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Topical Review

Amino Acid Transport in Isolated Rat Hepatocytes

Michael S. Kilberg

Department of Biochemistry and Molecular Biology, University of Florida, J. Hillis Miller Health Center, Gainesville, Florida 32610

Summary. Improvements in the collagenase perfusion techniques have made isolated rat hepatocytes a popular model in which to study hepatic function. Our knowledge of hepatic amino acid transport has been advanced as a result of this methodology. Translocation across the hepatocyte plasma membrane can, in some instances, represent the rate-limiting step in the overall metabolism of certain amino acids. Furthermore, regulation of amino acid uptake by hepatocytes appears to play a role in diabetes, and perhaps in malignant transformation. Comparisons between normal adult hepatocytes and several hepatoma cell lines show basic differences in amino acid transport. There are at least eight distinct systems in normal hepatocytes for transport of the amino acids. One of these, System A, transports the small neutral amino acids most efficiently and responds to a wide variety of hormones. Systems A and N exhibit enhanced uptake rates after the cells have been maintained in the absence of extracellular amino acids, a phenomenon termed adaptive control. Further studies using isolated hepatocytes will increase our basic understanding of membrane transport processes and their regulation.

Key words amino acids · transport · hepatocytes · hormones · adaptive control · liver

Metabolic Significance

The liver is the major site of amino acid metabolism in the body. Furthermore, it is known that most of the hepatic amino acid metabolism occurs in the hepatocytes rather than the nonparenchymal cells such as the Kupfer cells. In fact, the significance, if any, of the nonparenchymal cell fraction of the liver is usually ignored when discussing hepatic metabolism. The hepatocytes are responsible for converting amino acids into proteins, urea, carbon dioxide and water, and a broad spectrum of biologically important metabolites for export to the other tissues. Consequently, amino acid transport into the hepatocyte is not only of considerable interest with respect to hepatic metabolism in particular, but also becomes important when discussing the metabolic homeostasis of the entire animal.

One of the many important functions of the amino acids is to act as precursors in the gluconeogenic pathway. Although only a few amino acids are generally thought of as direct donors of carbon skeletons to *de novo* glucose synthesis, recognition of the glucose-alanine cycle (Felig, 1973) and extra-hepatic glutamine production (Marliss, Aoki, Pozefsky, Most & Cahill, 1971) demonstrates the involvement of many, if not most, of the amino acids in one capacity or another. The relationship between the glucose-alanine cycle and hepatic gluconeogenesis has been reviewed elsewhere (Snell, 1980). With respect to regulation of amino acid-dependent gluconeogenesis, it has long been recognized that amino acid supply may provide a control point in the overall process (Exton, Mallette, Jefferson, Wong, Friedmann, Miller & Park, 1970). Recently, it was shown that transport of alanine into isolated rat hepatocytes represents the rate-limiting step in alanine metabolism (Sips, Groen & Tager, 1980). These studies were performed in perifused hepatocytes from both fed and fasted rats using physiological concentrations of alanine (0.2 to 0.5 mM). McGivan, Ramsell and Lacey (1981) extended the work of Sips et al. (1980) to show that at alanine concentrations greater than 1 mM translocation of alanine across the plasma membrane was not metabolically rate-limiting. Furthermore, it was concluded that stimulation of alanine metabolism by exogenous cAMP could be limited by the rate of alanine transport at physiological levels of the amino acid, although the primary effect of the cAMP occurred on an intracellular reaction when the alanine concentration was above 1 mM (McGivan et al., 1981). Additional research in this area should yield valuable information regarding the importance of transport as a regulatory site for amino acid metabolism.

Amino Acid Transport Systems in Hepatocytes

Characterization of transport systems for the amino acids began with the investigations of the Ehrlich ascites tumor cell by Christensen and his coworkers

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Table 1. Amino acid transport systems in isolated rat hepatocytes

System	Substrate specificity	Selective substrate	Na^+ dependence	Regulation	Additional comments
A	Most neutral amino acids with some preference for those having small, unbranched sidechains	MeAIB	Yes	Adaptive and hormonal control	a) sensitive to pH values below 7.0 b) subject to trans-inhibition c) subject to noncompetitive inhibition
ASC	Neutral amino acids with small sidechains, especially those with an $-OH$ or $-SH$ group	L-cysteine	Yes	None	a) relatively insensitive to pH values below 7.0 but above 6.5 b) subject to trans-stimulation
Anionic (2 systems)	Dicarboxylic amino acids in the form having a net -1 charge	None	Yes (both)	Unknown	a) the model substrates L-cysteate and L-cysteine-sulfinate are transported by both systems b) the two systems can be separated kinetically
Gly	Glycine, sarcosine	None	Yes	Unknown	a) assayed by measuring Na^+-dependent, MeAIB-insensitive glycine or sarcosine uptake b) the velocity of the amino acid is related to the $[Na^+]^2$
L1	Neutral amino acids with large, branched, or aromatic sidechains	None	No	Possibly, but mechanism unknown (*see* text for details)	a) barely detectable in freshly isolated cells or during the first 12 h of primary cultures b) becomes much more predominant after 24 to 48 h of culture c) assayed by using the appropriate concentration of substrate (usually < 50 μM)
L2	Neutral amino acids with large, branched, or aromatic sidechains	None	No	No	a) predominant Na^+-independent system in freshly isolated cells and in primary cultures less than 24 hr old b) assayed by using substrate concentrations greater than 500 μM
N	Histidine, glutamine, and asparagine	Histidine	Yes	Adaptive control	a) sensitive to pH values below 7.0 b) only detected thus far in rat hepatocytes and H4-II-EC3 hepatoma

in the early 1950's (Christensen, Riggs, Fischer & Palatine, 1952). With the exception of a few, most of the transport systems for amino acids, particularly the neutral amino acids, have been described originally in the Ehrlich cell. In fact, this cell type still remains as the "standard" against which newly described systems in other cells are compared. These comparisons have proved beneficial in most cases. As will be discussed below, many of the features of Systems A, ASC, and L that were originally described for the Ehrlich cell have been shown to hold for the systems present in other cell types as well. The Ehrlich cell appears to lack Systems Gly, N, and those for the anionic amino acids and shows no observable regulatory properties. The relative simplicity of this cell's transport characteristics no doubt facilitated the original description of the transport systems present, but the Ehrlich cell must be considered atypical in many respects when one wishes to make comparisons to a "normal" eukaryotic cell. The differences between amino acid transport in the Ehrlich cell and the hepatocyte should serve to demonstrate the importance of characterizing the systems present in a given cell type. The amino acid transport systems which have been reported to exist in the normal adult rat hepatocyte are summarized in Table 1.

System Ly^+

Uptake of lysine by the Ehrlich cell was shown to be mediated by both neutral and cationic systems (Christensen & Liang, 1966). The agency responsible for transport of the cationic amino acids was designated System Ly^+ and later found to accommodate the charged form of lysine, arginine, and histidine. Interestingly, a neutral amino acid plus a sodium ion can competitively inhibit System Ly^+ and participate in exchange reactions with the ordinary substrates of this system (Christensen, Handlogten & Thomas, 1969). Hepatocytes, in primary culture or as freshly isolated cell suspensions, do not exhibit a significant amount of saturable uptake of arginine or its System

Ly^+-specific homolog, homoarginine (White & Christensen, 1982). Based on kinetic analysis, as well as inhibition analysis, it has been concluded that System Ly^+ is not expressed at appreciable levels in normal rat liver hepatocytes. Although the metabolic significance of arginine may lead one to wonder about the physiological importance of such a deficiency, it may be the lack of an exodus route (other than the nonsaturable component) which helps the hepatocyte maintain homeostasis with respect to organismal nitrogen metabolism in general and hepatic urea metabolism in particular. Circulating arginine apparently arises from dietary and extra-hepatic sources such as the kidney (Owen & Robison, 1963). In support of the observations indicating a lack of System Ly^+ transport activity by hepatocytes, it has been reported that, in humans, utilization of circulating arginine by the splanchnic bed is insignificant when compared to the total amino acid levels available (Felig & Wahren, 1971). In contrast to the hepatocyte, the hepatoma cell lines, HTC, 7777 and 8994, contain measurable rates of arginine uptake by System Ly^+ (White & Christensen, 1982). It has been proposed that the symbol Y^+ replace Ly^+ so as not to suggest a unique specificity of this system for lysine. The significance of System Ly^+ expression in the hepatoma as compared to normal hepatocytes is unclear; furthermore we do not know if all hepatoma cell lines contain Ly^+ activity.

Anionic Systems

In contrast to the Ehrlich cell, which does not have a specific system for the anionic amino acids (Garcia-Sancho, Sanchez & Christensen, 1977), isolated rat hepatocytes contain two saturable, Na^+-dependent transport systems for the anionic amino acids (Gazzola, Dall'Asta, Bussolati, Makowski & Christensen, 1981*a*). Cysteate and cysteinesulfinate represent excellent model substrates for anionic amino acid uptake because of the low value for the pK (about 1.5) of the side chain. Both of these compounds are taken up by both of the anionic systems present in normal hepatocytes (Gazzola et al., 1981*a*). On the other hand, cysteate and its homolog, homocysteate, appear to be specific substrates, one for each of the two systems that exist in the HTC cell (Gazzola et al., 1981*a*). Koch, Khalil and Lea (1980) have reported decreased uptake of aspartate and glutamate, relative to the host liver, by the rapidly growing hepatomas 7768, 5123C, 7288CTC and 7777. This decrease in uptake activity for the anionic amino acids is in sharp contrast to most of the neutral systems which are increased in activity upon transformation of liver tissue (Baril, Potter & Morris, 1969; Kelley, Becker & Potter, 1978; Kelley & Potter, 1979). In contrast to the hepatomas listed above, HTC cells appear to exhibit enhanced uptake of the anionic amino acids (M. Makowski, *unpublished results*). Beyond these differences between normal liver and the transformed tissue, regulation of anionic amino acid uptake has not yet been demonstrated in isolated hepatocytes.

System A

System A, originally described nearly two decades ago in the Ehrlich cell (Oxender & Christensen, 1963; Christensen, Oxender, Liang & Vatz, 1965) has received a great deal of attention because of its Na^+-dependence, the availability of specific substrates such as AIB[1] or MeAIB[1], and its regulatory properties. In general, the hepatic System A retains most of the characteristics of the system found in the Ehrlich cell. It is inhibited by pH values below 7.0 (LeCam & Freychet, 1977*a*; Kilberg, Handlogten & Christensen, 1980), it is quite stereo-specific (Kilberg et al., 1980), and it has the ability to accept N-monomethylated amino acid derivatives (Kilberg, Handlogten & Christensen, 1981). The ion-dependency of the System A present in hepatocytes may differ somewhat from that of the Ehrlich cell. It has been reported that Li^+ is unacceptable as a substitute for Na^+ in isolated hepatocytes (Edmondson, Lumeng & Li, 1979), whereas Li^+- for -Na^+ substitution is considered a characterizing feature of the System A present in the Ehrlich cell (Christensen & Handlogten, 1977). On the other hand, the ability of System A in cultured hepatocytes to accept Li^+ as a replacement for Na^+ becomes more apparent when the system is increased in activity, as in the case of adaptive control (Kilberg et al., 1981). The regulatory properties of the hepatic System A have generated a great deal of interest in this particular system, and as a result, more information has been obtained concerning the hormonal control of System A in liver than in any other tissue. Both adaptive and hormonal control of System A in rat hepatocytes will be discussed below.

System Gly

Recently, isolated rat hepatocytes have been shown to contain a glycine-specific system (Christensen & Handlogten, 1981), similar to that originally described in pigeon erythrocytes (Eavenson & Christensen, 1967) and rabbit reticulocytes (Winter & Christensen, 1965). The hepatic System Gly is Na^+-dependent and appears to transport two sodium ions for each glycine.

[1] *Abbreviations:* AIB, 2-aminoisobutyric acid; MeAIB, 2-(methylamino)-isobutyric acid; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid

Imler and Vidaver (1972) have shown a similar relation for the pigeon red blood cell. Glycine uptake by normal hepatocytes is not restricted to System Gly, however, as indicated by a sensitivity of a portion of its transport to inhibition by the System A-specific substrate MeAIB (Christensen & Handlogten, 1981). No significant stimulation of System Gly in normal hepatocytes is observed after treatment with insulin or glucagon or after amino acid starvation of the cells for 24 hr; these results aid in distinguishing System Gly from System A. Gelehrter and his coworkers have studied glycine transport in HTC cells in some detail, especially the regulation by hormones (Reichberg & Gelehrter, 1980). In this hepatoma cell, glycine transport is mediated by two distinct systems both of which are inhibited by glucocorticoid treatment.

System ASC

Until recently, the System ASC activity of rat hepatocytes had to be measured indirectly in the same manner as described for the Ehrlich ascites tumor cell (Christensen, Liang & Archer, 1967). The synthesis of N-(methylamino)-isobutyric acid (MeAIB) and the demonstration that its uptake was specific to the Na^+-dependent System A (Christensen et al., 1965), provided the means to establish that a portion of the Na^+-dependent uptake of alanine, serine, cysteine and other small neutral amino acids was not blocked by an excess of MeAIB (Christensen, Liang, and Archer, 1967). This residual uptake occurred by a process designated System ASC and by definition, characterized as Na^+-dependent, MeAIB-insensitive transport. LeCam and Freychet utilized this property to show that, in contrast to its reported System A specificity in the Ehrlich cell, Na^+-dependent AIB uptake was divided between Systems A and ASC in freshly isolated hepatocytes (LeCam & Freychet, 1976; 1977*a*). Others have also cautioned against the use of AIB as a System A-specific substrate without documentation for a given cell type (Kilberg et al., 1981). Similarly, Edmondson et al. (1979) demonstrated System ASC uptake of alanine by isolated hepatocytes in suspension through inhibition analysis using MeAIB sensitivity.

Kilberg, Christensen, and Handlogten (1979), using MeAIB inhibition as an indicator of System A mediation, found that Na^+-dependent cysteine uptake by hepatocytes in suspension, was totally insensitive to the inhibitor. These results suggested that all of the Na^+-dependent cysteine uptake was mediated by System ASC. Further research supported this hypothesis and demonstrated the usefulness of cysteine as a System ASC-specific substrate for rat hepatocytes (Hogberg & Kristoferson, 1979; Kilberg et al., 1981). Although Na^+-dependent cysteine transport occurs entirely by System ASC, when used as an inhibitor cysteine lacks specificity because of its noncompetitive inhibition of System A (Kilberg et al., 1981). Such noncompetitive inhibition makes it absolutely necessary to provide evidence that amino acids which inhibit the uptake of others do so competitively; without this information it is not possible to equate inhibition with substrate action (Kilberg et al., 1980; 1981). The ability to assay System ASC specifically with cysteine appears to also hold for the rat small intestine (Lancaster, Kilberg & Christensen, 1980), but in HTC cells threonine appears to represent a more specific System ASC substrate (Handlogten, Garcia-Canero, Lancaster & Christensen, 1981). Differences in hydrogen ion sensitivity and substrate specificity can also be used effectively to distinguish Systems A and ASC (LeCam & Freychet, 1977*a*; Kilberg et al., 1981). Using MeAIB-insensitive, Na^+dependent uptake of alanine or AIB, as well as the system-specific substrate, cysteine, several groups have shown that the hepatic System ASC does not respond to hormones (LeCam & Freychet, 1976; Kilberg et al., 1981) or adaptive control (Kelley & Potter, 1978; Kilberg et al., 1980).

As originally described in the Ehrlich cell, System ASC was considered to be a transport agency of relatively narrow specificity and hence perhaps of less physiological importance than Systems A and L. This misconception, along with the failure to identify a universal specific substrate, led to an interval of time during which there was an apparent lack of interest in System ASC. In isolated rat hepatocytes and many other eukaryotic cells, System ASC has a very broad specificity and for many substrates uptake by System ASC occurs at higher velocities than by System A. These findings have stressed the physiological importance of System ASC and generated renewed interest in its relation to hepatic metabolism.

System N

In addition to cysteine, the Na^+-dependent uptake of glutamine and histidine by isolated rat hepatocytes is also unaffected by the presence of a large excess of the System A-specific substrate MeAIB (Kilberg et al., 1980). By the "classical" definition of System ASC, these results would suggest that glutamine and histidine are taken up exclusively by this agency. It was discovered, however, that the System ASC-specific substrate, cysteine, did not competitively inhibit the Na^+-dependent uptake of glutamine. These and other results led Kilberg et al. (1980) to conclude that hepatocytes contained a Na^+-dependent transport system distinct from Systems A or ASC. Support for

such a system had already been reported by Joseph, Bradford and McGivan (1978) who had suggested that glutamine uptake did not occur by the same processes as alanine and serine. Those authors did not attempt to characterize fully the systems using specific properties or substrates, but did show that while alanine and serine were mutually competitive, glutamine inhibited the uptake of these amino acids in a noncompetitive manner. A more detailed study demonstrated the presence of a previously undetected transport agency which mediates the Na^+-dependent uptake of glutamine, histidine and asparagine (Kilberg et al., 1980). In fact, in fully repressed cells glutamine and histidine are specific substrates for the system, designated as System N to reflect an apparent affinity for amino acids containing nitrogen-bearing sidechains. Contrary to the original observation (Kilberg et al., 1980), glutamine uptake also shows a System A component in normal adult hepatocytes which are in a derepressed state (*see below*), thus decreasing its possible usefulness as a system-specific substrate even in that cell (Handlogten, Kilberg & Christensen, 1982*a*). Glutamine does retain complete specificity for System N in the hepatoma cell line H4-II-EC3 (also called H-35) as well as in hepatocytes isolated from rat fetuses (J.V. Vadgama and H.N. Christensen, *personal communication*). Histidine, on the other hand, retains the System N specificity in either amino acid-starved or hormone-treated normal adult hepatocytes, conditions which result in large increases in System A activity.

Interestingly, System N exhibits the ability to undergo derepression after starvation of cultured hepatocytes for amino acids (Kilberg et al., 1980; Handlogten et al., 1982*a*). This ability of System N also holds for the activity present in the hepatoma H4-II-EC3 and in the normal fetal cells (J.V. Vadgama and H.N. Christensen, *personal communication*). Although the increase in System N activity after derepression is not as great on a percentage basis as that for System A, the higher velocities for transport by System N suggest that even a two- to threefold increase in activity would alter the flux of System N substrates considerably. The importance of System N in relation to glutamine metabolism in the liver has not been established. The existence of System N activity in a tissue other than a form of liver tissue, either normal or transformed, has not yet been reported, although only a few cell types have been tested adequately. These limited studies suggest that system N is not present in human fibroblasts or rat intestinal segments (M.S. Kilberg, *unpublished results*), or in CHO cells (Shotwell, Jayme, Kilberg & Oxender, 1981). The possibility that System N is unique to the liver remains for the present.

Systems L1 and L2

The current picture of Na^+-independent neutral amino acid transport by normal adult hepatocytes is undergoing some extensive changes. One of the first amino acid transport systems described for eukaryotic cells was a Na^+-independent system termed System L (Oxender & Christensen, 1963). Since then, most investigators have somewhat arbitrarily assigned all saturable, Na^+-independent uptake to this activity, especially in those cases where the analog 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) was used, because of its reported specificity in the Ehrlich cell (Christensen et al., 1969). Thus, a putative System L activity was characterized in isolated hepatocytes (McGivan, Bradford & Mendes-Mourao, 1977) and shown not to be responsive to either adaptive control (Kelley & Potter, 1978) or hormonal stimulation (Harrison & Christensen, 1971; LeCam & Freychet, 1976; Kilberg & Neuhaus, 1977). This Na^+-independent transport activity also exhibited trans-stimulation (LeCam & Freychet, 1977*a*).

Recently, we have provided evidence which suggests the presence of a previously undetected Na^+-independent system for neutral amino acid transport by isolated hepatocytes (Handlogten, Weissbach & Kilberg, 1982*b*). In this cell the kinetics of leucine and BCH uptake in the absence of Na^+ are biphasic. Component I is a high affinity, low capacity agency with estimated K_m values of less than 200 μM, whereas component II is a low affinity, high capacity system with estimated K_m values between 2 and 5 mM. What little is known of the characteristics of these two components does not allow us to assign the term System L to either system. Although our initial thoughts (Handlogten et al., 1982*b*) were to relate component II to System L and component I to a recently described system (System T) in the red blood cell (Rosenberg, Young & Ellory, 1980), we have since accumulated new information[2] which makes such assignments seem premature. Accordingly, we have chosen to refer, temporarily, to the Na^+-independent components I and II of isolated hepatocytes as L1 and L2, respectively.

At a histidine concentration of 50 μM about 75% of the Na^+-independent transport occurs by L1 (*see* footnote 2); this uptake is completely inhibited by 5 mM cysteine, valine, BCH, isoleucine, tryptophan, tyrosine (2 mM), histidine, methionine, leucine and phenylalanine. It is not known if all of these inhibitors also represent substrates, because it has not been determined if the inhibition is competitive in all cases. System L2, on the other hand, can be assayed quite

[2] L. Weissbach, M.E. Handlogten, H.N. Christensen and M.S. Kilberg (1982) *J. Biol. Chem.* (*in press*).

reliably through the use of 500 μM leucine. At this concentration greater than 95% of Na^+-independent leucine transport occurs by System L2 (*see* footnote 2). Under these conditions leucine uptake is completely inhibited by 5 mM isoleucine, leucine, phenylalanine and BCH.

Systems L1 and L2 show an inverse relationship with respect to changes in activity during the initial 24 to 48 hr of hepatocyte primary cultures (Handlogten et al., 1982*b*; *see also* footnote 2). The activity of System L2 is relatively high in freshly isolated cells and declines rapidly during the first 24 hr of culture. In contrast, uptake by System L1 is barely detectable immediately after cell isolation, but increases from two to fivefold after an initial lag period of 12 to 24 hr (*see* footnote 2). What factors control these changes are unknown at the present time, but it has been shown that the increase in System L1 is dependent on *de novo* synthesis of both RNA and protein.

Hormonal Regulation

It has been recognized for many years that the uptake of neutral amino acids by liver tissue is responsive to a variety of hormones (Noall, Riggs, Walker & Christensen, 1957; Chambers, Georg & Bass, 1965, 1968). Most of the early studies dealing with the effects of hormones on hepatic amino acid transport were done *in vivo* or using isolated perfused liver or liver slices. As noted above advances in the various procedures used for isolation of rat hepatocytes have resulted in the widespread use of these isolated cells either in suspension or as primary cultures. In general, hepatocytes in culture or in suspension give comparable results, although there are indications that this should not be taken for granted (Gurr & Potter, 1980; Kilberg, Handlogten & Christensen, 1981). As discussed below, there is some disagreement about the role of certain hormones when tested for their effects on cultured or suspension cells. These results may be due to inherent differences in these two experimental systems. Table 2 is a representative list of the various hormones, growth factors, and second messengers which are known to stimulate transport by System A in rat liver. For each case one or two references are given which appear to best represent the majority of investigations. To list all of the studies reporting hormonal stimulation of hepatic amino acid transport would require a great deal more space. In the discussion below a few of these hormones have been chosen to describe in more detail their effects on amino acid transport in isolated hepatocytes, either in suspension or in primary culture.

Table 2. Stimulation of neutral amino acid transport in normal rat liver by hormones, growth factors, or second messengers

Effector	Methodology	Reference
cAMP	intact rat	Fuller and Baker (1975)
	liver slices	Tews, Woodcock and Harper (1970)
	isolated hepatocytes (suspension)	LeCam and Freychet (1976) McGivan, Ramsell and Lacey (1981)
	isolated hepatocytes (culture)	Pariza, Kletzien, Butcher & Potter (1976)
Catechol-amines	intact rat	Sanders and Riggs (1967)
	isolated perfused liver	Chambers et al. (1968)
	liver slices	Tews et al. (1970)
	isolated hepatocytes (suspension)	LeCam and Freychet (1978*b*)
	isolated hepatocytes (culture)	Pariza et al. (1977)
glucagon	intact rat	Fuller and Baker (1975)
	liver slices	Tews et al. (1975)
	isolated perfused liver	Mallette, Exton and Park (1969) Kilberg and Neuhaus (1977)
	isolated hepatocytes (suspension)	LeCam and Freychet (1976)
	isolated hepatocytes (culture)	Pariza, Butcher, Kletzien, Becker & Potter (1976)
gluco-corticoids	intact rat	Noall et al. (1957)
	isolated perfused liver	Chambers et al. (1965)
	isolated hepatocytes (suspension)	LeCam and Freychet (1977*b*)
	isolated hepatocytes (culture)	M.S. Kilberg, *unpublished results*
growth hormone	intact rat	Noall et al. (1957)
	isolated perfused liver	Jefferson et al. (1975)
insulin	isolated perfused liver	Chambers et al. (1965)
	isolated hepatocytes (suspension)	LeCam and Freychet (1978*a*)
	isolated hepatocytes (culture)	Kletzien et al. (1976)
thyroid hormones	isolated hepatocytes (suspension)	Kumar and Alli (1978)

Insulin

Insulin is an anabolic hormone which modifies specific cellular events and may enhance general protein synthesis as well. Considering the latter, it is entirely consistent to find that insulin stimulates neutral amino acid transport by isolated hepatocytes (Edmondson, Lumeng & Li, 1978; LeCam & Freychet, 1978*a*; Kilberg et al., 1980). Insulin's effect on System A occurs at physiological levels of the hormone, the effect beginning at about 10^{-11} M and attaining a maximal increase at 10^{-7} M (Kletzien, Pariza, Becker, Potter & Butcher, 1976; LeCam & Freychet, 1978*a*). The time required to observe the stimulation of transport by insulin varies somewhat between laboratories, but there appears to be a lag period of about 30 to 120 min prior to a detectable increase in activity (Kletzien et al., 1976; LeCam & Freychet, 1978*a*). The hormone can be removed, however, before the increase in transport is evident with only a moderate decrease in the degree of stimulation (LeCam & Freychet, 1978*a*). These results would suggest that insulin initiates a series of events which proceed without the presence of free insulin in the medium. In support of this conclusion, the same study showed that resuspension of ^{125}I-insulin-treated cells into insulin-free medium resulted in 70% of the cell-associated radioactivity being released from the cells. Fehlmann Morin, Kitabgi and Freychet (1981) showed that there is a parallel relationship between the amount of insulin bound and the degree to which AIB transport was stimulated. Whether or not insulin's action is mediated by a "second messenger" remains unanswered. It has been reported that the stimulation of System A by insulin is suppressed by disruption of hepatocyte microtubules (Prentki, Crettaz & Jeanrenaud, 1981), whereas accumulation of microfilaments appears to be related to a decrease in amino acid uptake (Mak & Pitot, 1981). Furthermore there are indications that internalization of insulin by isolated hepatocytes is not a prerequisite for stimulation of amino acid transport (LeCam, Maxfield, Willingham & Pastan, 1979).

Glucocorticoids

The relationship between glucocorticoid action and hepatic amino acid transport remains clouded by reports of conflicting data. One of the earliest investigations, using isolated perfused rat liver, showed that hydrocortisone stimulated AIB uptake and that the effects of hydrocortisone and insulin were additive (Chambers et al., 1965). In cultured hepatocytes, Potter and his coworkers reported that the glucocorticoid analog dexamethasone caused no effect on AIB uptake when given alone (Pariza, Butcher, Kletzien, Becker & Potter, 1976; Kletzien, Pariza, Becker & Potter, 1976; Kelley, Evanson & Potter, 1980). These investigators have shown that glucocorticoids may play a "permissive" role in stimulation of transport by other hormones such as glucagon or epinephrine (*see below*). In contrast, Bonney and Maley (1975), also using cultured hepatocytes, reported a decrease in AIB uptake by dexamethasone-treated cells. Freychet and collaborators find that dexamethasone causes a stimulation of AIB transport by freshly isolated hepatocytes assayed as cell suspensions (LeCam & Freychet, 1979*b*; Canivet, Fehlmann & Freychet, 1980). In this case, the effect followed a lag period of 1 hr and was primarily the result of a change in V_{max}. Cortisol was also stimulatory while deoxycorticosterone, a mineralocorticoid lacking glucocorticoid activity, was shown to be ineffective (Canivet et al., 1980). The hormone-induced stimulation of AIB uptake occurred by System A as shown by MeAIB inhibition of the enhancement and was abolished by the presence of cycloheximide or actinomycin D (LeCam & Freychet, 1977*b*). In contrast to the permissive effect observed in cultured cells (Pariza, Kletzien, Butcher & Potter, 1976; T.A. Vida, E.F. Barber and M.S. Kilberg, *unpublished results*), the effect of dexamethasone was found to be additive to that of insulin, glucagon, and the catecholamines in freshly isolated hepatocytes (LeCam & Freychet, 1977*b*).

Depending on which cell line is tested, AIB uptake by hepatoma cells is either stimulated or inhibited by dexamethasone treatment (Kelley et al., 1978). The glucocorticoid inhibition of Na^+-dependent glycine transport by HTC cells has been studied in some detail by Gelehrter and his colleagues (Gelehrter, 1980). The Na^+-dependent uptake of glycine in these cells occurs primarily by Systems A and Gly (Christensen & Handlogten, 1981) and both systems appear to respond similarly to the hormone (Reichberg & Gelehrter, 1980).

Glucagon

The effect of glucagon on hepatic amino acid transport has been studied extensively. Through the use of MeAIB, both as an inhibitor (LeCam & Freychet, 1976) and as a substrate (Kilberg & Neuhaus, 1977; Kilberg, Handlogten & Christensen, 1980), it has been shown that System A is responsible for the glucagon-mediated increase in transport. Kinetically the glucagon-induced uptake appears to be the result of an increase in the maximal velocity (Ayala & Canonico, 1975; Fehlmann, LeCam & Freychet, 1979; Kelley,

Campbell & Potter, 1982), although an alteration in K_m may also be possible under certain conditions (Kletzien, Pariza, Becker & Potter, 1976). The time required to observe the glucagon induction of transport may depend on the experimental procedure. Potter and his associates find that there is a lag period of 1 to 2 hr prior to any observable increase in AIB uptake by primary hepatocyte cultures (Pariza et al., 1976). Others report that little or no lag period is observed for freshly isolated cells in suspension (Freychet & LeCam, 1978; Edmondson & Lumeng, 1980). It is not clear at the present time if these differences are, in fact, due to culturing; however, rapid effects of glucagon are also seen in liver slices (Tews, Woodcock, Colosi & Harper, 1975) and perfused liver (Chambers et al., 1968; Kilberg & Neuhaus, 1977).

The stimulation of transport by glucagon in freshly isolated hepatocytes in suspension is independent of *de novo* protein synthesis for the initial 15–30 min, whereas after 30 min cycloheximide completely abolishes any further enhancement by the hormone (Edmondson & Lumeng, 1980). The mechanism by which glucagon increases amino acid transport prior to the involvement of protein synthesis is still unclear. It has been suggested (Edmondson & Lumeng, 1980) to be the result of a hyperpolarization of the plasma membrane, a well-recognized hepatic response to glucagon treatment (Friedmann & Dambach, 1980). Such a hyperpolarization could enhance uptake by Na^+-dependent systems given their electrogenic nature; yet one must explain the specificity of the glucagon stimulation for System A and not for the Na^+-dependent systems ASC, N and Gly (Kilberg et al., 1980; Christensen & Handlogten, 1981).

It is also known that the cells do not have to be incubated with glucagon for a long period to elicit a response. Although less effective than incubating the cells in the presence of the hormone for the entire experiment, glucagon-stimulated transport persists for several hours after only a 15-min incubation with the hormone (Pariza et al., 1976; Fehlmann et al., 1979). This result would indicate that, like insulin, glucagon initiates a series of events which once set in motion no longer require free hormone in the medium. In this regard, it has also been reported that there is not a parallel relation between the degree of receptor occupancy and glucagon stimulation of hepatic amino acid transport (Fehlmann et al., 1981). Thus, the half-maximal effect of glucagon on AIB uptake occurred with approximately 20% of the receptors occupied. A similar result has been shown for glucagon-stimulated cAMP accumulation in isolated hepatocytes (Sonne, Berg & Christoffersen, 1978).

Hyperglucagonemia is one of the major characteristics of the diabetic state (Unger, 1974; Unger, 1976). Given that glucagon stimulates System A transport, it was reasoned that if the liver remained responsive to the high plasma levels of the hormone found in diabetic animals, the uptake of neutral amino acids by System A would be increased in livers from diabetic rats. This response was observed both *in vivo* and in isolated perfused rat liver, demonstrating the usefulness of experimentally produced diabetic animals as a model to study hepatic transport regulation (Kilberg & Neuhaus, 1977). Those results were confirmed and extended by Samson, Fehlman, Dolais-Kitabgi and Freychet (1980) in isolated hepatocytes. Interestingly, those authors suggested that the stimulation of transport induced by experimental diabetes may be kinetically distinct from that induced by glucagon treatment *in vitro*.

Hormone Interactions

Potter and his associates have reported an important interaction between glucagon and the glucocorticoids with respect to hepatic amino acid transport. As mentioned above, in their system (i.e., hepatocyte primary cultures) glucocorticoids alone do not stimulate System A activity. If, however, the hepatocytes are maintained for several hours in the presence of dexamethasone and are then incubated with glucagon, the stimulation by glucagon is much higher in the cells previously exposed to the dexamethsone (Kletzien, Pariza, Becker & Potter, 1975; Pariza, Kletzien, Butcher & Potter, 1976; Kelley et al., 1980). We have confirmed those studies and extended them to show that cultured hepatocytes isolated from adrenalectomized rats are not competent to respond fully to stimulation of transport by glucagon (T.A. Vida, E.F. Barber and M.S. Kilberg, *unpublished results*). This permissive effect of the glucocorticoids has been reported to occur for other glucagon-stimulated processes as well (Gebhardt & Mecke, 1979; Stumpo & Kletzien, 1981). On the basis of their results, Potter and his coworkers have proposed a tentative model to explain their data (Pariza, Kletzien, Butcher & Potter, 1976). The key points of the proposal are: (i) the glucocorticoids cause the synthesis of an inactive or precursor form of the transport agency; (ii) glucagon by an unknown mechanism converts the inactive form to an active transport system; and (iii) glucagon itself inhibits the decay of the stimulated activity. There are also reports in both cultured (Vida et al., *unpublished results*) and suspension cells (LeCam & Freychet, 1977*b*; Canivet et al., 1980) to indicate that glucocorticoids alone can increase uptake by System A.

Freychet and coworkers have shown that for hepatocytes in suspension the glucocorticoids do not act synergistically with glucagon (Canivet et al., 1980).

In addition to the interaction between glucocorticoids and glucagon, the relationship between insulin and glucagon with respect to hepatic amino acid transport has also received some attention. Kilberg and Neuhaus (1977), using an isolated perfused rat liver preparation, showed that the addition of the proper concentration of insulin *in vitro* could reverse the stimulation of System A transport which had been enhanced *in vivo* by either administration of exogenous glucagon or induction of experimental diabetes. That study also showed that if an excessive concentration of insulin was added to the perfusate, rather than inhibition, a further stimulation occurred which was additive to that caused by the *in vivo* administration of glucagon. In contrast to these results, when glucagon and insulin are added to isolated hepatocytes, both at maximally effective concentrations, they show the antagonism generally associated with these two hormones with respect to glycogenolysis, but the same is not true for their combined effect on AIB uptake (LeCam & Freychet, 1978*a*; Fehlmann et al., 1979). In this case, adding the hormones simultaneously caused a stimulation that was approximately the sum of the increase when each was added alone. Cultured hepatocytes also show a similar response to the two hormones when they are added together (Kelley, Shull & Potter, 1980). This additive effect appeared to be independent of hormone concentration which was varied between 10^{-10} and 10^{-7} M for each. The apparent absence of any detectable antagonism of insulin and glucagon in isolated hepatocytes may depend on the experimental conditions. The investigations outlined above, which involved isolated hepatocytes, differed from those using the isolated perfused liver because in the former both hormones were added *in vitro* and at the same time, whereas in the liver perfusion tests the glucagon stimulation occurred *in vivo* and the hormone was not present when the insulin was added *in vitro*. When the liver perfusion experiments involving experimental diabetes are repeated, except that hepatocytes rather than intact livers are isolated from diabetic animals and then exposed to insulin, the elevation of AIB uptake is suppressed at a concentration of 10^{-9} M insulin, but is increased even further at insulin concentrations greater than 10^{-8} M (M.S. Kilberg, *unpublished observation*). Further experimentation will be necessary to determine if insulin action on hepatocytes which have been exposed to glucagon *in vitro* is the same as its effects on hepatocytes isolated from streptozotocin-induced diabetic rats.

Catecholamines

The catecholamines stimulate AIB uptake by isolated rat hepatocytes either in culture (Pariza, Butcher, Becker & Potter, 1977) or in freshly isolated cell suspensions (LeCam & Freychet, 1977*b*). The simulation of transport by epinephrine occurs predominantly via alpha-adrenergic receptors as shown by blockage of its effects by the alpha-blockers phentolamine and phenoxybenzamine, but not by the beta-antagonist propranolol (LeCam & Freychet, 1978*b*). Epinephrine was almost 5 times more potent than norepinephrine, and caused an increase in V_{max} without a change in K_m. The stimulation requires *de novo* protein synthesis and becomes apparent only after a lag period of 1 to 2 hr (Pariza et al., 1977; LeCam & Freychet, 1978*b*). Pariza et al. (1977) reported that either isoproterenol (6 μM) or epinephrine (6 μM) increased the levels of cAMP in cultured hepatocytes, while only the epinephrine resulted in a measurable increase in AIB uptake. Furthermore, propranolol blocked the epinephrine-induced increase in cellular cAMP, but not the increase in AIB uptake. On the basis of these and other results it was concluded that the epinephrine may stimulate transport by a cAMP-independent mechanism (Pariza et al., 1977). On the other hand, using hepatocyte suspensions, isoproterenol (100 μM) was shown to stimulate AIB uptake (LeCam & Freychet, 1978*b*).

Another area of apparent disagreement between these studies in cultured and suspension hepatocytes is the involvement of glucocorticoids in a permissive manner similar to that found for the interaction between glucagon and dexamethasone. Freychet and his coworkers have shown that for freshly isolated hepatocytes in suspension dexamethasone alone stimulates AIB uptake (LeCam & Freychet, 1977*b*; Canivet et al., 1980) and when added in combination with epinephrine the effects are additive (LeCam & Freychet, 1977*b*). In primary cultures of rat hepatocytes, Pariza et al. (1977) find that when dexamethasone-treated cells are incubated in the presence of catecholamines the stimulation is potentiated, thus showing the glucocorticoid-dependent permissive effect.

Adaptive Control of Hepatic Amino Acid Transport

The liver exhibits a second type of regulation for neutral amino acid transport, namely, a process termed "adaptive regulation" or "adaptive control". This phenomenon was originally described by Gazzola, Franchi, Saibene, Ronchi and Guidotti (1972) in chick embryo heart cells and, independently, that

same year by Riggs and Pan (1972) for rat uterus. [For a more extensive review of this subject *see* Guidotti, Borghetti and Gazzola (1978) and Gazzola, Dall'Asta and Guidotti (1981*b*)]. Although initial attempts to demonstrate the process in liver tissue were not successful (Guidotti, Gazzola, Borghetti & Franchi-Gazzola, 1975; Kletzien et al., 1976), it was later realized that hepatocytes in suspension (LeCam & Freychet, 1976) and in primary culture (Kelley & Potter, 1978; Kilberg, Schwass & Christensen, 1979) exhibit adaptive regulation. The process of adaptive regulation can be shown by incubating the cells in the absence of extra-cellular amino acids for periods of longer than 2 hr, after which the activity of System A increases with increasing incubation time for more than 24 hr (Kelley & Potter, 1978; Handlogten et al., 1982*a*). It is believed that adaptive control of System A is the result of a derepression of the synthesis of one or more of the proteins responsible for System A activity. Thus, both actinomycin D and cycloheximide effectively block the increase in transport when present during the amino acid-free incubation (Kelley & Potter, 1978; Handlogten et al., 1982*b*). One must demonstrate that the increased uptake is the result of adaptive regulation and not due to release from trans-inhibition (Kelley & Potter, 1978), although trans-effects appear to be less of a complication for normal hepatocytes than hepatoma cells (Kelley & Potter, 1979). The starvation-induced stimulation of System A activity appears to be the result of an increase in V_{max} with little or no change in K_m (Kelley & Potter, 1978). Derepression of the transport system in eukaryotic cells seems to require removal of all amino acids from the incubation medium, thus separating this phenomenon from the so-called "stringent response" in bacteria (Gallant, 1979). Furthermore, maintaining repressed cells in the presence of one amino acid, or adding back an individual amino acid to derepressed cells, is sufficient to cause repression of the transport activity. Whether or not this amino acid must be a substrate for the transport system which is being monitored remains questionable (Gazzola et al., 1981*b*; Handlogten et al., 1982*a*). It has been shown both by inhibition analysis (Kelley & Potter, 1978) and by using the specific test substrate, cysteine (Kilberg et al., 1979; Ehrhardt, 1980), that the hepatic System ASC does not respond to amino acid starvation.

Hepatocytes do contain a second neutral amino acid transport agency, System N, which undergoes adaptive control. System N activity has so far only been demonstrated in rat hepatocytes and the hepatoma cell line H4-II-EC3 as detailed in the section on System N. It is distinguished from System A by its intolerance to N-methylation and lack of hormonal stimulation, but is similar to System A with respect to adaptive control (Kilberg et al., 1979; Handlogten et al., 1982*a*). As mentioned above, on a percentage basis the increase in System N (two- to threefold increase in 24 hr) is not nearly as large as found for System A (10- to 15-fold) yet the relative velocities of the two systems must be considered. For example, the uptake rate of 0.05 mM MeAIB by System A in fully repressed cells is generally less than 10 pmol·mg^{-1} protein · 30 sec^{-1}, whereas the velocity of the same concentration of the apparent System N-specific substrate, histidine, is greater than 100 pmol·mg^{-1} protein · 30 sec^{-1}. In addition, the initial uptake rate of glutamine by System N is usually 200 pmol·mg^{-1} protein · 30 sec^{-1} or more (Handlogten et al., 1982*a*). Thus, a twofold increase of the basal activity for System N will have as much or more of an impact on the net flux of its substrates, as a 10-fold increase in System A activity would for its substrates. As a result of the physiologic importance of System N substrates, such as glutamine, the adaptive control of this transport system becomes a significant factor in hepatic amino acid metabolism and in evaluating the role of the liver in glutamine metabolism for the entire animal. Interestingly, System N can be repressed by a few amino acids (e.g., MeAIB) which show no apparent affinity for the system as substrates, yet other nonsubstrates, such as alanine or leucine, are not capable of such repression (Handlogten et al., 1982*a*).

Concluding Remarks

Although somewhat brief, it is hoped that this overview of hepatic amino acid transport depicts the status of our current knowledge. Clearly, this area of research is still in its infancy. Despite the fact that most of the investigations published to date have been descriptive and aimed at the cellular rather than the molecular aspects of the problem, we actually know very little about the physiological importance of the translocation process with respect to metabolism in general. We are just beginning to ask questions about how transport at the plasma membrane can regulate hepatic amino acid metabolism. If amino acid transport can be a rate-limiting step, how is it modified to accommodate the wide variety of metabolic changes that occur in the liver? Consider, for example, the post-absorptive period. What changes occur in hepatic amino acid transport in various disease states and how do these changes affect the pathophysiology of a given disease? The answers to these and other questions may come only after we have a much better perspective about how membrane transport and its regulation occurs at the molecular level. The

molecular aspects of amino acid transport by eukaryotic cells is an area which is just starting to develop. Isolation of the membrane proteins involved in translocation, an understanding of energetic coupling, insight into the molecular aspects of both adaptive and hormonal control, and a better appreciation of the regulation of transport by both RNA and protein synthesis are all areas of opportunity for future molecular and cellular biologists. This lack of knowledge should not be looked upon negatively, but seen instead as a challenge to those interested in research in the areas of membrane transport, hepatic physiology, or amino acid and carbohydrate homeostasis.

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