Primary cultures of adult rat hepatocytes were used to study the effects of 100 mM ethanol on various neutral amino acid transport systems. Ethanol exposure for 24 h selectively decreased amino acid uptake by the A and N systems by 40–70%, but had no significant effect on the ASC and L systems. The decrease in the A system was significant after 3 h of ethanol exposure, and the activity was not affected by the presence or absence of ethanol during the uptake measurements. Kinetic analysis showed that ethanol treatment affected predominantly the high-affinity component of A system activity by decreasing the apparent $V_{\text{max}}$ without significantly changing the apparent $K_m$. Ethanol treatment did not prevent the cells from increasing A system activity in response to insulin and glucagon, but the magnitude of hormone-stimulated uptake was reduced.

Various neutral amino acid transport systems of overlapping substrate specificity have been characterized in rat hepatocytes and in other cells (for reviews see Refs. 1 and 2). Experimentally, the different systems can be defined by their substrate specificity and by their responses to inhibitors, the ion composition of the medium, and treatment of the cells with hormones or nutritional deprivation.

The A system is sodium-dependent and hormone-responsive, and its activity increases in response to amino acid starvation. This system transports short-chain amino acids, including alanine, glycine, and the synthetic model amino acids, AIB\(^{1}\) and MeAIB. Sodium-dependent MeAIB uptake, and the portion of AIB uptake which is inhibited by MeAIB, are measures of A system activity (1). The ASC system is also sodium-dependent, but it does not respond to hormones or amino acid starvation. Sodium-dependent cysteine transport, in the presence of MeAIB to inhibit A system-mediated cysteine transport, is a measure of this system (3). The N system, which transports glutamine, can be assayed by measuring sodium-dependent glutamine uptake in the presence of MeAIB to inhibit that portion of glutamine uptake which is mediated by the A system (4). This system is sodium-dependent and increases in response to amino acid starvation, but not to treatment with hormones. The L system transports branched-chain amino acids, such as leucine, isoleucine, and valine. By contrast with the above systems, the L system is sodium-independent and is not hormone responsive, but does increase with leucine starvation. The L system is measured by inhibiting sodium-independent AIB uptake with BCH (1). The L system has more recently been divided into L1 and L2 (both sensitive to BCH) on the basis of selective inhibition characteristics (5).

Detailed kinetic studies of the A system have been carried out in hepatocytes, both in the basal state, and in cells which were activated by hormone treatment (insulin, glucagon, and $\alpha$-adrenergic agents) or by amino acid deprivation. Most investigations report that all such manipulations stimulate A system activity by increasing the apparent $V_{\text{max}}$ ($V_{\text{max}}$) without affecting the apparent $K_m$ ($K_m$) (1).

In a previous report from this laboratory (6), it was demonstrated that treatment of cultured hepatocytes with ethanol for 20 h reduced the uptake of AIB, presumably by the A system. In these studies, AIB uptake over a 30-min period was measured and the effects of ethanol on AIB transport were not kinetically characterized. Other studies showed that ethanol administration decreased in vivo AIB and valine uptake by placental and fetal tissues (7, 8). Ethanol exposure in vitro also decreased AIB and valine uptake by placental fragments (9, 10).

In this paper we present data showing that in cultured hepatocytes, ethanol treatment selectively decreases transport by the A and N systems. Kinetic analysis indicates that the effect of ethanol treatment on the A system predominantly reflects a decrease in $V_{\text{max}}$.

**MATERIALS AND METHODS**

**Isolation of Hepatocytes**—Hepatocytes were isolated from fed, male, Sprague-Dawley rats (200–300 g) using the collagenase perfusion method of Berry and Friend (11) as modified by Meijer et al. (12). The hepatocytes were plated in 35-mm plates (Primaria, Falcon Plastics, Oxnard, CA) in Williams’ Medium E (Flow Labs, McLean, VA) with 20 mM HEPES (Sigma), pH 7.4, in 5% fetal bovine serum (Whittaker, M. A., Biologicals, Walkersville, MD), 50 $\mu$g/ml of gentamycin, and 10$^{-4}$ M dexamethasone (Eli Lilly and Co., Indianapolis, IN). Three hours later, after attachment, the plates were routinely washed with PBS, pH 7.4, and the medium was replaced without serum. Viability, measured by trypan blue exclusion at the time of initial isolation, was 82–95%. Plating efficiency of viable cells was in excess of 90%.

**Ethanol Administration**—One-hundred mM ethanol was added to the cultures 3 h after plating for 20–24 h, or at other times as stated in the text. The concentration of ethanol was maintained by incubating the plates in a closed plastic box in which the atmosphere was saturated by constant exposure to 100 mM ethanol. The concentration of ethanol in the medium after a 20-h incubation was 85% of the initial concentration. The medium was routinely changed each morning, and fresh ethanol was added at this time. The concentration of ethanol was measured using alcohol dehydrogenase (15). Incubation...
with 100 mM ethanol for 20-30 h had no effect on the morphologic appearance of the cells or on their viability, as measured by trypan blue exclusion. Amino Acid Uptake—The uptake of various amino acids was measured at 37°C after 24-30 h in culture. The uptake buffer was a modified HEPES-Ringer containing 20 mM HEPES, 127 mM NaCl, 1.3 mM CaCl₂, 1 mM sodium phosphate, 2.35 mM KCl, 0.58 mM MgCl₂, and 138 mM dextrose at pH 7.4. In some experiments comparing Na⁺-dependent and Na⁺-independent uptake, the HEPES concentration was reduced to 5 mM and choline was used in place of sodium salts. The plates were washed three times with the same medium prior to uptake studies. Uptake was performed routinely in the absence of ethanol, except as noted in the text. Radiolabeled amino acids were added for a designated time (see text) and the uptake was stopped by removal of the radioactive medium and addition of PBS at 4°C, after which the plates were placed on ice. Subsequently, within 4 min, the plates were washed five times with cold PBS and dried.

Solubilization of the Cells—The cells were solubilized with 0.5 ml of 0.5% sodium lauryl sulfate in 2 N NaOH for 1 h. Samples were then taken for determination of protein using a modified Lowry procedure (14). Radioactivity was determined by dissolving a sample of the solubilized cell suspension in detergent-solve (Research Products International Corp., Mt. Prospect, IL) and counting in a Packard liquid scintillation counter.

Hormone Additions—Insulin and glucagon (Sigma) were added to cultures 6 h before uptake studies.

Chemicals—[14C]AIB and [14C]MeAIB were obtained from New England Nuclear, and [14C]glutamine and [14C]cysteine from American Corp. Collagase Type I was obtained from Sigma. All other biochemicals were obtained from Fischer.

RESULTS

The uptake of MeAIB (0.1 mM) by cultured hepatocytes was linear for at least 6 min (Fig. 1). A linear time course of MeAIB uptake was also observed for at least 4 min at higher substrate concentrations (up to 4 mM). The addition of 100 mM ethanol to the incubation medium during the uptake reaction had no significant effect on the accumulation of this amino acid. However, when the hepatocytes were incubated with 160 mM ethanol for 24 h, the uptake of MeAIB into the cells was reduced, even when ethanol was removed by washing the cells before the MeAIB uptake assay. The linearity of the uptake was not affected by ethanol treatment (Fig. 1). Since incubation with ethanol had no effect on the morphological appearance or viability of the cells, the effect of ethanol pretreatment is not due to a nonspecific deterioration of the hepatocytes. In all further experiments reported in this paper, the effect of pretreatment of the cells with ethanol was studied, and ethanol was removed from the cells by repeated washings before carrying out the amino acid uptake assay.

In the experiment shown in Fig. 1, extrapolation of MeAIB uptake to the time of substrate addition showed a small, but significant, intercept which was more pronounced with higher substrate concentrations (data not shown). This finding suggests a small contribution of nonspecific binding to MeAIB uptake, similar to the results of Kelley and Potter (15) with AIB. In our experiments, this rapid binding was unaffected either by acute ethanol addition or by pretreatment of the cells with ethanol (Fig. 1).

The time course of the effect of ethanol pretreatment on MeAIB uptake is shown in Fig. 2. No significant differences between control and ethanol-treated hepatocytes were observed after 1 h, but ethanol exposure for 3 h or more resulted in a progressively increasing inhibition of MeAIB transport. Addition of the protein synthesis inhibitor, cycloheximide (50 μM), also inhibited this transport system in a time-dependent manner (Fig. 2). Ethanol had no further inhibitory effect when added in the presence of cycloheximide.

We also studied the effects of varying ethanol concentrations. At concentrations between 25 and 50 mM, a significant inhibition of MeAIB uptake was observed, but the extent of inhibition was quite variable (results not shown). For this reason, all experiments were carried out with 100 mM ethanol, at which concentration significant inhibition of amino acid uptake was consistently observed. A serum level of 100 mM ethanol in man is in the high physiologic range. However, the liver may be exposed to even higher ethanol levels during gastrointestinal absorption.

Several different components contribute to the net uptake of MeAIB in hepatocytes (16). In addition to the sodium-dependent MeAIB transport, characteristic of the A system, there is some uptake of MeAIB in sodium-free medium (e.g. with choline replacing sodium). Fehlmann et al. (17) studied...
AIB and MeAIB uptake in freshly isolated hepatocytes. These authors provided evidence for a high affinity (apparent $K_m$ for MeAIB 0.27 mM) and a low-affinity (apparent $K_m$ for MeAIB 13.5 mM) sodium-dependent A system. However, other authors (15) have questioned the physiological significance of the low-affinity system.

The low- and high-affinity components of MeAIB uptake could be clearly distinguished both in a $V$ versus $S$ plot and in an Eadie-Hofstee plot (Fig. 3, A–C). Pretreatment of the cells with ethanol (100 mM) resulted in an inhibition of MeAIB uptake at all concentrations tested (Fig. 3A). In order to express the effect of ethanol pretreatment on the low-affinity component of MeAIB uptake in quantitative terms, we calculated an apparent first-order rate constant, $k'$, from the slope of the $V$ versus $S$ plots at higher substrate concentrations (between 0.2 and 4 mM). As shown in Table I, ethanol treatment significantly decreased this constant by about 40%. (Results obtained from five experiments were similar to that shown in Fig. 3, A–C.)

The effect of ethanol treatment on the high affinity system in the presence of sodium was examined over the MeAIB concentration range of 0.035–0.2 mM in a double reciprocal plot (Fig. 3B). The apparent $K_m$ and $V_{max}$ values are also shown in Table I. Ethanol treatment had no significant effect on the $K_m$, but decreased the $V_{max}$ by 40%.

These estimates are uncorrected for the contribution of the lower affinity system at the lower concentrations of MeAIB. Fig. 3C shows the analysis of the same kinetic experiment in an Eadie-Hofstee plot. The contribution of the low-affinity component to MeAIB uptake at lower substrate concentrations was estimated on the basis of the first-order rate constant obtained at high substrate levels (compare Table I). Fig. 3C shows that the corrected high-affinity MeAIB transport system was markedly decreased by ethanol treatment (apparent $V_{max}$, obtained by extrapolation to $V/S = 0$, was decreased by about 75% in this experiment), without a significant change in the slope of the curve. This finding indicates that the affinity of the translocator for MeAIB was unaffected by ethanol treatment. Essentially similar results were obtained in four other experiments. However, owing to the small contribution of the high-affinity component to the total MeAIB uptake in the basal state (19) especially in the ethanol-treated cells, the scatter in the experimental data was considerable, and statistically meaningful quantitative estimates could not be obtained by this analysis. Nevertheless, these data further emphasize the point that the ethanol pretreatment has its most pronounced effect on the high-affinity component of MeAIB transport. Also, owing to the intrinsically low level of MeAIB uptake by the high-affinity component in the basal state, it was necessary in the kinetic experiments described above to measure total (Na$^+$-dependent and -independent) MeAIB transport. As shown in Fig. 4, the effect of ethanol pretreatment is specific for the sodium-dependent component of high-affinity MeAIB uptake (the A system) and does not significantly affect the sodium-independent component.

The kinetic analysis of MeAIB uptake demonstrated that the effect of ethanol pretreatment is selective, affecting predominantly the high-affinity component of the sodium-dependent A system. It was therefore of interest to study the effect of this treatment on other neutral amino acid transport systems in the cell. The results shown in Table II demonstrate that, in addition to the effect on the A system, the glutamine transporting N system was selectively inhibited by ethanol treatment, whereas the ASC and L systems were not markedly affected. This result is of interest, since it is predominantly the A and N amino acid transport systems that are subject to regulation of their activity by hormonal stimulation or by nutritional influences, e.g. starvation (see “Discussion”).

The effect of ethanol treatment on the stimulation of the A system activity by insulin or glucagon is shown in Table III. The cells were treated for 24 h with ethanol, and maximally stimulating concentrations of the hormones were added 6 h before the uptake studies were carried out. In the control cells, insulin stimulated the A system activity 3.6-fold; the stimulation by glucagon varied widely in different cell preparations, from 3–16-fold. (Compare with Ref. 15.) Ethanol pretreatment inhibited the amino acid uptake in both the basal- and hormone-stimulated cells. It is of interest that the exposure of the cells to ethanol did not prevent the cells from increasing the A system activity in response to these hormones. However, the magnitude of hormone-stimulated uptake is less in cells pretreated with ethanol.

2 Since iteration of the correction was not carried out, this procedure may give an overestimate of the contribution of the low-affinity component (18).
Hepatocytes were cultured in serum-free medium 24 h in the presence or absence of 100 mM ethanol. Uptake was performed in HEPES-Ringer for 4 min at various MeAIB concentrations. Apparent first-order rate constant \( k' \) for the low-affinity system was obtained from the slope of \( V \) versus \( S \) plots, over the MeAIB concentration range of 0.2-4 mM. Kinetic constants for the high-affinity system were calculated from a double-reciprocal plot over the concentration range of 0.05-0.2 mM. Data represent total MeAIB uptake not corrected for Na\(^+\)-independent uptake. In individual experiments, uptake measurements were made in triplicate. Results are presented as the mean ± S.E. for five experiments. Statistical significance was calculated by paired t test.

<table>
<thead>
<tr>
<th>Low-affinity system</th>
<th>Control</th>
<th>Ethanol</th>
<th>Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k'(\text{mmol}/4\text{ min/mg/mm}) )</td>
<td>0.57 ± 0.03</td>
<td>0.35 ± 0.04</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>High-affinity system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_m (\text{mM}) )</td>
<td>0.37 ± 0.06</td>
<td>0.50 ± 0.10</td>
<td>NS*</td>
</tr>
<tr>
<td>( V_{\text{max}} )</td>
<td>0.73 ± 0.09</td>
<td>0.44 ± 0.10</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* NS, not significant.

**DISCUSSION**

A decrease in the uptake of AIB by cultured hepatocytes after ethanol treatment was demonstrated in a previous report from this laboratory (6). However, specific effects on the various systems for amino acid transport in the liver have not been documented. An effect of ethanol on the uptake of AIB in rat liver cells might reflect a decrease in one or more of several transport systems, since AIB is transported to varying extents by the A, ASC, and L systems (16).

The data reported here demonstrate that treatment with 100 mM ethanol selectively decreases the amino acid transport activity of the sodium-dependent A and N systems, while having little effect on the ASC and L systems. The ethanol-induced depression of the A system was shown to be due to a decrease in the \( V_{\text{max}} \), with no significant change in the apparent \( K_m \). Moreover, an apparent high-affinity and a low-affinity activity could be distinguished, both of which were diminished by ethanol pretreatment.

Our measurements were made with cell preparations under nonstimulated conditions and, therefore reflect the basal activity of the amino acid transport systems. This method avoids the possibility that ethanol treatment could interfere with the action of hormones on the cell. However, the intrinsically low level of A system activity of the unstimulated cells (19) made it difficult to obtain kinetic measurements of the Na\(^+\)-dependent component, which has to be calculated indirectly as the difference in uptake rates in the presence and absence of Na\(^+\). Therefore, all our kinetic experiments were carried out in a Na\(^+\)-containing medium. Our data show that under these conditions, ethanol decreases the \( V_{\text{max}} \) of MeAIB transport. As shown in Fig. 4, in the high-affinity range of substrate concentrations, the Na\(^+\)-independent MeAIB transport was not affected by ethanol treatment. This result indicates that the effects observed in the kinetic analysis are due only to changes in the Na\(^+\)-dependent MeAIB transport (i.e. the A system).

The contribution of the low-affinity system to the amino acid uptake at lower substrate levels can be significant (17). In Fig. 3C, where a correction for this contribution is made (see Ref. 18), it is shown that the effect of ethanol treatment is predominantly on the \( V_{\text{max}} \) of the high-affinity system, which was reduced about 70%. Fehlmann and co-workers (19) have shown that this high-affinity component is stimulated by hormones. In agreement with this observation, the data in Table III demonstrate that A system activity in stimulated cells is also diminished by about 70% after ethanol treatment.

There is considerable disagreement on the significance of the high- and low-affinity systems. Kelley and Potter (15) argued that the low-affinity component of AIB uptake is a methodological artifact, which could be due to two factors, namely, a rapid initial uptake or binding of AIB at high concentrations of substrate and a nonlinear time course over a 4-min time period. However, in the experiments of Kelley and Potter (15) on AIB uptake, the effects of the apparent low-affinity component were evident only at high substrate levels (>10 mM), and a correction for nonlinear AIB uptake appeared to have little effect on the kinetic constants obtained in the substrate concentration range of the high-affinity system. In our hands, no evidence of nonlinear uptake of MeAIB was found at the concentrations used (up to 4 mM), but we did observe a rapid uptake (or binding) component, even at 0.1 mM MeAIB (Fig. 1). We also find a decrease in the first-order rate constant, \( k' \), in the ethanol-treated cells in the low-affinity range of substrate concentrations (Table I). This observation suggests that in our cultured cells, MeAIB uptake is not due solely to an uncontrolled permeability of the cell membrane at high substrate concentrations. The fact that ethanol treatment affects both the high- and low-affinity components is also consistent with the hypothesis of Kelley and Potter (15) that uptake in both high- and low-substrate concentration ranges reflects the activity of the same transport system.

The possibility that ethanol treatment is nonspecifically damaging to the cells is not supported by our experiments. In the first place, cells treated with 100 mM ethanol show no morphological differences compared to control cells, and do not stain with trypan blue. Furthermore, the cells are able to respond to insulin and glucagon by increasing transport well above basal control levels (Table III). The magnitude of the response, especially to glucagon, varied widely in different
Ethanol Treatment Decreases Amino Acid Transport

Hepatocytes were isolated, plated in Williams E. medium with 5% serum, washed after 3 h with PBS, and cultured in serum-free Williams E. medium for 24 h with or without 100 mM ethanol. Sodium-dependent and -independent amino acid uptake was measured over a 1-min period in HEPES-Ringer solution containing 150 mM NaCl or choline Cl. The following conditions were used to distinguish different transport systems: A system, 0.1 mM MeAIB Na+-dependent uptake; ASC system, 0.1 mM cysteine, Na+-dependent uptake in the presence of 25 mM MeAIB; N system, 0.1 mM glutamine, Na+-dependent uptake in the presence of 25 mM MeAIB; L system, 0.1 mM AIB, Na+-independent uptake in the presence of 10 mM BCH. Results are presented as the mean ± S.E. of n experiments.

<table>
<thead>
<tr>
<th>Transport system</th>
<th>Substrate</th>
<th>Control</th>
<th>Ethanol</th>
<th>p</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 5)</td>
<td>MeAIB</td>
<td>0.0162 ± 0.0036</td>
<td>0.0086 ± 0.0035</td>
<td>&lt; 0.005</td>
<td>53</td>
</tr>
<tr>
<td>ASC (n = 4)</td>
<td>Cysteine</td>
<td>0.144 ± 0.018</td>
<td>0.122 ± 0.023</td>
<td>NS*</td>
<td>58</td>
</tr>
<tr>
<td>N (n = 5)</td>
<td>Glutamine (MeAIB)</td>
<td>0.211 ± 0.038</td>
<td>0.089 ± 0.016</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>L (n = 1)</td>
<td>AIB (BCH sensitive)</td>
<td>0.032</td>
<td>0.0281</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NS, not significant.

TABLE III

Effect of ethanol treatment on hormonal stimulation of the A system

Hepatocytes were isolated, plated in Williams E. medium with 5% serum, washed after 3 h with PBS, and cultured in serum-free Williams E. medium for 24 h with or without 100 mM ethanol. Sodium-dependent and -independent amino acid uptake was measured over a 1-min period in HEPES-Ringer solution containing 150 mM NaCl or choline Cl. The following conditions were used to distinguish different transport systems: A system, 0.1 mM MeAIB Na+-dependent uptake; ASC system, 0.1 mM cysteine, Na+-dependent uptake in the presence of 25 mM MeAIB; N system, 0.1 mM glutamine, Na+-dependent uptake in the presence of 25 mM MeAIB; L system, 0.1 mM AIB, Na+-independent uptake in the presence of 10 mM BCH. Results are presented as the mean ± S.E. of n experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Ethanol</th>
<th>p</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.090 ± 0.011</td>
<td>0.051 ± 0.012</td>
<td>&lt; 0.05</td>
<td>43</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.336 ± 0.051</td>
<td>0.125 ± 0.032</td>
<td>&lt; 0.01</td>
<td>62</td>
</tr>
<tr>
<td>Glucagon</td>
<td>1.10 ± 0.601</td>
<td>0.31 ± 0.11</td>
<td>&lt; 0.3</td>
<td>72</td>
</tr>
</tbody>
</table>

experiments, but this variability was observed in both control and ethanol-treated cells. Similar variations in the response to alcohol were observed by others (15). Third, the selectivity of the response to ethanol treatment argues against a generalized damage to the cell membrane. Other studies have also reported that cultured hepatocytes after 48 h in 80 mM ethanol are capable of selective enzyme induction in response to physiological stimuli (21).

The lack of any significant acute effect of ethanol, and the persistence of the effect after prolonged incubation, followed by removal of the ethanol during the uptake measurements, argue against a direct interference with the transport process, either by physical interaction of ethanol with the membrane lipids (21) or by its metabolism. The latter point is also supported by the lack of effect of 4-methylpyrazole, an inhibitor of alcohol dehydrogenase, on the ethanol-induced decrease in amino acid transport (6). Furthermore, effects of ethanol on amino acid transport have been shown in other tissues, e.g. placenta (Refs. 7-10, 22), which does not metabolize ethanol significantly.

The long-term effects described here, which are unaffected by washing, are different from those of Chang et al. (23), who used high concentrations (>0.5 mM) of ethanol to inhibit amino acid transport in everted intestine. The inhibition shown by Chang et al. (23) required only a few minutes to occur and was reversed after removing the ethanol by washing.

The time course of the onset of ethanol-induced decrease in A system activity suggests an effect on protein synthesis. Several authors have shown that ethanol has an inhibitory effect on protein synthesis, in vivo (24) and in vitro (25, 26). It is interesting that David et al. (27) showed that ethanol (100 mM) inhibits protein synthesis by about 35% by interfering with Leu-tRNA synthetase in a cell-free system from Chinese hamster ovary cells. This finding also argues for a direct effect of ethanol, independent of metabolism.

The effect of ethanol on the A system is similar to that of cycloheximide during the first few hours of incubation (Fig. 2). This observation supports the suggestion that inhibition of protein synthesis may be involved in the action of ethanol. After longer incubation times, the complete inhibition of protein synthesis by cycloheximide results in a more pronounced inhibition of MeAIB uptake, and ultimately leads to cell death. Indeed, cells incubated overnight in cycloheximide round up into single cells, whereas those incubated with ethanol are morphologically indistinguishable from control cells. It is possible that partial inhibition of protein synthesis by ethanol leads to a reduction in the number of transport units in the plasma membrane, without affecting the affinity of the translocator. The selectivity of the effect of ethanol could be explained by different turnover rates among the membrane proteins involved in the various transport processes.

It should be pointed out that a stimulatory effect of ethanol on protein degradation cannot be excluded on the basis of our data. Insulin and glucagon stimulate amino acid uptake by a process requiring protein synthesis (19). Ethanol treatment does not prevent the cell from responding to these hormones, although it decreases the absolute magnitude of the cellular response. These results are equally compatible with an effect of ethanol treatment on the rate of degradation of these transport proteins.

The net effect of ethanol exposure on amino acid transport is to reduce the total intake of amino acids by the cell (Table III). The physiological significance of the effect of ethanol on amino acid transport remains an open question. About 70% of alanine transport is mediated by the A system in the hepatocyte (28, 29). Several authors have suggested that at physiological alanine levels (1-2 mM), alanine transport can be a rate-controlling step in its metabolism (30, 31). This question is important in the liver, where alanine is a major precursor for gluconeogenesis. If transport is the limiting step in alanine metabolism, and alanine uptake is decreased after ethanol exposure, this decreased uptake may be reflected in a decreased capacity for gluconeogenic flux. Such a mechanism could contribute to the occurrence of hypoglycemia observed in some human alcoholics, under conditions in which ethanol is consumed during starvation (32). Glutamine is likely to be an important substrate for gluconeogenesis in the liver only under conditions of maximal stimulation of glutaminase (33); the possible regulatory contributions of glutamine transport under such conditions have not been evaluated. However, glutamine transport is important in other tissues, notably...
Ethanol Treatment Decreases Amino Acid Transport

kidney, where it is involved in acid-base regulations, and in the brain, where it may play a role in neurotransmission.

Acknowledgment—We thank Alice Wu for her skillful technical assistance.

REFERENCES