane, it follows that (as in the small intestine) system $y^*$ must provide the major route for cationic amino acid exit (or entry) at this pole of the cell. All of the other known cationic amino acid transporters seem precluded from playing a major role, since transport through them would be predicted to generate a depolarizing current (57, 105, 122).

---

![Figure 31](image-url)
Recently, Mora et al. (140) have shown that in a “renal proximal tubular” cell line (OK) there is a high-affinity Na⁺-independent heteroexchange transport for cationic and neutral amino acids (b⁰⁺) at the apical surface. This activity has similar characteristics to that induced by rBAT cRNA in oocytes. However, in cultured cells (Madin-Darby canine kidney) of presumed more distal origin from the nephron, Boerner et al. (23) showed evidence for a B⁰⁺⁺-like transport system in the apical membrane. This transporter was Na⁺ dependent and interacted with arginine, alanine, and leucine with comparable affinities; it only differed from B⁰⁺⁺ activity, as described in blastocysts (226), in its pH dependence. Expression of this system was strongly stimulated by confluence of the cells in culture and by prostaglandin E₂, presumably acting via adenosine 3’,5’-cyclic monophosphate generation. This observation might usefully be exploited in future molecular studies on this transport system.

A model for the reabsorption of cationic amino acids in the kidney was recently proposed by Chilarón et al. (40) and is shown in Figure 31.

3. Placenta

The placenta acts both as a polarized epithelium (with a brush-border surface at the maternal interface and a basal surface in contact with the interstitial fluid around the fetal circulation), as well as a specialized endothelium, lying as it does in direct contact with the maternal circulation in the intervillus space.

With regard to transplacental amino acid delivery to the growing fetus, it is known that the placenta generates a concentration gradient (fetal greater than maternal plasma concentration) for all the cationic amino acids (reviewed in Ref. 255); in vivo this ratio is 3.0 for lysine, 2.1 for arginine, 2.0 for ornithine, and 4.0 for histidine. Intracellular (trophoblast) concentrations are in all instances considerably higher than maternal and somewhat higher than fetal concentrations. This implies that entry across the brush border is against a chemical (but not necessarily an electrochemical) gradient and that exit across the basal surface is down a chemical (but not necessarily down an electrochemical) gradient. In fact, when appropriate values of membrane potential in this tissue (87) are taken into account, the data suggest that (as in other epithelia) entry will be driven by membrane potential (provided it occurs through an electrogenic system), whereas exit into the fetal circulation would be facilitated by a system that does not generate net current flow.

Two transport systems for lysine were described by Eleno et al. (72) in brush-border membrane vesicles from human placenta (Fig. 21). These systems have features characteristic of systems y⁺ and y’L. Thus, in the presence of Na⁺, the higher affinity system interacted with cationic and neutral amino acids (L-leucine, L-methionine, and l-glutamine) with comparable affinity (Kₐ ≈ 10–30 μM). The removal of Na⁺ reduced the affinity for neutral amino acids, but not the maximal inhibition. The other system showed very weak interaction with neutral amino acids, with 10 mM leucine inhibiting ~ 22% of lysine influx, whereas the Kₐ for leucine under optimal conditions (negative membrane potential) was 140 μM. This fraction of the flux was sensitive to NEM (71). All these properties are characteristic of system y’. The two systems have also been found in basal membranes, and in this case, system y’L activity predominates (N. Eleno and C. A. R. Boyd, unpublished data).

Distinct parallel pathways, both at the brush border and basal membrane of human placenta, have also been reported in other studies (79, 80, 128). In particular, Furesz et al. (80) have confirmed the presence, in brush-border membranes, of two transport systems for lysine that are compatible with systems y’L and y’L and have further shown that the high-affinity pathway (system y’L) appears to be very sensitive to changes in temperature, since it was apparent at 37°C but not at 22°C. Multiple transport systems have also been described by this group in basal membranes (79). The idea that distinct transporters participate in the transport of cationic amino acids in placenta is consonant with the data obtained by Wheeler and Yudilevich (236) in guinea pig placenta, showing different half-saturation constants for lysine at the maternal and fetal face.

In studies with rat trophoblast, Malandro et al. (128) described a Na⁺-dependent and broad-scope pathway in the microvillus membrane that increased in activity from 14 to 20 days of gestation. This system, which exhibits characteristics of system B⁰⁺⁺, was absent in basal membranes. In addition, two Na⁺-independent systems were described, which appeared to be present in both membranes. One of these showed the properties of system y’L for it was resistant to leucine inhibition and was inactivated by NEM. The evidence presented is insufficient to identify the leucine-sensitive component unambiguously.

As discussed in section IVB, the proposal that system y’L plays a role in placentar transport has received direct support from the observation of Fei et al. (73) that injection of mRNA obtained from a human choriocarcinoma cell line (JAR) into Xenopus oocytes induces y’L activity.

Because the syncytiotrophoblast of the human placenta develops from the blastocyst proper, transport properties expressed in this structure very early in development might reasonably be looked for in the mature placenta. Studies on the mouse blastocyst (224) showed that there were two systems, b⁰⁺⁺ and B⁰⁺⁺, present at this very early developmental stage. Because at term the systems found in the human appear to be y’ and y’L, it will be interesting for future studies to follow transporter expression during development, so as to relate embryonic to fetal events.
Both systems b_{0}^{+,+} and y_{L} may be important under conditions of fast growth, when their broad substrate specificity should allow a heterogeneous mixture of amino acids to be delivered to the proliferating cells. Given that these are transport systems that have only recently been described, they may be worth considering as potential targets for oncogene action and as regulators of cell growth (cf. Saier et al. in Ref. 185). This idea is supported by the observation that the 4P2 surface antigen appears to be highly expressed in most malignant human cells (183).

4. Genetic disorders of epithelial transport

There are two genetic disorders that have shed light on the normal physiology of renal and intestinal cationic amino acid transport: cystinuria and LPI (132). Cystinuria is a disease resulting from an inherited disorder of the transport of neutral and cationic amino acids at the apical membrane of both epithelia (82). Lysinuric protein intolerance is a (much rarer) genetic disorder of cationic amino acid transport at the opposite face of the cell (164). Because both are compatible with life, and given that lysine and histidine are essential amino acids, neither disease can be a disorder of the “ubiquitous” system y_{L} and each must result from the malfunction of an “epithelial-type” transport system.

A) CYSTINURIA TYPE I. In a major breakthrough, Calonge et al. (32) have shown that cystinuria (type I) is caused by mutations in the rBAT protein, thus establishing this transporter as a cystinuria-related gene product.

Cystinuria is one of the classical inborn errors of metabolism. It is an autosomal recessive disease characterized by excessive urinary excretion of cystine and of the cationic amino acids arginine, lysine, and ornithine. The disorder occurs as a result of the low solubility of cystine, which precipitates in the urinary tract. With the use of the data (for humans) given by Silbernagl (194) (a plasma cystine concentration of 30 \mu M and a clearance 1% that of glomerular filtration rate), it can be calculated that with normal hydration (i.e., a urinary flow rate of at least 1 ml/min), the urinary cystine concentration will be \sim 40 \mu M, i.e., roughly 6% of the concentration of a saturated aqueous solution of this amino acid. However, if the system(s) responsible for cystine reabsorption is impaired, so that the nephron reabsorbs <90% (rather than the normal 99%) of the filtered load of cystine, then with only modest oliguria, precipitation will occur in the renal tract. Cystinuria is responsible for 6–8% of renal stones in children, reflecting a heterozygote (carrier) frequency of \sim 1 in 60 in caucasians.

There appear to be three types of cystinuria differentiated either by the renal transport behavior of heterozygotes or by the function of the intestinal epithelium in homozygotes. In type I cystinuria, heterozygotes have normal renal and intestinal transport behavior; in contrast, heterozygotes in type II and in type III cystinuria, respectively, show cystinuria and dibasic amino aciduria. Additionally, it is only in type III cystinuria that homozygotes show a near-normal increase in plasma cystine after oral ingestion of the amino acid. Although earlier genetic evidence from families who were compound heterozygotes had suggested that these three forms of the disorder were probably the result of allelic mutations at the same locus (i.e., that the disease was a multiallelic monogenetic disorder) (see Refs. 169, 182, 192), recent evidence has shown this not to be the case (33). Rather, there is homogeneous linkage (see below) of type I cystinuria to mutations in the human rBAT protein (SLC3A1), whereas for type III cystinuria, there is no linkage to this locus (on chromosome 2p16.3). Very recent studies (22) have shown that type III cystinuria (and also probably type II cystinuria) is caused by mutations in genes carried on the long arm of chromosome 19. This means that cystinuria must now be considered as a heterogeneous disorder.

In their seminal paper, Calonge et al. (32) looked for mutations in rBAT proteins which, as discussed in section III, are known to induce system b_{0}^{+,+}-like activity following expression in Xenopus oocytes. They identified six mutations, all missense which were cystinuria (type 1) specific. The most frequent mutants (M467T and M467K), when reintegrated into cellular physiology by the observation that the 4P2 surface antigen appears to be highly expressed in most malignant human cells (183).

Fourteen additional rBAT mutations have now been described that also cause the disease (83, 138, 169, 159). All mutations, so far described, are in the extracellular domain of the single transmembrane segment model of this protein (see Fig. 10, B and C). Figure 32 shows how these mutations relate to the four-transmembrane model (141). It is interesting that the most common mutations M467T and M467K are, in this model, located in the third transmembrane domain.

The molecular work on cystinuria has recently been reintegrated into cellular physiology by the observation...
that in the renal cell line (OK cells) (140), expression of rBAT is associated with system b0,+ activity (see sect. III B). The activity was localized to the apical pole of confluent cells. This is completely in keeping with the fragmentary literature reviewed above, which indicates the presence of rBAT protein and high-affinity cystine transport in the proximal straight segment of the tubule (81, 167).

Based on their studies of the exchange properties of the systems related to rBAT and 4F2hc expression in X. laevis oocytes, Chillarón et al. (40) proposed that system b0,+ may participate in the uptake of cationic amino acids and cystine at the brush-border membrane, whereas system y+L is likely to serve as a route for cationic amino acid (but not cystine) exit across the basal membrane (Fig. 31). The authors pointed out that competition of lysine and cystine for reabsorption and heteroexchange of neutral for cationic amino acids at the brush border may explain the observation that, in dogs in vivo, infusion of lysine increases cystine secretion (27).

The observation that the transport of cationic amino acids in cystinuria type III is normal in both kidney and small intestine indicates that this disorder must be unrelated to abnormal functioning of any of the four transporters discussed in this review. The system involved in this case is probably a high-capacity transport system for cystine and located more proximally in the initial part of the tubule segment.

B) LYSINURIC PROTEIN INTOLERANCE. Lysinuric protein intolerance (also known as dibasicaminocaciduria type II) is an autosomal recessive disorder in which there is increased urinary excretion of lysine, ornithine, and arginine associated with intestinal malabsorption of protein-rich food. It is extremely rare in populations other than those from Finland. This genetic disorder causes impairment of cationic amino acid transport, specifically across the basolateral membrane of kidney and intestine (173, 174, 194). Although nothing as yet is known of the molecular basis of the defect, a plausible candidate gene for this disease would be that related to system y+L and, thus, to 4F2hc.

However, the very recent mapping of the LPI locus to the long arm of chromosome 14 (116) precludes the disease being caused by mutations in the 4F2hc protein itself, since this protein is encoded on chromosome 11 (166). This observation is of course compatible with the notion that 4F2hc may be a regulator of the protein encoded on the long arm of chromosome 14 and that it is this gene product (mutated in LPI) that is the y+L transporter itself.

The observation that LPI specifically affects the transport of cationic amino acids in both intestine and kidney, and that this is independent of whether the cationic amino acid enters the tissue across the brush border as free amino acid or as (a component of) a small peptide, reinforces the conclusion that this disorder must have the basolateral membrane transport system of these epithelial cells as its anatomic locus.

Early work on cationic amino acid transport in fibroblasts and red blood cells from patients with LPI was carried out by Smith and co-workers (199, 200) before...
there was knowledge of either the existence or properties of the various transport systems for cationic amino acids. These studies (purporting to show reduced trans-stimulation of 100 μM homoarginine efflux by 1 mM external lysine in fibroblasts, but not in red blood cells) should be repeated using the new experimental tools available to dissect out the contributions of the different pathways.

The ability of a general model such as that shown in Figure 31 to properly describe the epithelial transport of cationic amino acids can be tested by comparing its predictions with the observed phenotypic consequences of a specific monogenetic disorder. It is reassuring that as predicted from physiological studies on both tissues, in LPI the aberrant transport system both in the renal tubule (172) and in the small intestine (61) appears to be present in the basolateral membrane.

Whether currently identified cationic amino acid transporters are involved in other very rare genetic disorders such as histidinuria (184), in which there is selective impairment in both intestinal absorption and renal reabsorption of histidine, or in lysine malabsorption syndrome (156), in which again there is a selective deficit in the epithelial transport of a single cationic amino acid (lysine), is unknown. If they are, this would seem to require that the relevant mutations result in specific alterations in the properties of the substrate binding site of the affected transport protein.

C. Role of Cationic Amino Acid Transporters in Cells of the Immune System

As described in section III, the cDNA for the Tea, which initially was isolated as a partial sequence, was found to encode a cationic amino acid transporter [mCAT-2(B)].

What might be the function of this gene in T-lymphocytes? mCAT-2(B) transcripts have been shown to accumulate in activated murine T lymphocytes (124), and this accumulation follows a similar time course to that seen for the induction of lysine transport in human peripheral blood mononuclear cells (predominately T cells) after their in vitro activation by phytohemagglutinin (PHA) (28). Thus strong stimulation both of system y'-mediated transport and of Tea gene transcription was seen 8 h, but not 4 h, after PHA exposure, with maximal expression and transport at 18 h. Because system y'-L was activated with a slower time course and to a lesser degree, it was suggested that the Tea gene product was not associated with the y'-L phenotype.

MacLeod and colleagues (102, 126) have subsequently demonstrated that, after their maturation in the thymus, and having migrated to their target tissues as quiescent lymphocytes, these cells then downregulate CAT-2 gene expression until activated by mitogen (102, 126). Thus expression is biphasic, with a transient elevation at an early developmental stage; thereafter, the level of mRNA depends on the extent of mitogenic stimulation. Crawford et al. (60) showed that the activation of system y' transport is particularly prominent in one particular class of human T lymphocytes characterized by their expression of the CD45RA phenotype. These are thought to be "naive" T cells, a population important for T-helper cell function. T-cell proliferation is known to be strongly and specifically inhibited by NO (3, 38). Because the circulating T cells produce NO after their activation (Chen and Boyd, unpublished data), it is likely that subsequent proliferation of these activated cells will be modulated by negative feedback through NO. Hemoglobin is able to bind NO with extremely high affinity (84), and thus the concentration of NO depends on the extent of the hyperemia, associated with the inflammatory response. Increased inflammation would thus release the local NO brake on lymphocyte proliferation. Nitric oxide synthase II has been identified as the enzyme responsible for NO production in human T cells (175), and thus it is possible that, as suggested by Closs (52) for macrophages, there may be a requirement for extracellular arginine and that the activity of mCAT-2(B) may constitute a possible point of control of NO production by these cells.

Experiments using antisense oligonucleotides to inhibit expression of the CAT genes (39) support this model, since both in human peripheral blood mononuclear cells and in macrophages there is inhibition of NO production by antisense, but not by sense, oligonucleotides to the H13/CAT1 gene.

As Closs (52) points out, endothelial NO release is not dependent on extracellular arginine (160), and in this cell type, the mCAT-2(B) gene is not expressed. This raises the question of whether it may be possible, therapeutically, to target CAT-2(B) selectively to inhibit tissue production of NO differentially.

With respect to the proposed physiological role of mCAT-2(B) in the production of NO by T lymphocytes, and the possible function of this molecule in lymphocyte biology, it is interesting that in the subtractive hybridization experiments that led MacLeod et al. (126) to the discovery of the Tea gene [CAT-2(B)], high Tea expression (and, presumptively, NO production) was associated with the more differentiated cell line, whereas the Tea-negative cell line was rapidly growing and highly dedifferentiated.

A question that now needs to be addressed is how the integrated actions of arginine, CAT-2(B), and NO might alter and control the immune system, not with lymphocytes in vitro, but in intact lymphoid tissue, such as lymph node or Peyer’s patch. This challenging physiological problem is analogous to that encountered when considering the role of an inhibitory or excitatory amino acid at a synapse in vivo; it is also apparently relevant to the very recent observation that NO is a potent negative regulator of cell proliferation during Drosophila development,
controlling the balance between cell proliferation and cell differentiation (114).

The lymphocyte model of interactions between CAT-2(B) and NO release has shed light into the regulation of CAT transporter function by NO (38). Addition of an exogenous NO donor (such as S-nitroadenosylprusside; SNAP) inhibits lysine transport through system y^+, but not y^L. Endogenous production of NO [after activation of CAT-2(B) expression] causes further inhibition of lysine influx. This suggests that a classical metabolic negative-feedback loop exists in these cells, whereby the product (NO) inhibits a rate-limiting step (arginine influx) in the pathway of its biosynthesis. Nothing is known of the mechanism responsible for this effect; it could, for example, be the consequence of covalent phosphorylation of CAT-2(B) transporters via NO-sensitive guanosine 3',5'-cyclic monophosphate-dependent protein kinase; equally, it could be the consequence of target protein phosphorylation altering the rate of insertion of this transporter into, or retrieval from, the plasma membrane.

D. Regulation of Cationic Amino Acid Transport

The observation that some of the mCAT isoforms are tissue specific (Table 4) and, moreover, that their concentration in a given tissue varies according to environmental conditions, indicates that cationic amino acid transporters, like other amino acid transporters (reviewed by McGivan and Pastor-Anglada in Ref. 131), are subject to regulation (reviewed in Refs. 123, 127). This notion is supported by the finding of Finley et al. (75), showing that at least four widely spaced promoters are able to initiate mCAT-2(B) transcription.

There is also physiological evidence supporting the proposal that cationic amino acid transport is a regulated process. Recent information obtained in three different systems is reviewed.

1. Endothelium

In view of the central role that NO plays in endothelial physiology, it becomes important to understand the regulation of cationic amino acid transport in these cells. However, there is still controversy regarding which transport systems are present in endothelium. In human venous endothelial cells cultured in vitro, Mann et al. (130) originally described two distinct pathways for arginine (0.5 µM) influx; one of these was Na^+-independent (65% of total flux) and the other was Na^+- dependent (35% of total flux, choline substituting for Na^+); the specificity of these two components was not investigated. The authors also observed that ~20% of leucine (0.16 µM) influx, in the presence of Na^+, was inhibited by arginine and lysine (1 mM). These observations suggest that at least two systems must be involved in cationic amino acid transport in these cells. One of these systems is likely to be analogous to the Na^+-dependent system B^0,+ (see Tables 9 and 10).

More recent work, carried out by this group on the same cells (e.g., Refs. 201, 202), has been interpreted assuming that arginine influx occurs via system y^+ exclusively (the apparent K_m for arginine being 101 µM). The authors used Michaelis-Menten analysis of arginine influx as a criterion for homogeneity, which, as shown in section IV, it is not sufficient to rule out parallel pathways. The possible competition between arginine and typical substrates of system B^0,+ (BCO, alanine, valine) does not appear to have been investigated. The same assumption was made in studies of arginine transport in porcine aortic endothelium (24).

Other recent studies have, in contrast, shown there to be more than a single pathway for cationic amino acid transport in endothelium. Thus, in endothelial cells from porcine pulmonary artery maintained in culture on fibronectin-coated surfaces, Grene et al. (86) found convincing evidence for a B^0,+ -like system. This Na^+-dependent pathway (K_m, 62 µM) accounted for 30% of influx at 50 µM arginine. The system was shown to interact with cationic amino acids (lysine, ornithine) as well as with neutral amino acids (leucine, cysteine, alanine, glutamine); L^+ was not able to substitute for Na^+. The Na^+-independent component (70% at 50 µM arginine) was insensitive to pH changes and was completely inhibited by (10 mM) lysine, ornithine, or homoarginine and, thus, was ascribed to system y^+, although because the effect of additional amino acids was not tested, other possibilities have not formally been excluded. For reasons that are unclear, arginine (10 mM) did not saturate the transporter.

It is worth remarking that in vivo the endothelium is polarized and that the influence of polarity, on both regulation of expression and anatomic distribution of different transporters for cationic amino acids, has not been studied. This could contribute to understanding possible causes of apparently conflicting findings on cultured endothelial cells. Future experiments should include the study of arginine transport at each face of the cell in a “tight” capillary endothelium, such as at the blood-brain barrier or placental trophoblast.

Recent studies by Sobrevia et al. (201) show that cationic amino acid transport in endothelial cells is a regulated process. Bradykinin (100 nM) was found to increase K^+ permeability, via receptor-mediated Ca^{2+} mobilization and to hyperpolarize the endothelium (201). This was associated with a threefold increase in tracer arginine (20 nM) influx. This acute (2-min exposure to the peptide hormone) and indirect regulation of transport may be contrasted with that produced by arginine deprivation in the same study. After 24 h of selective deprivation of arginine, the intracellular concentration of cationic amino acids was found unaltered (the measured pool of arginine plus
lysin plus ornithine was 2.4 mM before and 2.6 mM after deprivation). Nevertheless, the rate of cationic amino acid influx (10 μM arginine, lysine, or ornithine) was increased significantly. The mechanism responsible for this interesting effect awaits further study. It may involve mechanisms similar to those underlying the effects of hyperglycemia and of insulin in stimulating arginine transport in normal endothelium (202). Both these stimuli appear to require protein synthesis, the former having a time constant of ~4 h, the latter being blocked by cycloheximide.

2. Smooth muscle

The transport of arginine into cultured rat aortic smooth muscle cells has been reported to have features characteristic of system y+ (69). Transport was Na+-independent, with a \( K_m \) of 125 μM. Arginine influx (50 μM arginine) was found to be inhibited by cationic amino acids (10 mM) but not by neutral amino acids. However, this experiment was carried out in the absence of Na+, so it does not completely preclude a contribution from system y^-L. Arginine (50 μM) influx into cells preloaded with a number of cationic or neutral amino acids showed that only the former \( transt \)-stimulated; again, the experiment does not rigorously exclude the possible contribution of system y^-L because of the low intracellular Na+ concentration.

This latter issue remains open, since in a similar cell preparation, interactions between the transport of lysine and neutral amino acids were found (121). Thus partial inhibition (~40% of total flux) of lysine (0.1 mM) influx was produced by methionine, leucine, isoleucine, valine, and tyrosine (all at 1 mM in Li^-containing medium). External leucine and phenylalanine (10 mM) did \( transt \)-stimulate lysine exit (in Li^- but not in choline medium). The Li^+ was more potent than Na^+, which was more potent than K^+, or choline, in activating \( transt \)-stimulation of lysine efflux by leucine. Similarly, with Li^+ in the external medium, \( cis \)-inhibition of lysine (0.1 mM) influx by leucine was already maximal at 1 mM leucine, whereas with K^+ as the external cation, the apparent affinity of leucine was at least 10-fold weaker. Consistently, preloaded leucine (low Na^+ environment) failed to produce stimulation of lysine influx (1 or 100 μM); this behavior is characteristic of system y^-L (Figs. 5 and 11; Tables 8–10). This is at odds with the conclusion in the paper that the findings were “entirely consistent with the uptake of lysine being mediated solely by system y^-.” This conclusion has been quoted by others in support of the notion that system y^- (and a diffusional/low-affinity component) accounts for total transport of arginine into rat isolated smooth muscle cells (240).

There is good evidence for regulation of cationic amino acid transport in smooth muscle cells. Wileman et al. (240) have demonstrated a striking effect of bacterial lipopolysaccharide (LPS), singly and in conjunction with interferon-γ on increasing the \( V_max \) of arginine influx. The effect was blocked by cycloheximide and required in a minimum of 8 h, implying that protein synthesis was required for the observed stimulation. Steroids (dexamethasone) did not antagonize the effect. It is interesting that increased expression of mCAT-2(B) transcripts has been noted in vascular smooth muscle (85) after stimulation by cytokines.

In addition, Low and Grigor (120) have recently shown transient induction by angiotensin II (at concentrations of 10^-10 M and above) of both mCAT-1 (10-fold at 2 h) and mCAT-2(B) (more weakly) gene expression in rat vascular smooth muscle in culture. This was accompanied by a doubling of the rate of entry of cationic amino acids 6 h after stimulation; this level of transport was then maintained. The effect of angiotensin II was mediated by an AT1 receptor and was completely blocked by prior inhibition of transcription (with actinomycin D) or translation (with cycloheximide). There was no effect of the hormone on the leucine-inhibitable component of arginine influx (which as discussed above we, but not the authors, identify as system y^-L). Angiotensin pretreatment (24 h) was shown to also increase (~5-fold) the efflux of labeled arginine from preloaded smooth muscle cells.

The authors point out that, in view of the mismatch between transient stimulation of gene expression and maintained stimulation of transport, their results indicate that there must be additional translational or posttranslational regulation. They speculate that the large size of the untranslated component of the transcript may indicate the presence, in the untranslated regions, of elements controlling translation and stability of the mRNA. The same group (85) has shown that individually two cytokines (interleukin-1β and tumor necrosis factor-α) stimulate arginine influx into the cultured smooth muscle cells and that these effects appear to be additive. This stimulation required de novo protein synthesis and was associated with increased expression of CAT-2(B), but not CAT-1 transcripts (in contrast to the angiotensin effect). Interestingly, cycloheximide in addition to abolishing the cytokine-induced stimulation also inhibited arginine influx into nonstimulated cells, suggesting that protein synthesis is required for transport of arginine under basal conditions. Because dexamethasone was also found to inhibit basal transport, it seems that this steroid may similarly alter mRNA stability or transcription.

In uterine smooth muscle, MacLeod and Kakuda (127) report the predominance of transcripts for mCAT-2(B), although both mCAT-1 and to a lesser extent mCAT-2A were detected (see Table 4).

In contrast to these results on smooth muscle, Simmons et al. (197) have shown in cardiac muscle (adult and neonatal rat ventricular myocytes in culture) that cytokine stimulation of cationic amino acid transport is associated...
with increased (and maintained) expression of CAT-1 as well as CAT-2(B) and (unexpectedly) CAT-2A transcripts. They also noted an increase in cationic amino influx in response to insulin (10⁻¹⁵ M); this was associated with a doubling of CAT-1, but no change in CAT-2A or CAT-2(B) steady-state mRNA levels. They concluded that stimulation of transport by insulin involves a pathway independent of that activated by the cytokines. This conclusion is interesting given that the authors concomitantly determined the influence of arginine transport on NO production and found that whereas the cytokine-induced production of NO was markedly dependent on arginine influx (being very substantially reduced by 10 mM extracellular lysine), that induced by insulin, in the presence of the cytokines, was virtually unaffected by lysine.

3. Macrophages

Marked stimulation (30-fold) of lysine influx into mouse peritoneal macrophages after in vitro stimulation by LPS has been described (187, 193). The effect was maximal after 12-h stimulation and then returned to resting levels. Both the induction of transport and the subsequent return of transport to resting levels were dependent on protein synthesis, since they were very substantially inhibited by cycloheximide added either coincidently with or 12 h after LPS. This is in keeping with the inhibitory effects of cycloheximide on transport into nonactivated smooth muscle cells reviewed above. The authors observed that in nonstimulated cells there was considerable inhibition (~70% of total flux) of lysine (50 µM) influx by glutamine (Kᵢ not determined but much lower than 1 mM). In retrospect, this must reflect the substantial contribution of system y’L to cationic amino acid transport by these cells in the unstimulated state. After stimulation, the contribution of the glutamine-insensitive pathway (presumably system y”) increased some 100-fold, becoming the dominant pathway (193). This activation of transport was followed by increased NO synthesis (measured by nitrite release) with a time lag of 6 h. However, arginine transport was stimulated at a concentration of LPS (half-maximal stimulation ~0.1 ng/ml), four orders of magnitude lower than required to stimulate nitrite release. Extracellular lysine competitively inhibited both LPS-induced transport and nitrite production (with 0.1 mM arginine, the Kᵢ was ~2 mM lysine); serine was without effect, in keeping with the conclusion that the pathway that is so strongly activated by LPS is system y”.

Stimulation of arginine (250 µM) influx after activation with either LPS or interferon-γ was also seen on a cultured macrophage cell line (J774) (25). This effect appeared to require protein synthesis, as evidenced by the time delay before stimulation and the increase in Vₘₐₓ. Subsequently, the same group (14) showed that cycloheximide blocked the stimulation of transport by LPS, whereas dexamethasone (in contrast to the effects of this steroid on cationic amino acid transport in smooth muscle) had no effect. These findings are of obvious interest given that sustained production of NO by these cells requires the presence of extracellular arginine (e.g., Ref. 67).

VI. CONCLUDING REMARKS

The evidence discussed in this review demonstrates that multiple transport systems are responsible for the passage of cationic amino acids across the plasma membranes of animal cells. Experiments performed during the last decade have indicated that at least three clearly distinct activities (B⁰⁺, b⁺⁺, y”L) must be considered, along with classical system y’, in the design and interpretation experiments, as well as in the elaboration of models regarding the mechanism or physiology of cationic amino acid transport. The four transporters are able to transport arginine, lysine, and ornithine but differ in their interactions with neutral amino acids and inorganic cations, and physiological evidence suggests that they are involved in quite diverse cellular processes. This quartet constitutes a unique model system for transport studies, which should attract the attention of those generally interested in the mechanistic and physiological aspects of transport.

Now that the identities of these transporters have been established, we may seek to understand how these biological machines function and in which ways they are relevant to cell physiology. For example, it is important to understand the differences in their transport sites [i.e., what is the “molecular design” that makes one of these transport sites (system y”) selective for cationic amino acids but allows the others to interact strongly with neutral amino acids]. It would also be interesting to find out why one of the transporters is Na’ dependent (B⁰⁺⁺), whereas another, which shows otherwise similar properties (b⁺⁺), can function optimally in the absence of Na’. Also, how do cations determine the substrate specificity of one of these transporters (system y”L)? Do they exert their effects by binding directly at the carrier site or by affecting carrier conformation from a separate site? From a physiological perspective, we would like to know where these different transporters play their respective roles and what process they affect, which are constitutive, and which are regulated. In essence, what is the significance of this diversity?

The strength of this model system has been reinforced by the recent molecular identification of two families of cDNAs that encode cationic amino acid transport proteins. Thus six different gene products have been identified (52, 127, 158): four of these [mCAT-1, mCAT-2A, mCAT-2(B) and rCAT-3] are cationic amino acid-selective transporters (system y” activity) and the other two (rBAT/D2 and 4F2hc) induce in the oocyte broad-scope...
transport activities (with characteristics of systems b₀,⁺ and y¹ L, respectively). It follows that the questions asked above can now be explored molecularly. It should be possible, for example, to engineer a site so as to transform a selective carrier into a broad-scope one, and vice versa, or to convert a Na⁺-dependent system into a Na⁺-independent one, and in the process of doing so, learn about the structural determinants of carrier function. Notable progress in this direction is to be found in the work with chimeras within the CAT family discussed in this review (55, 108).

The unusual structure of the proteins in one of these families (BAT), which are likely to possess a single (or at the most four) transmembrane domains, appears to be pointing toward new concepts in transport regulation. It has been proposed that these proteins may not be full transporters but that they may induce transport by stimulating endogenous activities in the oocyte. Interestingly, one of the transport proteins in this category (rBAT) has been shown to be the gene responsible for cystinuria, and the other (4F2hc, recently classified as CD98) is a surface antigen associated with proliferative states.

There is no doubt that whatever direction this field takes, future studies will require, in addition to a thorough understanding of transport kinetics, a full range of modern experimental approaches including cell biology and genetic manipulation, but more importantly, an open mind to accept that lessons in transport may be coming from unexpected and diverse disciplines. This has been the case with virology and immunology in recent years.

We are grateful to FONDECYT-Chile (1940561) for financial support.

REFERENCES

25. Bogle, R. G., A. R. Baydoun, J. D. Pearson, S. Moncada,


SATOH, O., Y. KUDO, H. SHIKATA, K. YAMADA, AND T. KAWA-


WANG, H. M. P. KAVANAUGH, R. A. NORTH, AND D. KABAT. Cell-


