The finding that the Na⁺-dependent uptake of cysteine into the rat hepatocyte is largely specific to one transport route allows us here to characterize directly System ASC for neutral amino acid uptake by that cell, without raising uncertainties about possible artifactual features that might conceivably be produced by adding an α-(methylamino) acid to limit transport to it. The pH sensitivity of ASC transport measured by cysteine again proves much less than that for System A. The uptake of cysteine is not increased by amino acid starvation, confirming the resistance of the ASC component to adaptive control. The consistency obtained, whatever method is used to observe System ASC transport, supports the validity of the earlier use of N-methylalanine derivatives for its discrimination. Another gain from the direct measurement of ASC uptake is an increased, although still equivocal, ability to predict to what degree amino acids may prove to be substrates from their inhibition of cysteine uptake. The converse use of cysteine, to demonstrate to what degree a given amino acid is transported by ASC from its susceptibility to cysteine inhibition, proves untrustworthy, however, because cysteine also inhibits System A. Kinetic studies avoiding use of methionylalanic acids confirm that at least two transport systems participate in the Na⁺-dependent uptake of the selected ASC substrate, serine. Although certain characteristics of the system identified as ASC are much the same from one occurrence to another, others are not. The differences include the substrate scope, which in the hepatocyte as in some other cells can include branched-chain and probably aromatic amino acids. We find instances where choline serves as an inferior substitute for Na⁺ for amino acid transport, a factor to be considered in discriminating the cation-dependent and -independent systems.

A heterogeneity in the Na⁺-dependent component of neutral amino acid uptake by the Ehrlich ascites tumor cell was previously shown to be best explained by a mediation by two distinct systems, System A (1) and System ASC (2, 3). Because System A transports N-methyl amino acids (2, 3), whereas these analogs are blocked to reaction with System ASC, and because no other Na⁺-dependent component with the same behavior could be detected, we were able to measure the latter system as the Na⁺-dependent uptake of such amino acids as alanine and serine retained in the presence of excess N-methylalanine or 2-(methylamino)isobutyric acid. For various red blood cells transport by System A was not detectable; hence, in that case no N-methyl amino acid had to be added to measure ASC activity (4, 5), although the presence of a system transporting glycine had to be taken into account. The similarity of the MeAIB⁻-resistant transport component observed in the Ehrlich cell to that seen in the red blood cells (2-5) ultimately persuaded us that System ASC is not an artifact of the presence of the N-methyl amino acid. Alanine, serine, cysteine, and all of their 4- and 5-carbon homologs so far tested are strong substrates for this system, a feature which we sought to evoke with the abbreviation ASC. In the Ehrlich cell we found that most of the branched-chain and cyclic amino acids as well as methionine and norleucine did not serve as substrates to a degree measurable by the rather insensitive procedure used, even though the latter two amino acids proved strong inhibitors of the ASC component (2, 5). Presumably their sidechains are too long to be accommodated through the transport process, at least at an appreciable rate (5). Branched chain amino acids appear to be transported by ASC-like mediators in several other cell types (for summary, see Ref. 6). In addition we have pointed out the similarity of the principal Na⁺-dependent system of the small intestine and kidney tubule, also observed without addition of an N-methyl amino acid, to System ASC rather than to System A (6, 7). This epithelial transport component handles a wide range of neutral amino acids.

Recently, however, for neutral amino acid transport insensitive to the addition of MeAIB or MeAla we have characterized another Na⁺-dependent system, “System N,” in the rat hepatocyte (8). This finding means that the general reliability of the procedure used previously for measuring ASC depended on our fortunate choice of a test substrate not reactive with the new system, or with any other unknown system sharing Na⁺ dependence and MeAla insensitivity with ASC. This reservation emphasizes a deficiency we have until recently been unsuccessful in correcting, namely our lack of a substrate restricted for uptake to System ASC. This lack of a model substrate restricted to ASC has led not only to an indirect measure of ASC but also to some incautious assignments to that system of all Na⁺-dependent transport retained in the presence of excess MeAIB or MeAla (6, 9). Also for identifying confidently the amino acids transported only slowly by System ASC, we need an inhibitor quite specific to that system.

Our discovery that cysteine can serve as an ASC-specific...
System ASC for Neutral Amino Acid Transport in Hepatocytes

substrate in the rat hepatocyte, reported in preliminary form (9), has enhanced the present characterization of System ASC for that cell. This substrate specificity of cysteine also aided the mentioned characterization of a new MeAIB-insensitive, Na⁺-dependent transport system (System N), specific so far as we know for glutamine, asparagine, and histidine (9). This rested justified our dissatisfaction with automatic assignments to System ASC of all Na⁺-dependent uptake not inhibited by N-methyl derivatives of alanine. Preliminary indications suggest that cysteine may serve in various other tissues as a specific substrate for System ASC, even though it does not in the Ehrlich cell. The present results also confirm the unreliability of 2-aminoisobutyric acid as a universal model substrate for System A (6, 9-11) and show its Na⁺-dependent uptake by the rat hepatocyte more or less evenly divided between Systems A and ASC. This result supports an unexpected inference by LeCam and Freychet that ASC transports AIB in these cells (12). Subsequently Kelley and Potter showed that System L also participates in AIB uptake by the hepatocyte (13).

Cysteine was shown to be concentrated into rat liver slices a decade ago, with its uptake divided between two routes (14). This finding is compatible with our observation here of a Na⁺-dependent and a Na⁺-independent component.

EXPERIMENTAL PROCEDURES

We have considered it advantageous to continue the study of both freshly separated and cultured hepatocytes for any information about transport regulation that may come from their comparison. Hepatocytes were isolated from male Sprague-Dawley rats as described previously (8) and now summarized briefly. The liver of an anesthetized rat was perfused in a retrograde manner by cannulating the inferior vena cava, severing the hepatic artery, and portal vein, and clamping the superior vena cava. The perfusate consisted of Swim's S-77 medium containing 125 units/ml of collagenase (Sigma No. C-2139). The dispersed cells were washed in either Krebs-Ringer bicarbonate buffer containing 1% bovine serum albumin (cells for immediate use in suspension) or Waymouth's medium (cells for culture). Cell viability after four washings was usually 90% as measured by impermeability to trypan blue. Cells were cultured on collagen-coated dishes (60-mm diameter) in the usual manner (8).

Amino acid uptake was initiated by the addition of 0.2 ml of the cell suspension (20 x 10⁶ cells/ml) to 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4 and 37 °C) containing 1% bovine serum albumin and the labeled amino acid. Bicarbonate-buffered solutions were maintained at pH 7.4 by aeration with 95% O₂, 5% CO₂. The assay was stopped at the appropriate time by pouring 10 ml of ice-cold Na⁺-free Krebs-Ringer bicarbonate buffer containing 0.2% sucrose into the incubation flasks. In the Na⁺-free buffer, 25 mM choline bicarbonate and 119 mM choline chloride replaced the corresponding Na⁺ salts, so that comparisons were made at the same sodium ion concentrations. The cells were quickly pelleted by centrifugation at 4 °C for 2 or 3 min at 2000 x g. The pellets were extracted with 1 ml of 5% sulfosalicylic acid after which aliquots of the cell extracts were assayed for radioactivity. The intracellular water (ICW) was determined by the difference between the total pellet water and the extracellular sucrose space (8). Ehrlich cells were produced in mice and amino acid uptake tested in cell suspensions (1, 2, 9) according to published procedures. All data from cell suspension experiments are expressed as nanomoles of test amino acid/g of ICW/unit of time.

Cultured cells were tested for transport 24 h after plating by adding 2 ml of Krebs-Ringer bicarbonate buffer containing the labeled amino acid to each dish. After removal of the medium by aspiration the cells were washed five times with ice-cold phosphate-buffered saline, pH 7.4. The cells were removed from the dishes by scraping with a rubber policeman in the presence of 1.5 ml of 0.2 N NaOH. Aliquots were taken for determination of both radioactivity and protein (15). The procedures for scintillation counting were as described previously (16). Data are expressed in picomoles or nanomoles of test amino acid/mg of protein/unit of time. The variance is recorded as the standard deviation.

New England Nuclear was the source of the labeled amino acids, whereas the unlabeled amino acids were purchased from Sigma or Calbiochem-Behring Corp. or synthesized in our laboratory by previously cited methods (6). Culturing media were obtained from Grand Island Biologicals Co.

RESULTS

Characterization of Cysteine Uptake—The time course of the Na⁺-dependent uptake of L-cysteine by freshly isolated hepatocytes is shown in Fig. 1. In 10 min the apparent distribution ratio of L-[U-¹⁴C]-cysteine, intracellular to extracellular, had reached a value of about 5 at an external 0.1 mM concentration, and a value of about unity at 10 mM. Evaluation of the actual distribution ratio for cysteine would of course require chemical analysis of the cellular content. To minimize possible discrepancies arising from the metabolism of cysteine, the cells had been treated with 1.0 mM aminoxyacetic acid at 4 °C for the 30 min prior to the uptake test.

Fig. 2 shows how cysteine saturates its own Na⁺-dependent uptake into freshly separated hepatocytes. The near linearity of the double reciprocal plot over the concentration range 0.1 to 20 mM provided support for the homogeneity of the agency serving for this uptake. The Kᵣ observed was 2.1 ± 0.19 mM, while the apparent Vₚ₅₀ was 900 nmol-g⁻¹ of ICW·min⁻¹.

Cation Specificity of Cysteine Uptake—Fig. 3 shows the relation between the rate of 0.1 mM cysteine uptake and the extracellular concentration of either Na⁺ or Li⁺. The half-maximally stimulating concentrations of Na⁺ and Li⁺ were 36 and 60 mM, respectively, while the corresponding maximal velocities of cysteine uptake were 77 and 22 nmol-g⁻¹ of ICW·min⁻¹. In contrast 0.1 mM glutamine uptake via System N is only moderately handicapped by Li⁺ substitution for Na⁺, the effect in that case corresponding to a halving of its apparent affinity for transport.

Antipodal Specificity of Cysteine Uptake—For the Ehrlich cell, Systems A and L proved much less selective between D and L isomers of cysteine than System ASC (1, 3, 17). The hepatocyte uptake of cysteine is quite stereospecific, as shown in Fig. 4, but in this cell the relatively sharp discrimination between D and L isomers holds also for the System A and N

![Fig. 1. Time course of 0.1 or 10 mM L-cysteine by freshly isolated hepatocytes in suspension.](image-url)
any case, the present result does not establish ASC transport for S-carbamylcysteine since the inhibitory action could be an effect unrelated to its transport by the same agency as cysteine.

Because excess cysteine decreased uptake of 0.1 mM cysteine below the level of 8% of the uninhibited rate predicted from its $K_a$ value, we cannot conclude from the data of Table I that S-ethylcysteine and serine are necessarily weaker inhibitors than the substrate itself.

**Evidence That the Na⁺-dependent Uptake of Cysteine Probably Occurs by a Single Mediator**—Although MeAIB fails to inhibit cysteine uptake by hepatocytes, cysteine paradoxically does inhibit MeAIB uptake (9). Fig. 5 shows by a Dixon plot the effect of increasing levels of external cysteine components (8). It will be interesting to see whether the low antipodal specificity of the Ehrlich cell may apply to other tumor cells.

**General Survey of Inhibitory Action on the Transport System Mediating Cysteine Uptake**—The uptake of 0.1 mM cysteine was tested for possible inhibition by various amino acids, some naturally occurring and others artificial (Table I). As we showed earlier (9), the Na⁺-dependent uptake of cysteine appears entirely MeAIB-insensitive, thus ruling out transport by System A. S-Monomethylation or S-monoethylation of the cysteine molecule produced little effect on its inhibitory action on cysteine uptake, whereas an S-carbamyl group rendered the amino acid distinctly less inhibitory (Table I). Since the carboxamide group prevents glutamine and asparagine from reacting with System ASC in the hepatocyte, it would not be strange if the S-carbamyl group had a similar effect. We have not excluded a possible partial hydrolysis of S-carbamylcysteine to cysteine before or during the test. In

![FIG. 2. Lineweaver-Burk plot of kinetics of cysteine uptake into freshly isolated hepatocytes. Uptake of cysteine at 0.1 to 20 mM was tested for 1 min at 37 °C. The observed velocities in the absence of Na⁺ have been subtracted so that only the contribution by System ASC is shown. The data are the averages of three determinations from 1-min uptakes at 37 °C.](image2)

![FIG. 3. The relation between the velocity of 0.1 mM cysteine uptake and the extracellular concentration of either Na⁺ or Li⁺. The concentration of the cation was varied between 12.5 and 119 mM. The buffer used throughout was 25 mM choline bicarbonate. Choline chloride was used to bring all of the assays up to 119 mM in cation chloride concentration and the rate in absence of either Na⁺ or Li⁺ (choline chloride = 119 mM) has been subtracted. The data are the averages of triplicate determinations from 1-min uptakes at 37 °C.](image3)

**TABLE I**

| Inhibitor tested, 25 mM | Per cent of control |
|------------------------|---------------------|
| None                   | 100                 |
| MeAIB                  | 106                 |
| L-Cysteine             | 0                   |
| S-Methyl-L-cysteine    | 0                   |
| S-Ethyl-L-cysteine     | 0                   |
| S-Carbamyl-L-cysteine  | 10                  |
| L-Penicillamine        | 32                  |
| L-Alanine              | 16                  |
| L-Serine               | 6                   |
| L-Threonine            | 33                  |
| L-Proline              | 54                  |
| L-Hydroxyproline       | 60                  |
| L-Phenylalanine        | 74                  |
| L-Leucine              | 39                  |
| L-Valine               | 57                  |
| L-Isoleucine           | 64                  |
| L-Melphanol           | 17                  |
| BCH                    | 94                  |
| L-Cysteine sulfinate   | 222                 |
| L-Cysteate             | 198                 |

*Velocity is 33.0 nmol·g⁻¹·ICW⁻¹ min⁻¹.*
values that allow the presence of a substantial fraction of it in a potentially reactive form. For hepatocytes in culture we also measured the Na+-dependent uptake of cysteate at various pH values. As favorable pH, Fig. 1 compares the ability of cysteine to inhibit the pH of the assay medium was raised, inevitably increasing the Na+-independent uptake by systems for which they are not substrates (2, 3, 5, 22) (Fig. 5). System ASC may be particularly vulnerable to such effects. Therefore, in Table II we measure the partition of uptake of 15 natural or artificial amino acids among Systems A, ASC, and the Na+-independent component, using each amino acid as a labeled transport substrate and accepting resistance to MeAIB inhibition as the characteristic that defines ASC uptake (2). The Na+-independent fraction includes the nonsaturable component as well as System L uptake. The partition between A and ASC uptake must be taken as approximate and not absolute for three reasons: 1) the actual rate for each amino acid varies somewhat among cell preparations; 2) the ratio between the A and ASC components fluctuates because of variable stimulation of A activity by hormones and by amino acid starvation-induced derepression; 3) the particular division shown in Table II applies only for the test amino acid at 0.1 mM.

Although cysteine is a specific substrate here for System ASC, it is not, to the disadvantage of its full analytical use, specific to ASC as an inhibitor, as we have shown by its action on the uptake of both BCH (as discussed above) and MeAIB (Fig. 5). Hence, we still lack a model analog that can be used cleanly to inhibit ASC as we used MeAIB to block System A in Table II. We cannot eliminate uptake of substrates by System ASC with cysteine without also decreasing their uptake by System A. Furthermore, the column showing ASC take by System A. Furthermore, the column showing ASC take not occurring by System A, includes uptake by System L. Finally, we still lack a model analog that can be used solely for the test amino acid at 0.1 mM.

We do find an effect of cysteine on at least one of two Na+-dependent transport systems for anionic amino acids, as measured by the uptake of L-[3,3-3H]cysteate or L-[3,3-3H]cysteinesulfinate (20, 21). Cysteine occurs in solution to some extent as an anion and might be transported in that form at a favorable pH. Fig. 6 compares the ability of cysteine to inhibit the Na+-dependent uptake of cysteine at various pH values. As one might predict, the inhibition by cysteine increased as the pH of the assay medium was raised, inevitably increasing the fraction of cysteine present in the appropriate anionic form bearing the charges +, -, -., the same charges carried by glutamate, aspartate, and cysteate. These results indicate that cysteine can react with the anionic transport system(s) at pH values that allow a substantial fraction of it to be in a potentially reactive form. For hepatocytes in culture we also find the converse effect, namely that 5 mM cysteate can inhibit uptake of 0.1 mM cysteine (40% at pH 8.9, but not measurably at pH 7.4). The inappreciable effect at pH 7.4 indicates that this Na+-dependent component of cysteine uptake will be negligible under most physiological conditions.

These results fail to detect any further Na+-dependent component of cysteine uptake, beyond the system taken to be ASC.

**Survey of the Partition of Amino Acids among Mediating Systems, Particularly Na+-dependent Systems**—Unfortunately we cannot safely determine how rapidly various amino acids are taken up by System ASC from their inhibition of cysteine uptake, as in Table I. In too many cases amino acids inhibit uptake by systems for which they are not substrates (2, 3, 5, 22) (Fig. 5). System ASC may be particularly vulnerable to such effects. Therefore, in Table II we measure the partition of uptake of 15 natural or artificial amino acids among Systems A, ASC, and the Na+-independent component, using each amino acid as a labeled transport substrate and accepting resistance to MeAIB inhibition as the characteristic that defines ASC uptake (2). The Na+-independent fraction includes the nonsaturable component as well as System L uptake. The partition between A and ASC uptake must be taken as approximate and not absolute for three reasons: 1) the actual rate for each amino acid varies somewhat among cell preparations; 2) the ratio between the A and ASC components fluctuates because of variable stimulation of A activity by hormones and by amino acid starvation-induced derepression; 3) the particular division shown in Table II applies only for the test amino acid at 0.1 mM.

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of Systems A and L in the hepatocyte. Table II also shows the more or less equal participation of System A and ASC in AIB uptake into hepatocytes, not seen in the Ehrlich cell. As for other cells, the hepatocyte appeared to transport BCH almost entirely by System L, whereas cycloleucine showed both Na+-dependent components (Table II). Presumably as in other cells, uptake occurred also by System L.

In contrast to the hepatocyte, more than 75% of the rapid Na+-dependent uptake of cysteine by the Ehrlich cell can be identified with System A (Table III). In that cell, glutamine serves as an inhibitor (Table III) and as a substrate of System ASC. We found glutamine uptake at 0.1 mM partitioned as follows among 3 systems: A, 552; ASC, 145; and L, 23 mmol-1 of ICW-30 s-1.

Features of the Mediator of Cysteine Transport Identifying It with System ASC in Other Cells—We have become reluctant to consider the insensitivity of this component to MeAIB inhibition (Table II) as a sufficient identification with System ASC. Its noninducibility also corresponds, however, with the behavior of ASC in other cells. In connection with our tests of Li+ substitution for Na+, we found a small but definite Li+-supported alanine uptake even in hepatocytes from fed animals.

(23), and which contributes also in ordinary hepatocytes. The apparently high ASC uptake shown by glycine in Table II therefore includes a component due to System Gly. Unfortunately we do not have in cysteine a means of measuring ASC uptake by direct inhibition, for example, to generate in Table II a column of figures corresponding to those in Column 3 for System A, the latter obtained by use of MeAIB inhibition. Instead, Column 2 merely contains the residual Na+-dependent uptake surviving MeAIB inhibition. Although glutamine and glycine illustrate the risk of assigning ASC activity with System ASC in Other Cells cultured in either amino acid-rich or amino acid-free medium

After 24 h in primary culture, the medium was changed to either amino acid-rich (Waymouth's medium) or amino acid-free (Krebs-Ringer bicarbonate medium) solutions and the cells were incubated for an additional 6 h. One-min uptake rates in the presence of either 119 mM Na+ or 119 mM Li+ were tested at 37 °C. The buffer component in each case was 25 mM choline bicarbonate, pH 7.4. System A activity was monitored as the Na+-dependent alanine uptake inhibited by the presence of 30 mM N-methyl-DL-alanine, while the remaining Na+-dependent uptake was taken as a measure of System ASC activity. The velocity in the absence of sodium was 89.9 ± 4.5 and 106 ± 4.6 pmol-mg-1 of protein-min-1 for cells cultured in amino acid-rich and amino acid-free media, respectively. These rates have been subtracted from the values found in the presence of the indicated cation to obtain the velocities shown. The results are the averages ± S.D. of at least five determinations.

### Table II

| Test amino acid, 0.1 mM | Na+-dependent uptake | Total Na+-independent uptake |
|------------------------|----------------------|-----------------------------|
|                        | ASC                  | A                           |
| [1-4C]MeAIB            | 20.7                 | 0                           |
| t-[3,3-3H]Cysteine     | 0                    | 33                          |
| [Me-4C]Methyl-L-cysteine| 4.1                  | 12.3                        |
| L-[U-14C]Alanine       | 9.5                  | 7.5                         |
| L-[U-14C]Serine        | 16.7                 | 11.1                        |
| L-[U-14C]Homoserine    | 11.5                 | 35                          |
| L-[U-14C]Proline       | 5.4                  | 3.0                         |
| L-[G-H]Hydroxyproline | 2.9                  | 18.4                        |
| L-[U-14C]Theonine      | 5.1                  | 23                          |
| L-[Me-4C]Methionine    | 3.1                  | 1.4                         |
| L-[U-14C]Glycine       | 8.3                  | 9.4                         |
| L-[U-14C]Phenylalanine| 0                    | 3                            |
| L-[U-14C]Leucine       | 4                    | 8                            |
| [1-14C]AIB             | 1.9                  | 5.1                         |
| [1-14C]Cycloleucine    | 7.5                  | 3.0                         |
| [1-14C]BCH             | 0                    | 191                         |

### Table III

| Inhibitor, 25 mM | Na+-dependent velocity of cysteine uptake | Na+-independent velocity of cysteine uptake |
|-----------------|-------------------------------------------|-------------------------------------------|
| None            | 555 (569, 541)                            | 108 (109, 107)                            |
| MeAIB           | 127 (134, 120)                            |                                            |
| L-Glutamine     | 0                                          |                                            |
| L-Cysteine      | 0                                          |                                            |
| BCH             | 24 (24.3, 23.1)                            |                                            |

### Table IV

| Transport system tested | Cation present during assay | Amino acid-rich | Amino acid-free | Ratio of amino acid-rich to amino acid-free |
|-------------------------|-----------------------------|----------------|----------------|--------------------------------------------|
|                        | Alkali-ion dependent rate   |                 |                 |                                            |
|                          | pmol-mg-1 protein-min-1     |                 |                 |                                            |
| A                       | Na+                        | 34.7 ± 10.3     | 261 ± 13.4      | 7.5                                        |
| ASC                     | Na+                        | 90.5 ± 7.3      | 69.2 ± 5.4      | 0.8                                        |
| A                       | Li+                        | 15.2 ± 8.6      | 52.2 ± 4.7      | 3.4                                        |
| ASC                     | Li+                        | 10.9 ± 8.4      | 7.9 ± 5.4       | 0.7                                        |
mals, contrary to the conclusion of Edmondson et al. (24) that hepatic System A fails to accept Li⁺ for co-transport. This activity increased proportionally with increases in the Na⁺-sustained System A activity seen in cells isolated from fasted rats (9). Table IV shows the ability of System A to accept Li⁺ and the effect of a 5-h incubation either in amino acid-rich or in amino acid-free medium on this acceptance. The ASC component scarcely changed on amino acid deprivation, however, whether assayed in the presence of Na⁺ or of Li⁺ (Table IV; see also Ref. 8).

Table II also shows the characteristic effect of a distal hydroxyl group in intensifying ASC transport (e.g. homoserine, hydroxyproline; Refs. 25 and 26). Note also that hydroxyproline shows six times the rate observed for proline, even though it was scarcely more effective than proline as an inhibitor of cysteine uptake (Table I), another quantitative failure of inhibition to predict substrate action in this system. Although the 3- and 4-carbon linear amino acids are as usual among the best substrates indicated for System ASC (Table II), the uptake of leucine attributed here to System ASC differs from the experience with the Ehrlich cell (2). Even phenylalanine appears likely to be an ASC substrate, although a weak one. The results of Table II support our rising suspicion (6) that the substrate scope of System ASC is rather wider in its various occurrences than was originally observed for the Ehrlich cell (2). This difference has intensified our concern in studying the resemblance of the transport system under study to System ASC in other cells.

The sensitivity of cysteine uptake in the hepatocyte to pH changes shows as in other cells a difference from System A. In the Ehrlich cell we could help differentiate Systems A and ASC by the greater sensitivity of the first to increases in H⁺ concentration (2). We have already used pH modification to identify provisionally cysteine inhibition of cysteate uptake with an anionic form of cysteine. Our tests using cysteine as substrate have the advantage (along with the tests of Fig. 2 in Ref. 8) that the effects of pH can be applied without adding any amino acid at above 0.1 mM. In two separate experiments with fresh hepatocytes neither uptake attributed to A nor to ASC was slowed by more than 20% when the pH was lowered to 6.0, whereas System N activity as monitored by 0.1 mM glutamine uptake was sharply decreased (the Na⁺-dependent velocities at pH 7.5, 7.0, 6.5, and 6.0 were 259 ± 13, 78.5 ± 4.5,

![Fig. 7. A Dixon plot showing the inhibition of AIB by MeAIB and vice versa. The 2-min rates of uptake at 37 °C of either AIB or MeAIB (0.1 mM) were tested for inhibition by the other analog at increasing concentrations. The Na⁺-dependent rates were obtained after subtraction of the Na⁺-independent values of 11.0 ± 0.06 and 9.8 ± 0.18 for AIB and MeAIB, respectively. The results are the averages of three assays.](image)
of the heterogeneity of the Na+-dependent uptake of serine and related amino acids, also in the hepatoma cell line HTC, the heterogeneity of its Na+-dependent uptake by the hepatocyte in the derepressed condition. This problem appears not to exist for pigeon erythrocytes and rabbit reticulocytes, where there appears to be no System A uptake to account for the prevalence of its Na+-dependent uptake by the hepatocyte (12, 28-32). This difference from the behavior of AIB in the Ehrlich cell (35) has been documented elsewhere (8, 12, 13).

**DISCUSSION**

We originally concluded in favor of the separate existence of System ASC in the Ehrlich cell only after substantial attention to the possibility that MeAIB addition had somehow generated an artifactual heterogeneity in an otherwise homogeneous Na+-dependent route. Because of the generally larger contribution by System A, this problem is more difficult in the Ehrlich cell and in the hepatocyte in the derepressed condition. This problem appears not to exist for pigeon erythrocytes and rabbit reticulocytes, where there appears to be no System A uptake to need blocking with MeAIB. An important advantage of our results on the properties of System ASC uptake obtained here with cysteine (e.g. stereospecificity and relative insensitivity to pH lowering) is that they were obtained without the equivocal consequences of adding an excess of an N-methyl amino acid. The heterogeneity of the Na+-dependent uptake of AIB and serine, on the one hand, and the evidence for homogeneity of the uptake of MeAIB and cysteine, on the other hand, support the separate contributions of these two systems to uptake.

Gazzola et al. (34) showed that the uptake of labeled alanine or serine retained by embryonic chick heart cells in the presence of 5 mM concentrations of unlabeled BCH and MeAIB (taken therefore to occur via System ASC) fails to show the usual stimulation during a prior 2 h of amino acid starvation. It would be remarkable for MeAIB inhibition during the transport test to spare only a constant portion of the Na+-dependent uptake of serine or alanine, and none of the incremental part, if that uptake really occurred by a homogeneous System A. On the basis of such experiments, it was concluded that only System A, and not ASC or L, shows adaptive regulation. Other studies have supported the same selectivity of adaptive regulation in various tissues. In Table V we show this same distinction applies to the two components of Na+-dependent alanine uptake in the hepatocyte. In this cell, MeAIB and glutamine serve to represent transport systems (namely A and N) responsive to amino acid starvation, whereas cysteine represents the unresponsive System ASC (Fig. 5 in Ref. 8). Differences in regulatory response, including that to the pancreatic hormones (also seen in Ref. 8), appear then to provide the strongest support for the reality of the A-ASC heterogeneity in various tissues. These differences in turn provide support for the validity of the distinction provided by the N-methylated model amino acids. That validity may, however, deserve reexamination in any new context.

The wide scope and biological importance of transport System ASC for neutral amino acids is reinforced by the present findings for the isolated rat hepatocyte. Elsewhere we have called attention to the greater similarity of the Na+-dependent absorption of neutral amino acids in the small intestine and in the kidney to System ASC than to System A (6, 7). The possibility that these so-called "iminoglycine" system of these tissues is a variant of System A has also been considered elsewhere (35). A recent preliminary communication from this laboratory outlines a transport system accounting for much of the Na+-dependent uptake of neutral amino acids by segments of rat small intestine (36). For this system also, cysteine appears to serve as a specific substrate, no significant portion of its Na+-dependent uptake by intestinal segments in vitro being inhibited by either AIB or MeAIB (36). Similarly, Sepúlveda and Smith (37) have pointed out that the principal Na+-dependent system for neutral amino acids in the rabbit ileum is quite unlike System A. It transports serine and threonine rapidly and is not inhibited by either AIB or MeAIB. Furthermore, inhibition tests suggest that this system may transport phenylalanine, leucine, and other amino acids with bulky sidechains, in approximate agreement with the broad substrate specificity reported here for ASC in hepatocytes and now also found in isolated segments of rat intestine (36). Mircheff et al. (38) have reported uptake of alanine into vesicles derived from rat intestinal basal lateral membrane consistent with participation of Systems L, A, and ASC. A personal report from Yudilevich (cf. Ref. 39) indicates that System ASC in the salivary gland has a similarly wide scope. Note also that a Na+ stimulation of renal tubular resorption of BCH has been shown, although not well categorized (40). Conceivably initial rates of BCH transport were not secured with this preparation. A test to see whether System L might conceivably become Na+-dependent on preparation of vesicles from the Ehrlich cell has given negative results (41).

It appears likely that in its various occurrences System ASC varies somewhat as to its reactivity with such amino acids as phenylalanine and leucine. As suggested already, the significant inhibition of cysteine uptake by phenylalanine in the hepatocyte (Table I) may mean that it also is a substrate with a very low V_{max} for uptake. The total Na+-dependent uptake of phenylalanine (Table II) was not large enough, however, to make convincing the partition shown. For the same reason, System ASC of the Ehrlich cell may well also transport phenylalanine and leucine at rates too low to have been observed. These and all other ordinary neutral amino acids

![Graph](https://via.placeholder.com/150)
inhibited the ASC component in that cell, supporting a view that all might be substrates but many of them at very low maximal velocities (2). Several of the subsequent conclusions in favor of the contribution of System ASC to amino acid uptake in other tissues were made without exploring its scope with reference to bulkier-chained amino acids. Note also that proline was found to act as an ASC substrate in the Ehrlich cell (2) and in red blood cells (4, 5), despite its extra space requirements. The vigorous uptake of the trans isomers of the hydroxyprolines (26) shows that the structure of proline can be well accommodated. A similar effect of a substituent on a ring structure is indicated by comparison of phenylalanine and melphalan action on cysteine uptake (Table 1). Melphalan is much more inhibitory. Its cytotoxic action as an alkylating agent, as well as the inhibition pattern for the uptake of this widely studied anticancer agent by a cultured lymphoblast line (42, 43) and by a murine leukemia cell (44), has indicated that melphalan uptake occurs both by System L and a Na+-dependent system resembling ASC. The observation that leucine and phenylalanine inhibited the Na+-dependent component of melphalan uptake no longer argues against identification of this component with System ASC, or in favor of its identification with a conceivable but unprecedented Na+-dependent variant of System L. Although we should like to reexamine the substrate scope of System ASC in the Ehrlich cell, unfortunately we do not yet have an amino acid with the properties needed to identify reliably by its inhibitory action small proportions of the migration of other amino acids occurring by that system.

A transport system for rat brain slices reported by Tews et al. (45) underwent inhibition by various amino acids in the following sequence of decreasing strength: 2-amino-n-butyric acid > threonine, homoerine > alanine > serine > leucine > phenylalanine > AIB, tryptophan. Retaining the usual reservation that positive results for inhibition do not necessarily reflect substrate action, we take these results to suggest the operation of System ASC in brain slices. To complete the identification, MeAIB or MeAla should be added to the list of inhibitors tested. A MeAla-insensitive, Na+-dependent component of amino acid uptake by mouse brain slices was demonstrated by Sershen and Lajtha (46). A transport system with broad substrate specificity present in lectin-activated pig lymphocytes has been considered to correspond to System ASC, although that conclusion is also based on the pattern of amino acids inhibiting it, rather than of those acting as substrates (47). Both this component and one corresponding to System A were enhanced in activity by phytohemoglutinin treatment. In the cultured Chinese hamster ovary cell, the MeAIB-insensitive portion of the Na+-dependent uptake of phenylalanine is also larger than the MeAIB-inhibitable component, and here the ASC component is almost as large as the System L component.5 Na+-dependent uptake of neutral amino acids by these cells showed kinetic evidence for two routes in the absence of an added N-methyl amino acid (cf. Fig. 8).

Whereas cysteine serves fairly well as a defining substrate in the ordinary rat hepatocyte studied here, in the hepatoma cell, HTC, threonine proves to take its place. Besides this difference, we have mentioned a special pH sensitivity for threonine uptake in HTC,4 similar to that seen for a system for anionic amino acids.5 These differences persuade us not to count on precise correspondences of transport systems in HTC to those seen in the untransformed hepatocyte. Nevertheless, System ASC in HTC resembles ASC systems in general, and its study gives even stronger support for the reality of the A/ASC dichotomy. This result, along with the results of the two preceding paragraphs, does not, however, support a uniform substrate selectivity or H⁺ reactivity for System ASC in its various occurrences. The agencies producing this transport accordingly appear not the same, and even the mediating influence of pH may vary from species to species. Strictly speaking, one could name them all as different, a tendency initiated provisionally by calling the system in the pigeon red cell by the abbreviation, ASCP (4). Instead of proliferating such abbreviations, however, it seems preferable first to recognize provisionally a generic nature for each system and its name, e.g. ASC, A, etc., and then to qualify that name in words, e.g. System ASC as observed in hepatoma cell line HTC.

Our results suggest that an analog inhibition which does not prove competitive, especially if it shows a Ki rather higher than the Ka for its uptake under similar conditions, should be suspected of not corresponding to shared transport. We see this phenomenon in Fig. 5, and also in the relation between glutamine and MeAIB in the hepatocyte. In contrast to the Ka value of about 1 mM for glutamine uptake assigned to System N, this amino acid also shows a Ki of about 12 mM for its noncompetitive inhibition of uptake by System A (8). Presumably this behavior points to a reaction with the mediating system at a site other than the receptor site for transport. Possible regulatory functions then come to mind, although the Ki values are rather high for that function.

The ionization of the sulphydryl group of cysteine is a complex subject which has been studied intensely (48). The pK₂ value of about 8.38 observed by titration does not apply entirely to either the sulphydryl group or the amino group. At any pH in this range about two-thirds of the cysteine molecules assuming the anionic form are deprotonated at the sulphydryl groups and thus assume the charge distribution appropriate to the cysteate- and glutamate-transporting agency or agencies; the other one-third of the molecules deprotonated have lost a proton at the amino group and should be unsuitable for any known transport system. We recognize that combination of cysteine with the receptor site could enhance the sulphydryl dissociation. Enough of the appropriate form of cysteine is already present in free solution, however, to make plausible the inhibition seen. Although these estimates can account for the inhibitory action of cysteine on cysteate uptake (Fig. 4), they are at the same time compatible with a comparatively insignificant uptake of cysteine by that route until the pH is brought substantially higher than neutrality. On test, the transport reactivity of cysteine as an anion at pH 7.4 or lower appears to add an insignificant complication to its use as a System ASC model substrate.

Although the results of Table II show that the fraction of the uptake of such amino acids as phenylalanine and leucine occurring by System A in the hepatocyte can be small, nevertheless their total uptake by Na+-dependent systems is large enough to deserve attention in evaluating the possible cooperation between Na+-dependent and Na+-independent systems in determining steady states of uptake as discussed elsewhere (6). Only in red blood cells, where the system has had the most study, has System ASC been shown apparently to be locked into an exchange function (4, 23, 49, 50). In other occurrences net transport is likely to be more typical.

The characteristically specific reactivity of cysteine with System ASC described here intensifies interest in the Na+-independent "cysteine system" found by Young and Ellory in sheep erythrocytes (51). These authors note the similarity of

5 M. A. Shotwell and D. L. Oxender, personal communication.
6 H. N. Christensen and M. E. Handigoten, unpublished results (1980).
7 M. Makowske, unpublished doctoral thesis (1980) University of Michigan.
this system to ASC in respects other than Na\textsuperscript{+}-dependence. One might speculate that the activity they observed is due to a new cation-independent system resembling but not identical with System L, or even due to a Na\textsuperscript{+}-independent variant of System ASC. First, however, we might consider the possibility that in their tests another cation may have partially fulfilled the role ordinarily played by Na\textsuperscript{+}. Organic cations such as choline and the methylguanidinium ion can serve as inferior substitutes for Na\textsuperscript{+} in its role in certain transport systems (5, 36). Young and Ellory (51) used choline as the osmotic replacement for the omitted Na\textsuperscript{+}.

For isolated segments of rat intestine cysteine uptake supported by choline, relative to a mannitol control, was about 20% of that obtained in a normally Na\textsuperscript{+}-rich medium (36). Similar results are obtained for serine uptake in the hepatocyte (data not shown). Conceivably the so-called cysteine system might prove inconspicuous in, for example, a mannitol medium. A high-affinity, Na\textsuperscript{+}-dependent system transporting cysteine has been observed in adult human red blood cells (52). Alanine shares this transport with cysteine, but otherwise the scope of the system remains undefined. An emphasis by the authors of that study on the similarity of the \(K_m\) for cysteine transport (about 20 \(\mu\text{M}\)) to the plasma concentration of this amino acid may be misplaced, unless alanine and other abundant substrates of that system have \(K_m\) values so high they cannot as one would expect overwhelm cysteine in saturating the system under physiological conditions.

We originally proposed the abbreviation ASC to avoid the implications inherent in a briefly considered alternative designation, "System A." The three-letter abbreviation has shown an unforeseen tendency to focus excessive attention on only three amino acids, a mistaken emphasis that becomes steadily less appropriate. We suggest that the letters ASC be replaced by System L, or even due to a Na\textsuperscript{+}-independent system to ASC in respects other than Na\textsuperscript{+}-dependence.

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REFERENCES
1. Oxender, D. L., and Christensen, H. N. (1963) J. Biol. Chem. 238, 3696-3699
2. Christensen, H. N., Liang, M., and Archer, E. G. (1967) J. Biol. Chem. 242, 5297-5296
3. Christensen, H. N., Oxender, D. L., Liang, M., and Vatz, K. A. (1965) J. Biol. Chem. 240, 3609-3616
4. Eavenson, E., and Christensen, H. N. (1967) J. Biol. Chem. 242, 5396-5396
5. Thomas, E. L., Christensen, H. N., and Handlogten, M. E. (1969) Biochim. Biophys. Acta 193, 229-230
6. Christensen, H. N. (1979) Adv. Enzymol. 49, 41-101
7. Christensen, H. N., and Handlogten, M. E. (1977) J. Membr. Biol. 37, 193-211
8. Kilberg, M. S., and Handlogten, M. E., and Christensen, H. N. (1980). J. Biol. Chem. 255, 4011-4019
9. Kilberg, M. S., Christensen, H. N., and Handlogten, M. E. (1979) Biochim. Biophys. Res. Commun. 88, 744-751
10. García-Sanchez, J., Sanchez, A., and Christensen, H. N. (1977) Biochim. Biophys. Acta 404, 285-292
11. Kilberg, M. S., and Neuhaus, O. W. (1971) J. Supramol. Struct. 6, 191-204
12. LeCam, A., and Freychet, P. (1977) Biochem. Biophys. Res. Commun. 73, 893-901
13. Kelley, D. S., and Potter, V. R. (1978) J. Biol. Chem. 258, 9009-9017
14. Crawhall, J. C., and Davis, M. G. (1971) Biochim. Biophys. Acta 225, 326-334
15. Lowry, O. E., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
16. Pal, P. R., and Christensen, H. N. (1961) J. Biol. Chem. 236, 894-897
17. Oxender, D. L. (1965) J. Biol. Chem. 240, 2976-2982
18. Christensen, H. N., Handlogten, M. E., Lam, L, Tigner, H. S., and Zand, R. (1969) J. Biol. Chem. 244, 1510-1520
19. Tager, H. S., and Christensen, H. N. (1972) J. Am. Chem. Soc. 94, 966-972
20. Gazzola, G. C., Dall'Asta, V., Bussolati, O., Makowske, M., and Christensen, H. N. (1961) J. Biol. Chem. 256, in press
21. Handlogten, M. E., Christensen, H. N., Gazzola, G. C., Kilberg, M. S., Schwass, D. E., and White, M. E. (1979) Fed. Proc. 38, 283
22. Thomas, E. L., and Christensen, H. N. (1971) J. Biol. Chem. 246, 1682-1688
23. Thomas, E. L., and Christensen, H. N. (1970) Biochim. Biophys. Res. Commun. 40, 277-283
24. LeCam, A., and Freychet, P. (1977) J. Biol. Chem. 252, 148-156
25. Tews, J. K., Colosi, N. W., and Harper, A. E. (1976) Life Sci. 18, 739-750
26. Kletzien, R. F., Pariza, M. W., Becker, J. E., Potter, V. R., and Butcher, F. R. (1976) J. Biol. Chem. 251, 1504-1509
27. Jefferson, L. S., Schriver, C. M., and Tolman, E. L. (1976) J. Biol. Chem. 251, 197-204
28. Chambers, J. W., Georg, R. H., and Bass, A. D. (1968) Endocrinology 83, 1185-1192
29. Harrison, L. E., and Christensen, H. N. (1971) Biochim. Biophys. Res. Commun. 43, 119-125
30. Christensen, H. N. (1969) Adv. Enzymol. 35, 1-20
31. Gazzola, G. C., Franchi-Gazzola, R., Ronchi, R, and Guidotti, G. G. (1973) Biochim. Biophys. Acta 311, 292-301
32. Christensen, H. N. (1975) Curr. Top. Membr. Transp. (1975) 6, 227-238
33. Lancaster, K. T., Kilberg, M. S., and Christensen, H. N. (1980) Fed. Proc. 39, 1712 (abstr.)
34. Sepulveda, F. V., and Smith, M. W. (1978) J. Physiol. 282, 73-90
35. Mircheff, A. K., van Os, C. H., and Wright, E. M. (1980) J. Membr. Biol. 52, 83-92
36. Yudilevich, D. L., and Eaton, B. M. (1980) Biochim. Biophys. Acta 599, 315-319
37. Ullrich, K. J., Ruzmich, G., and Kiss, S. (1974) Pfleger's Arch. Ges. Physiol. 351, 49-60
38. Kilberg, M. S., and Christensen, H. N. (1980) Membr. Biochem. 3, 155-168
39. Goldenberg, G. J., Lam, H. P., and Begleiter, A. (1979) J. Biol. Chem. 254, 1057-1064
40. Begleiter, A., Lam, H. P., Grover, J., Froese, E., and Goldenberg, G. J. (1979) Cancer Res. 39, 353-359
41. Vistica, D. T. (1979) Biochim. Biophys. Acta 550, 300-317
42. Pews, J. K., Good, S. S., and Harper, A. E. (1978) J. Neurochem. 31, 581-589
43. Sersen, H., and Lajtha, A. (1979) J. Neurochem. 32, 719-726
44. Bozetti, A. (1976) J. Physiol. 247, 527-532
45. Kallen, R. G. (1971) J. Am. Chem. Soc. 93, 6227-6230
46. Wheeler, K. P., and Christensen, H. N. (1967) J. Biol. Chem. 242, 3782-3788
47. Koser, B. H., and Christensen, H. N. (1971) Biochim. Biophys. Acta 241, 9-19
48. Young, J. D., and Ellory, J. C. (1977) Biochem. J. 162, 33-38
49. Young, J. D., Woloszyk, M. E., Jones, S. E. M., and Ellory, J. C. (1979) Nature 279, 800-802