SYNTHESIS OF METABOLISM-RESISTANT SUBSTRATES FOR THE TRANSPORT SYSTEM FOR CATIONIC AMINO ACIDS; THEIR STIMULATION OF THE RELEASE OF INSULIN AND GLUCAGON, AND OF THE URINARY LOSS OF AMINO ACIDS RELATED TO CYSTINURIA

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SUMMARY

Several amino acids have been synthesized as model transport substrates building on the piperidine and cyclohexane rings. Only when the distal N atom is part of an unambiguously cationic structure are these compounds transported predominantly by the cationic amino acid system. These amino acids in labeled form are excreted rather slowly in unmodified state, very little $^{14}$CO$_2$ being released. Those which are unambiguously cationic (including also homoarginine) led to a greatly increased excretion of arginine, lysine, ornithine and citrulline. Those which might be expected to act as lysine analogs had little effect on the excretion of the basic amino acids, although the excretion of citrulline and the sum of glutamine plus asparagine was accelerated. Certain of the analogs intensified the excretion of citrulline in dissociation from effects on resorption of the basic amino acids, also in dissociation from effects on cystine resorption. These results indicate citrulline resorption does not occur principally by the same agency serving for the basic amino acids, nor by the agency serving for cystine, despite the observed interactions for resorption. The injection of either of three transport analogs for arginine into the rat leads to early increases in the circulating levels of immunologically reactive insulin and glucagon.

INTRODUCTION

To study some rather mystifying interactions between basic and neutral amino acids (including particularly cystine) for renal tubular transport, and to study other aspects of the transport of cationic amino acids, we have synthesized a series of non-metabolizable amino acid analogs built on the piperidine ring system. The following features seemed to us desirable for inclusion in a model substrate for study of a general transport system for basic amino acids: (1) An unambiguously cationic

Abbreviations: PA, 4-aminopiperidine-4-carboxylic acid; CPA, 4-amino-1-carbamoylpiperidine-4-carboxylic acid; GPA, 4-amino-1-guanylpiperidine-4-carboxylic acid; PG, 4-piperidinoglycine; MPA and $M_2$PA, 4-amino-1-methyl- and 4-amino-1,1-dimethylpiperidine-4-carboxylic acid, respectively.
group removed by three or more carbon atoms from α-amino group. (2) Several
methylene groups to produce high affinity. The optimal distribution of these groups
in space is not well known. (3) To minimize metabolic attack, a cyclic structure,
thereby avoiding any chain-ending methyl or methylene group, and at the same time
avoiding the presence of a hydrogen atom on the α-carbon. We expected to find,
however, a disadvantage to reactivity from the presence of two β-carbon atoms,
because L-aminocyclopentane carboxylic acid has proved a weak inhibitor of the
uptake of basic amino acids in the presence of Na⁺ (ref. 4). That is, two β-carbon
atoms do not appear to be accommodated freely at this transport site in the cells we
have studied, even if these two carbon atoms are part of a ring structure. (4) A cyclic
structure to fix the position of the terminal cationic group in space with reference to
the α-amino and carboxyl groups. Here again, the absence of detectable inhibition
of lysine uptake by proline in the presence of Na⁺ (ref. 4) generated doubt that the
otherwise promising aminoprolines or aminopipeolic acids could be accommodated
at the site to provide evidence (such as that obtained for the topography of Site
ASC* for the position in space of the structure recognizing the terminal basic group
at Site L.*)

Heretofore we have used homoarginine as a model substrate for System L*. Although it includes only the first two features listed above, it is metabolically more
stable than arginine. In the present work, an amino and a carboxyl group were
introduced on C-4 of piperidine to generate a lysine analog. 4-Piperidinoglycine was
prepared as another lysine analog.

In studies of the transport of these compounds into the Ehrlich cell reported
elsewhere we find that the nitrogen atom of the piperidine ring, or the amino group
of an amino acid built on the cyclohexane ring, fails to fulfill the role of the desired
distal cationic group. On the contrary, several amino acids containing these struc-
tures served as substrates of System L* as if their sidechains were uncharged. This
behavior was especially conspicuous when the piperidine N was monomethylated, or
when the 4-amino group of 1,4-diaminocyclohexane carboxylic acid had the
orientation to the α-amino group.

This behavior suggests that the protonation of the piperidine N or other
terminal amino group is sufficiently repressed at the receptor site for transport so
that those compounds behave to a major extent as neutral amino acids. This repression
might arise from an apolar environment at the appropriate region of the receptor site.
It seemed to us more likely, however, that the behavior reveals a decreased availability
of the H⁺ near the external surface of the membrane, because exodus of these amino
acids from the Ehrlich cell is extremely slow, as though the receptor site at the internal
surface of the plasma membrane sees these amino acids, as expected, in an unsuitable
state of protonation. Furthermore, the uptake of these amino acids generally is
remarkably rapid and highly concentrative, permitting us to show unequivocally the
strong uphill operation of transport System L, and its sensitivity to inhibition both
by 2,4-dinitrophenol and by oligomycin. The implications of these results are dis-

duced elsewhere.

Even lysine itself acts to some extent as a neutral amino acid in transport so
that it cannot be trusted as a model substrate for cationic amino acid transport

* The several transport systems mentioned are defined and summarized in ref. 5.
The tendency to act as a neutral amino acid in transport is greatly enhanced in \(\alpha\beta\)-diaminobutyric acid\(^9\) and thialysine\(^8\), both showing a value of 8.4 for their second \(pK_a\), as against the value of 9.5 seen for lysine. For the first of these two amino acids it is System \(A\) rather than System \(L\) that accounts for a very high maximal velocity for uptake. For the second, both systems show high maximal velocities\(^8\). Hence these effects of a rather weakly basic distal amino group in producing unusually rapid uptake against strong concentration gradients are not limited to a single transport system.

To obtain unambiguously cationic amino acids, we then introduced the guanyl group on the piperidine N, so that the compounds 4-amino-1-guanylpiperidine-4-carboxylic acid (GPA) and 1-guanylpiperidine glycine (GPG) in Fig. 1 were made available as arginine analogs. These substances were fairly reactive with the transport system for cationic amino acids in all tested occurrences, but not with the systems for neutral amino acids.

Proceeding in the opposite direction, we converted one of the lysine analogs into an unambiguously neutral amino acid, namely 4-amino-1-carbamoylpiperidine-4-carboxylic acid (CPA) which is a citrulline analog.

One of our purposes in preparing these compounds was to test their ability to mimic the action of arginine and lysine in stimulating the release of insulin and glucagon from the pancreas, in parallel to the action of 2-aminonorbornane-2-carboxylic acid in mimicking the action of leucine in stimulating the pancreatic release of insulin\(^{10,11}\) but not that of glucagon\(^{12}\). The effects of leucine and its non-metabolizable analog differ from those of arginine and lysine in several important respects. Although the action of arginine cannot be accounted for by the effect of the ornithine to which it is converted, the view that it is the basic amino acid \textit{per se} that is effective would be greatly strengthened if a non-metabolizable analog could be

![Fig. 1. Structures and abbreviations of the amino acid analogs used. PA = 4-aminopiperidine-4-carboxylic acid (the "piperidine amino acid"); CPA = 4-amino-1-carbamoylpiperidine-4-carboxylic acid (the "carbamoylpiperidine amino acid"); GPA = 4-amino-1-guanylpiperidine-4-carboxylic acid; PG = 4-piperidineglycine; GPG = 1-guanylpiperidine glycine. 4-Amino-1-methyl- and 4-amino-1,1-dimethylpiperidine-4-carboxylic acid (MPA and M\(\text{2}PA\)) may be visualized by replacing one or both of the hydrogen atoms on the piperidine N of PA with one or two methyl groups.](image-url)
shown also to produce this effect. We find and are now reporting that the much more slowly metabolized homoarginine also stimulates an increase in the immunologically reactive insulin of the plasma in the rat without appreciably raising the plasma lysine level; but more significantly GPA and GPG also increase the circulating level of insulin, and at the same time, that of glucagon. Preliminary reports of both of these findings have appeared\(^1\)-\(^2\). Subsequently, similar results have been obtained on intravenous infusion into the dog, as reported also in a preliminary way\(^1\)-\(^2\). In the meantime, GPA has also been shown in the laboratory of Hellman to stimulate the release of insulin from the pancreatic islet *in vitro*, using tissue microdissected from obese, diabetic mice\(^1\). The present communication reports also on the metabolic stability of some of these amino acids on injection into the rat, and on their effects on the excretion into the urine of the ordinary amino acids associated with clinical cystinuria.

**EXPERIMENTAL SECTION**

*Synthesis of analogs. Precursor ketones for Strecker or Bucherer–Libe synthesis*

*p-Toluenesulfonate salt of N-dimethyl-4-piperidone.* 40 mmoles of *N*-methyl-4-piperidone (Aldrich) in 70 ml dry benzene was treated with 7.44 g of redistilled *p*-toluenesulfonic acid methyl ester, added dropwise. On standing overnight the solution became semi-rigid with almost colorless crystals. After filtering off 25 mmoles of product, the supernatant was refluxed 25 min. On cooling 6 mmoles more of light yellow product were collected.

*Toeryl derivatives of 4-piperidone and 4-aminocyclohexanone.* 4-Hydroxy-piperidine·HCl was converted to *N*-p-toluenesulfonfyl-4-piperidone, (m.p. = 125–126 °C) according to the procedure of Arndt and Kalischek\(^1\). Similarly 4-aminocyclohexanol, 100 mmoles, was dissolved in 35 ml 4 M NaOH and treated at 50 °C with 23.8 g (125 mmoles) *p*-toluenesulfonfyl chloride added in 8–10 portions, stirring until each portion dissolved before adding the next. Additional NaOH was introduced to keep the pH distinctly alkaline.

(The product could be obtained in two isomeric forms, one crystallizing from aqueous solution and purified by crystallization from chloroform, m.p. 166–167 °C, presumably the *trans* form. The other form could be extracted from the aqueous mother liquor with chloroform. This extract was taken to a small volume and the material remaining in solution crystallized by adding benzene. It melted at 113–114 °C. For our purposes, however, separation of the isomers was avoided.)

The product was extracted from the aqueous solution with chloroform, the chloroform solution taken to a small volume, and an excess of benzene added. The crystalline precipitate, 17.5 g, was oxidized in glacial acetic acid solution with chromic oxide\(^1\), to yield 4-*p*-toluenesulfamidocyclohexanone, yield 82% of theoretical, melting point of crystals from glacial acetic acid, 115 °C.

*Strecker synthesis. Typical procedures frequently used in this laboratory*

(1) To 10 mmoles of *N*-dimethyl-4-piperidone as the *p*-toluenesulfonate salt in a test tube were added a solution of 10 mmoles each of KCN and NH\(_4\)Cl in 3.3 ml water. The tube was sealed in a flame, and the ketone dissolved by shaking. The tube was then held at about 65 °C for 4 h. To the cooled contents of the tube was
added in a hood a large excess of concentrated HCl, and the resulting solution refluxed 5 h to hydrolyze the aminonitrile. The hydrolysate was taken to dryness, and the residue dissolved in water and poured onto a column of Amberlite IR-120 resin in the protonated form, using a 4-fold excess or more of the resin. After washing the resin bed with about 500 ml water, the amino acid was displaced by frontal elution with 0.5 M aqueous NH₃. Collection of eluate was begun when its pH rose sharply into the neutral range, and continued until only a weak color was obtained with alcoholic ninhydrin applied to test spots on paper. The eluate was then taken to dryness in vacuo, and the residue taken up in boiling ethanol. Successive crops of crystals were taken to a total yield of 40% of theoretical. The product on heating decomposed at 225–230 °C with an amine-like odor. It was somewhat hygroscopic and samples developed a yellow color in a few months on the shelf.

(2) 10 mmoles of 1-p-toluenesulfonyl-4-piperidone was dissolved in 13.3 ml methanol, and then a solution of 10 mmoles each of KCN and NH₄Cl together in 6.7 ml of water was added rapidly with mixing, and a glass stopper fastened firmly to close the flask. The resulting clear solution was held for 5 h at 50 °C. A few dense crystals appeared during the heating, and then half filled the solution on holding 48 h at 5 °C. The presumed aminonitrile, 2.33 g, m.p. 137–139 °C, was collected by filtration and washing with 2:1 aqueous methanol. The intermediate was hydrolyzed by refluxing in a large excess of constant-boiling HCl for 8 h beyond the time needed to obtain a clear solution. The amino acid was then separated in its isoionic form on an IR-120 resin column as described above, combining only the eluate portions that gave a copper-colored spot with ninhydrin, discarding any later portions of eluate that gave a light blue ninhydrin reaction, probably because of incomplete hydrolysis. After evaporating the ammoniacal eluate to dryness in vacuo, the residue was dissolved in a minimal quantity of hot 1:1 aqueous ethanol, and crystallization produced by adding an equal volume of ethanol. Two subsequent crops were taken at smaller volumes of the same solvent mixture, to give a total yield 42% of that theoretically possible from the quantity of the piperidone taken. Similar results were obtained, yield 54% of 4-aminopiperidine-4-carboxylic acid (PA), from Strecker synthesis in water solution from 1-acetyl-4-piperidone, which subsequently became commercially available. The product on heating sintered at 285–295 °C and decomposed gradually at above 305 °C.

(3) 10 mmoles each of N-benzylpiperidone, KCN and NH₄Cl were dissolved in 3 ml water plus 5 ml methanol. This solution was held 6 h at 60 °C in a sealed tube. The reaction mixture was hydrolyzed with HCl as in the first example, and the amino acid separated with an IR-120 resin column, using for elution aqueous NH₃ at only 0.1 M to compensate for rather low solubility of the product. After evaporating the ammoniacal solution, the residue was recrystallized from water in 25% yield. Elution of this rather insoluble amino acid from the resin column may well have been quite incomplete. On storage several months, yellowing was observed. On heating the amino acid decomposed at 225–230 °C.

When the Strecker synthesis was run on N-methyl, N-butyl or N-phenethyl-piperidone, these aminoketones were half-neutralized with HCl before adding the KCN, and the reaction run as usual. This neutralization was not considered necessary for N-benzylpiperidone because its low apparent pKₐ (7.2 in water–methanol 1:1, v/v) means that it has little effect on the pH of NH₄CN solutions. Water served as
the solvent for the Strecker reaction with \(N\)-methylpiperidone; for the \(N\)-butyl and \(N\)-phenethyl derivatives, a methanol–water (2:1, v/v) mixture was used. For the phenethyl derivative, the aminonitrile was crystallized, presumably as the dihydrochloride, on adding excess HCl after the addition reaction. After hydrolyzing this intermediate, the amino acid dihydrochloride was titrated in solution to pH 5 or 6 with the resin Amberlite IR-4B, the monohydrochloride crystallized, and then recrystallized as fluffy needles from ten portions of water, yield 40\% of that theoretically possible from the ketone. On heating sintering led to loss of crystal structure without clear melting at 255–260 °C. The yield in the case of the 4-amino-1-methylpiperidine-4-carboxylic acid was 43\% of the theoretical after two crystallizations from absolute alcohol. This compound melted at 243–245 °C without obvious decomposition. Sintering was observed to begin at 200 °C during the test. Gratifyingly, this the simplest to prepare of the three piperidine amino acids (the 1-dimethyl, the 1-monomethyl, and the unmethylated) proved also the most interesting for transport study.

(4) 10 mmoles each 4-p-toluenesulfonamidocyclohexanone, NH\(_4\)Cl and KCN together were shaken up with 5 ml water to dissolve the principal portion of the inorganic salts. Rapid addition of 10 ml of methanol with shaking caused essentially complete dissolution of the remaining solids before crystallization began, the temperature rising to perhaps 40 °C. The solution largely filled with crystals while it was held, firmly stoppered, at 40 °C in a hood for 24 h. Further crystallization was encouraged by holding at 5 °C overnight. The mass of the presumed aminonitrile, m.p. 132–137 °C, suggested a nearly quantitative yield. It was dissolved in hot glacial acetic acid in a test tube, 5 ml for each g. An equal volume of 12 M HCl was then added, the tube sealed in a flame, and held overnight at 124 °C in a steam autoclave. After evaporating the solvent, the product was separated as usual by frontal elution with aqueous NH\(_3\) from IR-120 resin, and crystallized from a near-minimal quantity of water by adding two volumes of ethanol.

(5) Strecker synthesis of \(\alpha\)-(methylamino)isobutyric acid. This amino acid has proved so useful as a model substrate for System A that we should record that its Strecker synthesis is carried out in the usual way\(^{14}\) at 94 °C during 10 h in a solution containing 3 mmoles of each reactant (acetone, KCN and methylamine hydrochloride) per ml of water. The amino acid is crystallized from absolute alcohol, the solubility of residual methylamine hydrochloride in alcohol being sufficient to facilitate its elimination, with a yield of 18\%. A reaction temperature of 100 °C for 20 h led to less satisfactory crystallization. The amino acid sintered and sublimed at 226–230 °C under the conditions of the melting point determination.

\textit{Bucherer–Libe reaction with 4-p-toluenesulfonamidocyclohexanone}

2.5 g (NH\(_4\))\(_2\)CO\(_3\) was dissolved in 10 ml water. 10 mmoles of the powdered ketone was added, and rubbed up with a stirring rod. 10 ml of ethanol were then added with mixing and rubbing up of the solid phase. Finally 0.80 g of finely crystalline KCN was added all at once, followed by vigorous shaking. This procedure permitted essentially quantitative dissolution of the reactants before crystallization of the product began. After three days at room temperature the preparation was chilled overnight, and the crystallized hydantoin washed with 2:1 aqueous alcohol. The yield was 90\% of that theoretically possible.

The hydantoin was hydrolyzed in glacial acetic acid plus HCl by the procedure
just described for hydrolyzing the aminonitrile, and the corresponding diamino acid separated in 50% yield. Higher yields appeared distinctly possible. Hydrolysis was also obtained by heating in 60% H₂SO₄ terminating at 140 °C when gas evolution was complete.

Separation of isomers of 1,4-diaminocyclohexane-1-carboxylic acid

The crystalline product by the Strecker route typically contained 95–99% of a form a migrating more rapidly on eluting with pH 5.28 citrate from the amino acid analyzer (Table II), and a few percent of another form emerging some 44 ml later. When longer hydrolysis was used, 60% of isomer a and 40% of isomer b was obtained, with a lower overall yield. The Bucherer route, followed by HCl hydrolysis, led to an approximate inversion of these proportions, typically about 80% of the product being in the more slowly migrating form. The isomers could be purified by repeated crystallization from aqueous alcohol, b being only about one-third as soluble in water as a.

The unexpectedly low water solubility of the copper salt of isomer b permitted a simpler separation. A 0.2-M solution of the amino acid was boiled up with a small excess of basic cupric carbonate until the volume was decreased to one-fifth. The precipitate, collected after chilling overnight, was washed repeatedly by rubbing up thoroughly with water on a sintered-glass filter. Washings after the first two or three were discarded. The soluble and the insoluble copper salts were acidified with HCl and the resulting solutions freed of Cu²⁺ with H₂S and taken to near dryness. Addition of absolute alcohol led to crystallization of the amino acid dihydrochloride of isomer b, the purity of which was confirmed with the amino acid analyzer. Isomer a decomposed on heating at 263–268 °C, whereas isomer b decomposed at about 290 °C, after sintering between 275–285 °C.

The formation of an unusually insoluble copper salt by isomer b led us to suspect that it was the cis diamino acid, both amino groups of which can undoubtedly coordinate with Cu²⁺. We have confirmed that supposition by showing that only the a isomer, in the form of its methyl ester hydrochloride, cyclizes spontaneously to the bicyclic lactam, 4-amino-2-azobicyclo[2.2.2]-octan-3-one, on treatment with an excess of freshly precipitated hydrous silver oxide.

\[
\begin{align*}
\text{H} & \hspace{1cm} \text{C} & \hspace{1cm} \text{O} \\
\text{N} & \hspace{1cm} \text{H} & \hspace{1cm} \text{O} \\
\end{align*}
\]

This behavior, corresponding to formation of the cyclic lactam by ornithine, cannot occur when the 4-amino and the 1-carboxyl group are trans to each other. Hence the two amino groups must be cis to each other in the b isomer, which shows far stronger transport than isomer a by System L of the Ehrlich cell. The bicyclic lactam is, as expected, however, without obvious effect on amino acid transport. A close precedent for the formation of the lactam bridge in this case was presented in 1916 for p-aminohexahydrobenzoic acid, which on dry distillation forms 2-azobicyclo[2.2.2]-octan-3-one. The present product reverted to the amino acid on hydrolysis in boiling HCl. It showed a single pKᵃ = 7.5 on acid–base titration. The infrared spectrum was fully consistent with the assigned structure.
Conversion of basic amino acids to guanidinoamino acids

1-Guanyl-4-amino-piperidine-4-carboxylic acid (GPA). 20 mmoles of 4-amino-piperidine-4-carboxylic acid (PA) in 100 ml water was boiled up for 10 min with 1.2 g basic cupric carbonate, and the suspension filtered hot, with liberal washing of the insoluble residue. A solution of 20 mmoles of O-methylisourea hydrogen sulfate was treated under cooling with 10 ml 2.5 M NaOH. The two cooled solutions were then mixed. An additional 6 ml of 2.5 M NaOH served to bring the pH to 10.5. After several h the volume was reduced to 20 ml. After five days at room temperature, 30 ml of 2 M HCl were added, Cu$^{2+}$ was removed with H$_2$S and the CuS washed thoroughly with hot water. After evaporating the filtrate to 10 ml, a major crop of the slightly soluble sulfate was collected after chilling overnight, to give 60–68% of the theoretically possible yield. This amino acid decomposed on heating at 279–283 °C.

Even when the preparation of the precursor amino acid used had contained about 5% of an unidentified contaminant, the sulfate of the guanyl derivative was chromatographically pure, and showed an equivalent weight of 281 (calculated for C$_7$H$_{18}$N$_4$O$_4$S, 284) on titration with 0.25 M Ba(OH)$_2$ solution. No Ba$^{2+}$ remained in the solution when the titration was terminated at pH 10. After removing BaSO$_4$, the filtrate was concentrated until the crystals forming could barely be redissolved at the boiling temperature. The crystals were collected after chilling overnight. The yield from this step was essentially quantitative.

The same procedure was used for guanylation of the $\alpha$ isomer of 1,4-diaminocyclohexane carboxylic acid. The product crystallized as the isoionic amino acid in 60% yield, after removing anions by titration to pH 10 with Dowex 1-hydroxide resin. Recrystallization from water was needed to eliminate a trace of the precursor diamino acid. The crystals decomposed gradually on heating to 288–298 °C.

1-Guanyl-4-piperidineglycine (GPG). Guanylation was carried out on the copper salt of 5-piperidineglycine in the same way except that 0.2 M Ba(OH)$_2$ was substituted for NaOH. After a few h the suspension was taken to about one-third of its volume by vacuum distillation at less than 30 °C. After standing five days, the BaSO$_4$ was filtered from the solution, and one equivalent of HCl added per mole of amino acid used. Copper was removed with H$_2$S, and the filtrate taken to a thick sirup in vacuum. After taking up the residue in water, it was titrated batchwise from pH 1.5 to 4.5 with Amberlite IR-4B resin in the free amine form. The filtrate plus abundant washings from this resin treatment was again taken to a sirup, and crystallization initiated by scratching the interior of the flask with a glass rod. A crystalline form presumed to be the monohydrochloride separated as needles, and was recrystallized from water to give a 55% yield. Titration of a solution of this salt to pH 11 caused crystallization of the isoionic form of the amino acid. On heating the amino acid decomposed at about 305–310 °C.

4-Amino-1-carbamoylpiperidine-4-carboxylic acid (CPA). In a 0.2–0.25 M solution of 1–20 mmoles of PA as its copper salt, was dissolved in an open beaker 1.1 molar equivalents of potassium cyanate$^{18}$. By passing 5% CO$_2$ through the solution, either very slowly or else intermittently during three days the pH was kept in the approximate range 9–10. Two treatments with CO$_2$ per day sufficed. A copper salt began to crystallize after about two days in early runs, but in some later runs crystallization began within an hour. After four or five days or more, the gleaming blue crystals were filtered off and washed with water. The product was dissolved to roughly 0.05 M in
water containing at least an equivalent of HCl. CuS was precipitated as usual at room temperature and washed well with hot water. After removal of H₂S the solution was concentrated in vacuo to about 0.5 M. Crystals formed during the evaporation. After chilling the suspension overnight, the well-washed crystals represented a 50–60% yield. On heating, the amino acid decomposed at 278–281 °C. The above preparation of CPA was quite as convenient when we used 1 mmole of the [¹⁴C]-carboxyl precursor amino acid.

**Radioactive labeling of amino acids**

Radioactive preparations of several of the other substances were made using K¹⁴CN in either the Strecker or the Bucherer–Libe reaction, without significant change except for the obvious scaling downward of quantities. In the cases of 4-amino-1-methylpiperidinecarboxylic acid (MPA), and 4-piperidinoglycine (PG), the final products were purified by chromatography on Whatman No. 3 paper, extracting only the band of paper showing the characteristic maximum of radioactivity on strip counting. A 100-ml sample of PG was tritiated for us by the Wilzbach procedure by the New England Nuclear Corporation. The product was guanylated as above and crystallized in isoionic form.

**Characterization.** Melting behavior as described above was examined with the use of the Fischer–Johns melting point block. Table I records analytical values for the products. The amino acids were titrated at 25 °C in aqueous solution, 0.05–0.1 M, NaCl added to I=0.15. The following values were observed for pK'₂: PA, 7.5; its derivatives: 1-methyl (MPA), 7.2; 1-dimethyl (M₂PA), 7.0; 1-butyl, 7.2; 1-benzyl, 6.7; 1-phenethyl, 7.1; and 1-guanyl (GPA), 8.0; also cis-1,4-diaminocyclohexane-1-carboxylic acid, 8.4. pK'₃ values were approximately as follows, MPA, 9.7; the corresponding 1-benzyl amino acid, 9.3; the 1-phenethyl amino acid, 9.4. The change in the ultraviolet spectrum of the benzylpiperidine amino acid on acid–base titration

<table>
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<tr>
<th>TABLE I</th>
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<tr>
<td><strong>ANALYTICAL VALUES FOR PREPARATIONS</strong></td>
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<tr>
<td>Calculated</td>
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<tr>
<td>4-Aminopiperidine-4-carboxylic acid, C₇H₁₂O₂N₂ (PA)</td>
</tr>
<tr>
<td>4-Amino-1-methylpiperidine-4-carboxylic acid, C₇H₁₄O₂N₂ (MPA)</td>
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<tr>
<td>4-Amino-1-guanylpiperidine-4-carboxylic acid, C₇H₁₄O₂N₄ (GPA)</td>
</tr>
<tr>
<td>4-Amino-1-carbamoylpireridine-4-carboxylic acid, C₇H₁₅N₃O₃ (CPA)</td>
</tr>
<tr>
<td>1-Guanylpiperidinoglycine, C₇H₁₆O₂N₄ (GPG)</td>
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<tr>
<td>1,4-trans-Diaminocyclohexane-4-carboxylic acid monohydrate, C₇H₁₅N₂O₃</td>
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<tr>
<td>Inner lactam of same, C₇H₁₅O₃N₂</td>
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is recorded elsewhere. Table II records the volumes at which the amino acids emerge from the ion-exchange resin columns of the amino acid analyzer (Phoenix). The same table also records the chromogenicity of the amino acids with ninhydrin for this analyzer, and the retardation factors of the piperidine amino acids on paper chromatography.

**TABLE II**

**VOLUME OF ELUTION AND CHROMOGENICITY OF AMINO ACIDS ON THE AMINO ACID ANALYZER**

$R_F$ values on paper chromatograms. Columns 2A (10 cm) and 2 (20 cm) were filled with resins designated XX8-10-I and XX8-20-I, and eluted with 0.35 M sodium citrate buffer, pH 5.28. Column 1 (60 cm) was filled with the resin designated XX8-60-I, and was eluted with 0.2 M sodium citrate buffer at pH 3.25 for the first 93 ml, and then at pH 4.25, all at 50 °C and at a flow rate of 80 ml/h. The column used, when not 2A, is indicated in parentheses in the central column of the table. The color constants were calculated as the height times the width of the peak for 570 nm wavelength, per micromole of amino acid, the color constant for lysine being 17.7. Paper chromatograms were developed with a solvent composed of 40 vol. ethanol, 2.5 vol. concentrated aqueous ammonia and 7.5 vol. water.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Migration on chromatography</th>
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<tbody>
<tr>
<td></td>
<td>Ion-exchange column</td>
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<td></td>
<td>Column 2A</td>
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<tr>
<td>4-Aminopiperidine-4-carboxylic acid (PA)</td>
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<td>1-Methyl derivative (MPA)</td>
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<tr>
<td>1-Dimethyl derivative (M2PA)</td>
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<td>1-Carbamyl derivative (CPA)</td>
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</tbody>
</table>

* At 30 °C, elution was at 93 ml.
** The absorbance at 570 nm was 6.7 times that at 440.

**Test of behavior of the synthetic amino acids and homoarginine in the intact rat**

L-Homoarginine hydrochloride, containing 0.05% lysine, and (L-cysteine)-α,ε-diaminopimelic acid were obtained from Calbiochem, and 2-amino-3-ureido-propionic acid from Cyclo Chemical Company. Otherwise we used the synthetic amino acids described above.

Labeled amino acids were administered at doses of 1–16 μmoles (1–10 μCi) to fasted 50-g male rats. The animal was subsequently kept in an all-glass apparatus under an air stream. The air drawn through the container was passed through two successive towers containing glass beads covered with a methanolic Hyamine hydroxide solution for collection of expired CO₂.

Male albino rats of 100–300 g were kept without food for 16–18 h and about 10 ml of an isoosmolar or a slightly hyperosmolar solution of the synthetic amino
acids then injected intraperitoneally at doses of 5–10 mmoles/kg body weight. Urine collections were arranged as described previously. Control animals received an equal volume of 0.15 M NaCl solution by the same route.

Endogenous amino acids present in the urine (and in some cases in the plasma) were measured by ion-exchange chromatography using a Phoenix amino acid analyzer. Details appear in the legends to the tables. Radioactive tracers were measured by liquid–scintillation counting in ethanol–toluene, using a standard phosphor and a Packard scintillation spectrometer.

*Tests for increases in circulating insulin and glucagon in rats receiving the cationic amino acids*

8 mmoles of the amino acid (10 mmoles of GPG) per kg body weight were administered as just described. The rats weighed 50–140 g for the insulin studies, 100–200 g for the glucagon studies. Control animals again received 0.15 M NaCl solutions. 27 min later, blood samples were taken from the superior vena cava with the animal under ether anesthesia. Serum samples were then separated and subjected to a double-antibody radio-immunoassay as applied elsewhere for one or the other hormone. Because the immunizing hormones were not homologous, the levels observed in the rat are considered relative rather than absolute. These assays were made for us through the kindness of Dr Stefan Fajans and Dr Sumer Pek of the Endocrinology and Metabolism Unit, Department of Medicine. The results with rats stimulated us to synthesize GPA in the quantities required for studies in the dog, for the collaborative test to be described shortly.

**RESULTS**

*Apparent metabolic release of $^{14}$CO$_2$ from the carboxyl-labeled amino acids*

After administering 1–16 µmoles (1–10 µCi) intraperitoneally to 50-g rats, the expired $^{14}$CO$_2$ collected in Hyamine hydroxide during 24 h was 0.07–0.12% of the administered dose for PA, MPA, GPA and CPA. A slightly higher figure of 0.35% observed for M$_2$PA seems likely from the moderately greater difficulties of purification to reflect a slightly lower purity of the preparation, rather than a higher vulnerability to metabolic attack, whereas the release of 2.4% from PG is likely to represent significant if slow metabolic attack. The presence of an α-H in it is of course a source of vulnerability. Note that attack by intestinal microorganisms, as well as imperfect purity, is a plausible source of the very slowly released $^{14}$CO$_2$ from the preparations bearing the amino and carboxyl group on the piperidine ring. Accordingly we question whether any attack of most of these compounds occurs in the organism of the rat, *per se*.

No radioactivity was found on paper chromatograms of the urine samples collected during these tests except at the $R_f$ for the administered amino acid. An anthracene scintillation flow-cell showed that 1.1% of the urinary $^{14}$C administered to the rat as [1-$^{14}$C]GPA emerged from the column of the amino acid analyzer just before glutamic acid, rather than at the position characteristic of this analog.

*Renal excretion of $^{14}$C-labeled amino acids administered parenterally to the rat*

Fig. 2 compares the rates of the early urinary losses of the model amino acids tested at low dosages in this study, before the subsequent phase of an exponential
rate of loss was established. From 43–63% of the test dose was lost during the first 6 h. During this time the amino acid largely passes out of the peritoneal space, a competition being set up between uptake by various tissues and loss into the urine. Because several rate constants must contribute, the shapes of the curves during this interval are not easily analyzed. We may note, as a gross effect, that adding the 1-methyl group to the piperidine amino acid about halves the rate of loss during the first 2 h; the 1-guanyl group is somewhat less effective in this respect.

We may note for contrast with the new amino acids that during the same 6-h period the urinary losses of α,α-dicyclopropylglycine and α,α-diethylglycine, two amino acids designed for minimal transport, were 90% or more. Nevertheless some accumulation of the latter by liver and some uptake of it by brain slices have been observed. An opposite contrast is presented by the minimal loss of a much more effective renal transport model, α-(methylamino)-isobutyric acid, as shown by dashed curve at the bottom of Fig. 2.

A somewhat clearer comparison of renal behavior with respect to these model amino acids is provided by an exponential phase established after about 24 h (illustrated for GPA and PA in Fig. 3) and continuing as long as excretion remained measurable. This phase concerns a retained 20–50% of the administered amino acid dose. The linearity seen in Fig. 3 would be expected if a constant proportion of the amino acid retained in the body is present in the plasma and if a constant proportion of that contained in the filtered plasma is not resorbed. The half-times thus indicated for renal loss are as follows: GPA, 46 h; M2PA, 31 h; PG, 19 h; CPA, 17 h; PA, 10 h; MPA, 7 h. If we recall that l-aminocyclopentane-carboxylic acid shows a biological half-life of 22 days for renal excretion by the mouse (3.5 days in man) we may conclude that acceptance of these piperidine amino acids by the renal transport mechanisms is

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**Fig. 2.** Cumulative urinary excretion of 14C-labeled amino acid analogs during the first day after injection. Dosages were 1 μmole in 0.5 ml 0.15 M NaCl into a 45–50-g rat, except that 11 μmoles of CPA and 16 μmoles of GPA were injected as indicated in parentheses. (The results are probably not dose-dependent at these low dosages.) The curves marked DCG (α,α-dicyclopropylglycine), DEG (α,α-diethylglycine) and MeAIB (α-(methylamino)-isobutyric acid) are taken from ref. 19.

**Fig. 3.** Decline in rate of urinary excretion of 4-amino-1-guanyl-piperidine-4-[14C]carboxylic acid (upper two lines) and 4-aminopiperidine-4-[14C]carboxylic acid (lower line) during several days after intraperitoneal injection into the rat. 40–50-g animals received 3–16 μmoles (1–5 μCi) of the test amino acid in 0.5 ml 0.15 M NaCl. The slopes of the principal linear plots correspond, in the order shown, to half-times of retention in the animal of 39, 44 and 9.4 h.
only fair, even though most of the filtered amino acid must be resorbed. The lower resorption rates did not prevent our establishing sufficient concentrations of them to largely saturate the relevant transport agencies, as described in the next section.

**Effects of test compounds on renal excretion of endogenous amino acids in the rat**

Table III summarizes the principal effects of the model amino acids under study, after intraperitoneal injection of large doses into the rat. The numbers in the body of the table indicate by what factor the urinary excretion of the amino acid named at the head of the column was typically increased. For example, the injection of homoarginine, 5 mmoles/kg of body weight, led to an increase of citrulline excretion by 104-fold, of cystine excretion by 44-fold, of lysine excretion by 168-fold, and so on. No significant change was seen in the excretion of neutral amino acids except as mentioned below. Although lysine is a likely intermediate in the slow metabolism of homoarginine, homoarginine administration led to no perceptible increase in the plasma lysine. Homoarginine is known to be much more resistant to arginase action and other metabolic attack than arginine.22,23

Although the guanylpiperidine amino acid shows somewhat lower transport affinities than arginine or homoarginine in all systems tested, and indeed very little in *Escherichia coli* and *Salmonella typhimurium*, the injection of doses of 5 mmoles/kg body weight always strongly intensified the urinary excretion of the same five endogenous amino acids. The abbreviation "obsd" in Table III indicates that the

**TABLE III**

**EFFECTS OF THE INTRAPERITONEAL INJECTION OF AMINO ACID ANALOGS INTO THE RAT ON THE URINARY EXCRETION OF FIVE NATURAL AMINO ACIDS**

<table>
<thead>
<tr>
<th>Administered</th>
<th>Citrulline</th>
<th>Cystine</th>
<th>Lysine</th>
<th>Ornithine</th>
<th>Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine analogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homoarginine</td>
<td>104</td>
<td>44</td>
<td>168</td>
<td>36</td>
<td>43</td>
</tr>
<tr>
<td>GPA</td>
<td>12</td>
<td>23</td>
<td>217</td>
<td>43</td>
<td>25</td>
</tr>
<tr>
<td>GPG*</td>
<td>17</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>obsd</td>
</tr>
<tr>
<td>Citrulline analog</td>
<td>obsd</td>
<td>13</td>
<td>114</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>CPA</td>
<td>obsd</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supposed lysine analogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PG (8 mmoles/kg)</td>
<td>29</td>
<td>0</td>
<td>obsd</td>
<td>obsd</td>
<td>0</td>
</tr>
<tr>
<td>(Lysine)</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Aminoisobutyric acid**</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Note dose of L form only 2.5 mmoles/kg. Tests at 8 mmoles showed citrulline still most affected. Increases in the other basic amino acids and cystine were associated with rises in the neutral amino acids.

** Doubled doses gave large increases in cystine, lysine and excretion of many neutral amino acids, but not of citrulline.
chromatographic peak for the indicated amino acid was completely obscured by the test amino acid. For example, the presence of the citrulline analog unfortunately obscured the analytical result for citrulline itself, although its administration stimulated the excretion of all four of the other endogenous amino acids tabulated.

GPG at the test dose of 5 mmoles/kg (undoubtedly including only 2.5 mmoles of the L isomer) had an effect largely limited to citrulline excretion; but at a dose of 8 mmoles/kg the losses of all of the same five amino acids were affected. The supposed lysine analogs, PA and PG, had effects largely limited to citrulline excretion, although smaller effects were seen on the losses of some other neutral amino acids, in particular on the sum of asparagine and glutamine (not shown in Table III). For example 5 mmoles/kg of PA increased this sum to 3.4-fold, and 8 mmoles increased it to 9.4-fold, in each case more than for any other neutral amino acid. The same dose of MPA doubled glutamine plus asparagine excretion. The effects on renal amino acid excretion are discussed in a later section.

**Stimulation of increases in the circulating levels of immuno-reactive insulin and glucagon**

Table IV summarizes our observations of the changes on the apparent levels of the circulating hormones in the rat 27 min after administration of the amino acids. This time interval had been selected on the basis of only a few tests of shorter and longer times. If the effects in the rat had been as transient as those seen subsequently in the dog at a dose of about 1 m mole of GPA per kg body weight, no significant effects would have been seen at the time interval selected for the rat.

A general screening of GPA for significant pharmacological effects, other than that reported here, has been carried out with largely negative results under the direction of Dr Robert A. Maxwell of the Burroughs-Wellcome Research Laboratories, Research Triangle Park, N. C. These results support the conclusion that the actions are direct ones on the endocrine tissue, and not a result of other disturbances.

**TABLE IV**

**APPARENT LEVELS OF CIRCULATING IMMUNORESPONSIVE INSULIN AND GLUCAGON IN THE RAT 27 min AFTER ADMINISTRATION OF MODEL AMINO ACIDS**

Preliminary reports of these findings were made earlier. The control population received an equal volume of NaCl, the observations being interspersed among the experiments.

<table>
<thead>
<tr>
<th>Administered</th>
<th>Insulin (μunits/ml ± S.D.)</th>
<th>Glucagon (ng/ml ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15 M NaCl</td>
<td>4.5 ± 1.7 (19)</td>
<td>0.39 ± 0.17 (9)</td>
</tr>
<tr>
<td>GPG</td>
<td>10.7 ± 2.1 (7)</td>
<td>1.05 ± 0.15 (6)</td>
</tr>
<tr>
<td>GPA</td>
<td>9.4 ± 2.7 (13)*</td>
<td>0.90 ± 0.32 (8)</td>
</tr>
<tr>
<td>Bis-α,β-hydroxymethylglycine**⁴</td>
<td>16.0 ± 0.7 (4)</td>
<td>0.51 ± 0.06 (2)</td>
</tr>
<tr>
<td>Homoaarginine</td>
<td>17.8 ± 4.6 (3)**</td>
<td>1.17 ± 0.70 (3)</td>
</tr>
<tr>
<td>Arginine</td>
<td>14.5 ± 3.4 (6)</td>
<td>0.84 ± 0.28 (3)</td>
</tr>
<tr>
<td>α-N-Methylarginine</td>
<td>4.6 ± 0.46 (2)</td>
<td>0.26 ± 0.03 (2)</td>
</tr>
<tr>
<td>CPA</td>
<td></td>
<td>0.42 ± 0.20 (2)</td>
</tr>
</tbody>
</table>

* Effect confirmed by similar increases in 8 rats, 100–200 g.
** Effect confirmed by slightly smaller increases in 7 rats of 100–200 g.
The same conclusion is supported by the subsequent finding that GPA stimulates the release of insulin from pancreatic islets isolated from an obese, diabetic strain of mice.  

**Discussion of the effects of the administered amino acid on urinary excretion of other amino acids**

According to the results of Table III, citrulline now joins cystine as a neutral amino acid associated with the renal tubular resorption of the cationic amino acids. The possibility that citrulline excretion occurs in cystinuria received consideration some years ago. Traces of this amino acid were observed by Dent and Rose in the urine of human cystinuric patients, but they attributed this finding to bacterial attack on arginine after collection of the urine. Treacher found citrulline in the urine of cystinuric dogs, but because he failed to observe citrulline in the plasma, he proposed a renal origin. Others have, however, not encountered difficulty in measuring plasma citrulline in the dog. Asatoor et al. found citrulline in the uncontaminated urine of cystinuric patients who showed no evidence of kidney infection, especially after arginine administration. They concluded, however, that the citrulline was formed from unabsorbed arginine by bacteria in the alimentary tract, and that the citrullinuria was of an "overflow" type. Accordingly, we find no well supported prior evidence that the renal resorption of citrulline may be subnormal in association with the otherwise well-known aminoaciduria of the cystinuric patient. The present observations appear to be the first of mutual inhibition of renal resorption between citrulline and the amino acids principally lost in cystinuria.

Administration of the arginine analogs raised the excretory rate for the endogenous basic amino acids, for cystine and for citrulline. Conversely, administration of the non-metabolizable citrulline analog stimulated the excretion of the basic amino acids and cystine, citrulline itself being obscured from analysis in this case. Furthermore lysine administration also stimulated a citrullinuria. The supposed lysine analogs PA and PG had effects largely limited to citrulline excretion, the basic amino acids being little affected.

Experiments in the Ehrlich cell have indicated that the membrane receptor sites tend to "see" these lysine analogs with the piperidine N unprotonated, even though the protonated species is abundant in free solution. In the Ehrlich cell their effects on cationic amino acid transport are weak, unless the piperidine N is rendered unambiguously cationic by dimethylation or by guanylation, much as observed here for renal transport. The circumstances that lysine clearance and lysine excretion are increased in Hartnup's syndrome suggests either that a substantial proportion of the renal resorption of lysine also occurs by a neutral amino acid system, or that one or more of the neutral amino acids accumulating in the tubular urine in this disorder interferes substantially with lysine resorption.

These results show that the resorption of citrulline can be inhibited independently and hence has a point of vulnerability not shared with basic amino acids or with cystine. Furthermore, at low doses GPG increased principally citrulline excretion. These results are not compatible with a migration by the same transport system as citrulline and the basic amino acids, even though at sufficient levels an interaction for transport does occur between citrulline and its analogs, on the one hand, and arginine and its analogs, on the other.
Furthermore cystine resorption has a point of vulnerability not shared with citrulline. Although α-aminoisobutyric acid at a dose of 5 mmoles/kg body weight does not provoke increased loss of the endogenous amino acids under discussion, at 10 mmoles/kg a general aminoaciduria is provoked including cystine and the cationic amino acids, but not citrulline, a point we checked repeatedly.

![Diagram](image)

**Fig. 4.** Interpretation of the interaction among the cationic amino acids, citrulline and cystine for renal tubular resorption. Dashed arrows indicate inhibition without transport. It has not been proved that the transport site marked No. 2 is different from the one marked No. 3, although Nos 1 and 3 must be different.

Fig. 4 offers a model to present these relations. It becomes necessary to conclude that citrulline and the basic amino acids plus cystine are not resorbed by the same transport system. Hence we must picture a mutual inhibitory interaction between citrulline and the cationic amino acids not based on a shared route. That is, the site that recognizes and transports citrulline cannot be the same one that recognizes and transports the cationic amino acids. The dotted lines of Fig. 4 therefore indicate inhibition of a transport system without significant accompanying transport, an action that occurs here in both directions. Substantial precedent for inhibition of transport without shared transport has been obtained between amino acids of like charge as well as with amino acids of unlike charge. Such effects may well tend to be maximized in the renal tubular system, because of the tendency of a poorly resorbed solute to reach very high levels in the tubular urine.

The temptation arises at this point to lump cystine and probably glutamine together with citrulline into a single transport system requiring a nitrogen-bearing sidechain without net charge. But the transports of citrulline and cystine can be independently inhibited, so evidently they also cannot be transported by the same agency. By analogy with the case for citrulline, the most conservative position remaining is to propose that cystine migrates by a third transport system which can be inhibited by either citrulline analogs or arginine analogs. We have not, however, so far been able to inhibit cystine resorption independently of cationic amino acid resorption. Therefore we cannot yet refute unequivocally Dent's placement of cystine in the same family with the basic amino acids for tubular resorption, based on the pattern of amino acids lost together in cystinuria, although for the present Dent's hypothesis becomes less likely. Thier and Segel have reviewed other evidence not obviously consistent with the Dent hypothesis.

In Fig. 5 we see for comparison structures for two of the basic amino acids, also for cystine and for citrulline. The trouble with placing cystine in the cationic
amino acid transport family is what to do with the charged distal carboxylate group, a difficulty often rendered unobvious by the poor practice of drawing the structural formulas of amino acid in their non-zwitterionic forms. We can detect no transport system in isolated animal cells that will accept such a two-headed amino acid. The environment into which this carboxyl group enters would need to be very apolar indeed to permit it to be stabilized in the protonated form, unless a chemical group at the receptor site participates in stabilizing it in that form. The analogous effects of the citrulline analog on basic amino acid transport might permit us to picture H⁺ as being transferred from the distal amino to the distal carboxylate group on combination of cystine with the receptor site. The analogy of the cystine structure with that of the basic amino acids would then be substantially weakened, and cystine would be seen rather plausibly instead as an analog of citrulline and glutamine. Apparently any such tendency is not so strong, however, as to permit cystine and citrulline to share mainly the same transport process.

It is even less likely that the ureido structure of citrulline could come to be protonated in combining with the receptor site to make it an analog of lysine and arginine. The more plausible explanation is that citrulline and glutamine, and perhaps also asparagine, constitute a newly identified family, transported by a heretofore undescribed system, and that their interaction with cationic amino acids for renal tubular transport, as illustrated in Table IV, is based on a fit with the receptor site sufficient to produce inhibition of transport although the complexed structure is not such as to permit transport to follow binding. The same relation is suggested for cystine with, however, insufficiently clear support for the present for the separateness of the transport of this amino acid.

We need amino acid structures closely analogous to cystine and glutamine to complete the discrimination of these systems. These compounds should lack the notable biological instability of the two natural amino acids, especially the susceptibility of cystine to reductive cleavage. For example, in *E. coli* α,ε-diaminopimelic acid is transported by a system serving for cystine34. It is significant that diaminopimelic acid administration provoked increased lysine excretion in the rat (Table V), although the circumstance that arginine and ornithine excretion did not rise leaves
LYSINURIA IN THE RAT AFTER ADMINISTRATION OF (L+meso)-x,e-DIAMINO-PIMELIC ACID

(10 mmoles/kg body weight). Illustrative results. The numbers show the factor by which the rate of urinary excretion of histidine and lysine was increased. Urinary arginine and ornithine were not increased; other urinary amino acids obscured. Plasma lysine was not increased.

<table>
<thead>
<tr>
<th>Time after administration (h)</th>
<th>Number of times by which urinary excretion was increased</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Histidine</td>
</tr>
<tr>
<td>2</td>
<td>5.9</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>5.1</td>
</tr>
</tbody>
</table>

equivocal the interpretation of that result. Since plasma lysine was not increased, the lysinuria was not prerenal. Our efforts so far indicate some difficulty in designing stable structural analogs of cystine that show cystine-like behavior in the renal tubule.

ACKNOWLEDGEMENTS

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