# Renal Tubular Transport of Proline, Hydroxyproline, and Glycine

## III. GENETIC BASIS FOR MORE THAN ONE MODE OF TRANSPORT IN HUMAN KIDNEY

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ABSTRACT Impaired renal tubular transport of proline, hydroxyproline, and glycine was inherited as an autosomal recessive trait in two Ashkenazi-Jewish pedigrees and one French-Canadian family; the heterozygotes for the trait exhibited hyperglycinuria only. Intestinal transport of imino acids and glycine was not impaired in homozygotes. It is possible that more than one mutant allele may occur at a locus controlling tubular transport of the imino acids and glycine, since one subject with the imino–glycinuric phenotype had one parent who was not hyperglycinuric.

More than 60% of the specific tubular transport function is still available in homozygotes for absorption of imino acids and glycine at endogenous substrate concentrations; however, this persistent transport is already saturated at these concentrations in contrast to the large capacity available in normal subjects. Furthermore, the glycine portion of this persistent transport is noninhibitable by imino acids in contrast to the normal situation. The imino acids can inhibit each other's uptake in mutant and normal phenotypes. Two modes of transport for the imino acids and glycine are proposed to explain these observations: (*1*) a common system with high capacity, and (*2*) two additional systems, each with low capacity (one-tenth or less of the common system). One of these systems is apparently shared by proline and hydroxyproline. The mutant allele(s) observed in this investigation occur at the locus for the common system.

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The in vivo infusion procedures performed in this investigation were approved by a properly constituted review committee. Informed consent was also obtained from the three subjects. All procedures were performed on the author first before other subjects participated.

*Received for publication 27 October 1967 and in revised form 4 December 1967.*

## INTRODUCTION

The mammalian kidney absorbs the imino acids, L-proline and 4-hydroxy-L-proline, and the amino acid glycine from the glomerular filtrate by at least one tubular transport agency, which is common to these particular solutes (1–4). The specificity of the transport system is made apparent only when the concentrations in plasma are raised above their normal endogenous levels; the system is saturable and either imino acid will competitively inhibit the uptake of the other two substrates. Any transport agency with this capacity for discrimination presumably has a structural specificity to account for its function. This specificity will be genetic in origin and, hence, mutations affecting it may be anticipated.

This report describes three unrelated pedigrees in which the homozygous form of a recessively inherited phenotype shows impaired tubular absorption of the imino acids and glycine; the heterozygous phenotype shows hyperglycinuria alone.

It was noted that the homozygote nonetheless retains considerable absorptive activity towards the imino acids and glycine under the usual endogenous conditions; the most likely explanation for this paradox is that more than one type of transport exists for imino acids and glycine in human kidney, only one of which is affected by the mutation (5). In the interval, after preliminary publication of the results found in one pedigree (5, 6), two additional pedigrees have been discovered. New considerations of the genetic basis of this transport mutation, additional studies of the kinetics of transport in normal and mutant subjects, and information on the organization of membrane transport systems, whereby more than one mechanism is apparently available to a single substrate, are presented in this communication.

## METHODS

### Subjects

PEDIGREE A

The propositus (A,II,2) of this pedigree (Fig. 1A) is a healthy Ashkenazi Jewish white male, whose mother (A,I,2) died with cirrhosis and malignant hepatoma (7). Investigations of the family for a possible metabolic disorder led to the discovery of imino-glycinuria in the propositus. All living individuals studied in this pedigree are healthy.

PEDIGREE B

The propositus (B,II,1) for this pedigree (Fig. 1B) is a white Caucasian male infant of French-Canadian origin; the presenting diagnosis was cystinosis. Investigation of the family led to the incidental discovery of specific hyperaminoaciduria in the mother, who is otherwise healthy, and in other relatives.

PEDIGREE C

The propositus (C,III,2) of this pedigree (Fig. 1C) is a five month old Ashkenazi Jewish male infant discovered through routine screening to have cystathioninuria and hyperglycinuria. Investigation of the family revealed that his mother has imino-glycinuria; she is otherwise healthy.

*Materials.* Chromatographically pure L-proline and hydroxy-L-proline (obtained from Mann Research Laboratories, New York) were used for the i.v. infusions. Purity was confirmed by chromatographic methods and the solutions were prepared for infusion as described previously (1, 2). L-proline, hydroxyproline-free (obtained from Nutritional Biochemical Corp., Cleveland, Ohio), which was used for the loading tests was given by mouth.

*Techniques.* Amino acid content and endogenous clearance rates of amino acids were estimated in the overnight-fasted state in the manner previously described (1, 2, 8); random or 24-hr urine collections were not used. L-proline was infused i.v. into subjects A,II,2, A,II,3, and B,I,4, and hydroxy-L-proline was infused separately into subjects A,II,2 and A,II,3, in order to achieve an estimate of their respective maximum rates of tubular absorption (Tm) (1, 2). Intestinal absorption of L-proline was evaluated in subjects A,II,2 and B,II,7, and in two healthy adult males matched for weight and age. After an overnight fast and withdrawal of a control sample of venous blood, L-proline was given by mouth (100 mg/kg), after which venous blood was obtained at hourly intervals; the concentration of proline was measured in these samples. All samples were prepared for analysis by procedures described elsewhere (1, 2, 8).

ANALYTICAL METHODS

*Qualitative.* The amino acid content of urine aliquots, equivalent to 250 $\mu$g of total nitrogen, was evaluated by ascending partition chromatography on 10-inch square No. 4 Whatman filter paper, developed in two dimensions, first in phenol, then in lutidine (9). The chromatograms were stained with a ninhydrin-isatin mixture (10) and were read immediately with transmitted light, and again 24 hr later. The chromatograms were then overstained with Ehrlich's reagent to enhance the detection of hydroxyproline (11). The presence (abnormal) or absence (normal) of iminoaciduria (proline and hydroxyproline) in human subjects beyond early infancy is reliably determined under these conditions.

The criteria for "hyperglycinuria" are more difficult to define, since glycinuria is a normal phenomenon; the diagnosis of "hyperglycinuria" was considered if three experienced observers considered the glycine spot on the partition chromatogram to be excessive. The quantitative criteria for "hyperglycinuria" in the fasted state were: a concentration in urine exceeding 0.16 $\mu$mole/mg of total nitrogen; or an endogenous clearance rate exceeding 8.6 ml/min per 1.73 $m^2$. These maxima were defined in earlier investigations and publications (1, 8). The amino acid content of feces was examined on filter paper by a method employing combined high voltage electrophoresis and partition chromatography (12).

*Quantitative.* Urine and plasma samples were analyzed quantitatively for acidic and neutral amino acid content by elution chromatography on a 55 cm column of PA-28 spherical cation exchange resin with a Beckman-Spinco Model 120 amino acid analyzer (13). The proline concentration in the samples of plasma obtained during loading tests with L-proline were analyzed by a modified protocol for rapid analysis on a short (23 cm) resin column (14). Inulin in urine and plasma was determined by the method of Bojesen (15).

## RESULTS

*Inheritance of the imino-glycinuric phenotype.* More than one form of hyperaminoaciduria appears in each of the pedigrees (Fig. 1). A dominantly inherited hyperglycinuric trait is evident

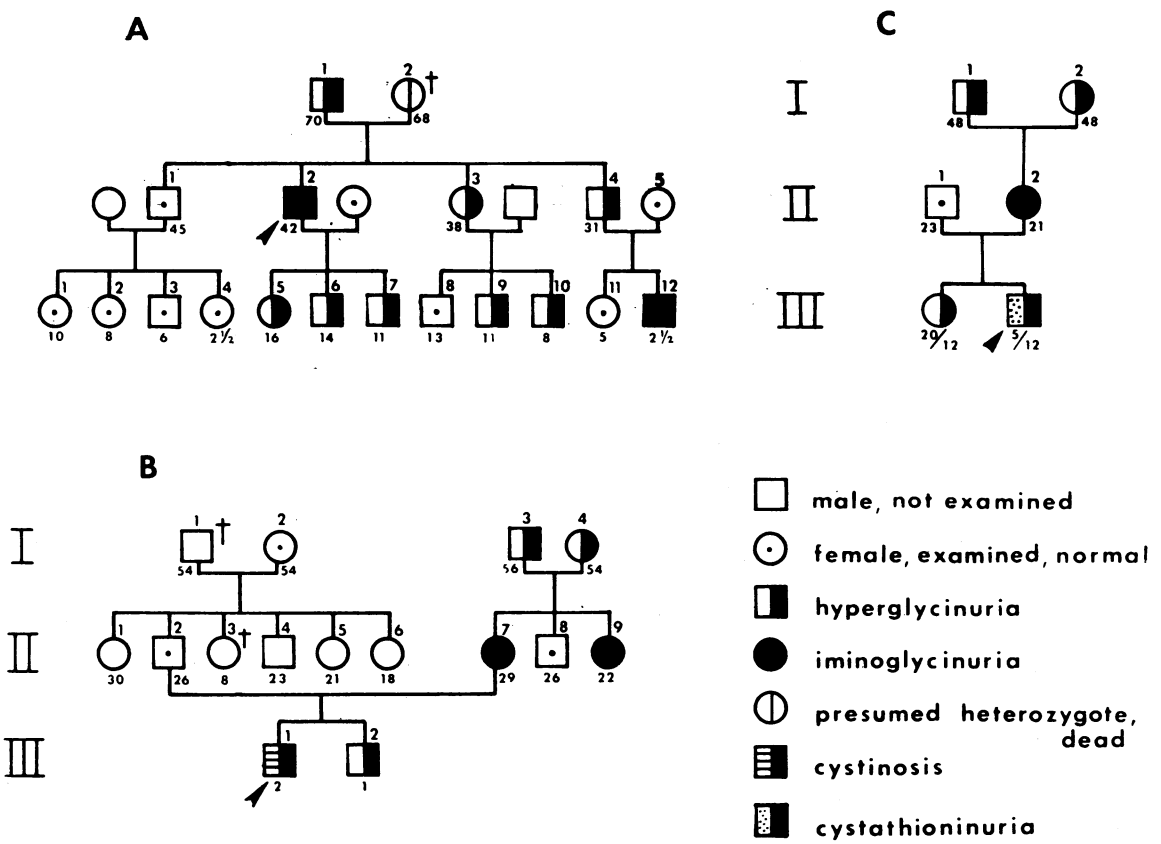


FIGURE 1 Illustrations of three pedigrees, which contain otherwise healthy members, with impaired tubular transport of proline, hydroxyproline, and glycine (iminoglycinuria). The phenotype is inherited in an autosomal recessive manner. The concentration in plasma of the affected amino acids is normal; the function of a specific tubular transport system common to them (1–4) is presumably impaired by the mutation. Subjects A,II,2, B,II,7, and C,II,2 each have parents and offspring with hyperglycinuria, but no iminoaciduria. The latter is therefore considered to be the heterozygous phenotype; iminoglycinuria presumably represents the homozygous phenotype. One subject (A,III,-12) has only one hyperglycinuric parent; he is possibly heteroallelic for two different mutations at one locus controlling the imino–glycine transport system.

in each of the pedigrees, reminiscent of a similar trait found in an Ashkenazi–Jewish pedigree by DeVries, Kochwa, Lazebnik, Frank, and Djaldetti (16). In each of our pedigrees a second trait is represented by abnormal urinary excretion of glycine and the imino acids, proline, and hydroxyproline. All offspring of imino–glycinuric subjects are hyperglycinuric; the parents of these individuals are also hyperglycinuric (Pedigrees B and C, Fig. 1 and Table I). The segregation of the two traits is, therefore, such as to suggest that the hyperglycinuric subjects are the heterozygotes for a mutant allele which in the homozygous form is accompanied by an imino–glycinuric phenotype. Glycine clearance rates were measured wherever possible; this was of importance primarily in subject A,I,1 who should exhibit the hyperglycinuric trait, but did so unequivocally only in terms of his glycine clearance rate.

One patient raises important interpretative difficulties. Subject A,III,12 exhibited consistent imino–glycinuria (comparable to subjects A,II,4, B,II,7, and C,III,2) on three occasions within a 1 yr period (2½–3½ yr of age). His father (A,II,4) has the hyperglycinuric trait; however, his mother (A,II,5) has normal aminoaciduria. She was studied on three occasions during the same period (Table I); concentration of glycine in her plasma was 0.2 mmole/liter, which is sufficient for her to manifest the hyperglycinuric trait if she were indeed a typical heterozygote; nonetheless, there was no abnormality in her excretion of glycine (Ta-

TABLE I

*Aminoaciduria in Subjects of Three Imino-Glycinuric Pedigrees*

| Subject | Age | Sex | Assigned genotype | No. of exam. | Amino acids excreted in urine | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Proline | Hydroxy-proline | PPCgly | Cgly | UVgly |
| A,II,2 | 42 | M | Homozygote | 4 | +(4/4) | +(4/4) | 4/4 | 17.0, 29.7, 33.6 | |
| A,III,12 | 2½ | M | "Homozygote"(Var)* | 3 | +(3/3) | +(3/3) | 3/3 | 20.3 | 0.78 |
| A,I,1 | 70 | M | Heterozygote(Obl) | 3 | 0 | 0 | 1/3 | 8.6 | 0.13 |
| A,III,5 | 16 | F | Heterozygote(Obl) | 2 | 0 | 0 | 2/2 | 13.3 | |
| A,III,6 | 14 | M | Heterozygote(Obl) | 2 | 0 | 0 | 1/2 | 10.9 | |
| A,III,7 | 11 | M | Heterozygote(Obl) | 2 | 0 | 0 | 1/2 | | 0.25 |
| A,II,3 | 38 | F | Heterozygote | 3 | 0 | 0 | 3/3 | 18.6, 10.0 | |
| A,II,4 | 31 | M | Heterozygote | 2 | 0 | 0 | 1/2 | 10.8 | |
| A,III,9 | 11 | M | Heterozygote | 2 | 0 | 0 | 1/2 | 10.0 | |
| A,III,10 | 8 | M | Heterozygote | 2 | 0 | 0 | 2/2 | 10.4 | |
| A,II,5 | 29 | F | "Heterozygote"(Var) | 3 | 0 | 0 | 0/3 | 3.9 | 0.063 |
| A,II,1 | 45 | M | Normal | 2 | 0 | 0 | 0/2 | | 0.13 |
| A,III,11 | 5 | F | Normal | 2 | 0 | 0 | 0/3 | | 0.04 |
| B,II,7 | 29 | F | Homozygote | 4 | +(3/4) | +(3/4) | 4/4 | 19.4, 19.8, 34.9 | |
| B,II,9 | 22 | F | Homozygote | 2 | +(1/2) | +(1/2) | 2/2 | | 0.81 |
| B,I,3 | 56 | M | Heterozygote(Obl) | 2 | 0 | 0 | 2/2 | 19.4 | |
| B,I,4 | 54 | F | Heterozygote(Obl) | 2 | 0 | 0 | 2/2 | 14.7, 25.9 | |
| B,III,1 | 2 | M | Heterozygote(Obl)‡ | 2 | +‡ | +‡ | 2/2 | 44.9‡ | |
| B,III,2 | 1 | M | Heterozygote(Obl) | 3 | 0 | 0 | 2/3 | 16.4 | |
| B,II,8 | 26 | M | Normal | 1 | 0 | 0 | 0/1 | | |
| B,II,2 | 26 | M | Normal | 2 | 0 | 0 | 0/2 | | |
| C,II,2 | 21 | M | Homozygote | 1 | + | + | 1/1 | | 1.27 |
| C,I,1 | 48 | M | Heterozygote(Obl) | 1 | 0 | 0 | 1/1 | | 0.24 |
| C,I,2 | 48 | F | Heterozygote(Obl) | 1 | 0 | 0 | 1/1 | | 0.52 |
| C,III,1 | 20/12 | F | Heterozygote(Obl) | 1 | 0 | 0 | 1/1 | | 0.20 |
| C,III,2 | 5/12 | M | Heterozygote(Obl) | 3 | 0 | 0 | 3/3 | 20.9 | 0.18, 0.40 |

+, Iminoaciduria detected on chromatogram.
PPC, paper partition chromatography.
Cgly, endogenous renal clearance of glycine; normal range = 1.2–8.6 ml/min per 1.73 m² (8, 23).
UVgly, urinary excretion rate of glycine; maximum normal value = 0.16 μmole/min per 1.73 m²; recalculated from previous data (8).
Obl, obligatory heterozygous genotype.
Var, probable variant of usual mutant heterozygous genotype found in this pedigree,
*, Probable heteroallelic homozygous genotype.
‡, Patient has cystinosis with renal tubular failure.

ble II). Therefore, it is possible that this is a "silent" trait in this woman which represents a second mutant allele only made apparent in her son, A,III,12. If this is the case, the son could be considered heteroallelic for two mutations at the same locus on the gene.

*Mechanism of the hyperaminoaciduria.* The endogenous renal clearance rates of proline, hydroxyproline, and glycine are usually greatly increased in the homozygote (Table II), although the concentration of each compound in plasma is normal; diminished net tubular reabsorption is thus evident (Table II). Nonetheless, the loss of tubular absorption of these three solutes is *not* complete; the majority of the endogenous filtered load is still absorbed in the mutant homozygous phenotype (Table II).

Tubular absorption of both imino acids is complete in heterozygotes. The renal clearance of glycine is greater than normal in these subjects, but

TABLE II

*Renal Excretion of Imino Acids and Glycine by Normal and Mutant Phenotypes*

| Amino acid | Phenotype | Plasma | Clearance | Tubular absorption |
|---|---|---|---|---|
| | | *μmole/ml* | *ml/min per 1.73 m²* | % |
| Proline | Normal* | 0.07–0.30 | 0–0.3 | 99.8 |
| | Heterozygote‡ | 0.15 (0.08–0.22) | 0–0.2 | 99.8 |
| | Heterozygote(Var)§ | 0.16 | 0 | 100 |
| | Homozygote‖ | 0.17 (0.14–0.26) | 8.0 (0.6–19.6) | 77–99 |
| | "Homozygote"(Var)¶ | 0.30 | 2.2 | — |
| Hydroxyproline | Normal* | 0.010 | — | 100 |
| | Heterozygote‡ | 0.010 | 0 | 100 |
| | Heterozygote(Var)§ | 0.010 | 0 | 100 |
| | Homozygote‖ | 0.010 | 13 (1–33.6) | 65–99 |
| | "Homozygote"(Var)¶ | 0.010 | 23.7 | — |
| Glycine | Normal* | 0.11–0.35 | 1.2–8.6 | 93–99 |
| | Heterozygote‡ | 0.25 (0.11–0.55) | 14.6 (8.6–26.2) | 82–95 |
| | Heterozygote(Var)§ | 0.19 | 3.9 | — |
| | Homozygote‖ | 0.20 (0.17–0.32) | 25.7 (17.0–34.9) | 63–77 |
| | "Homozygote"(Var)¶ | 0.24 | 20.3 | — |

* Data on normal subjects compiled from investigation of adults (23) and children (8).
‡ 13 observations on 11 subjects in A, B, and C pedigrees showing mean and range.
§ Subject A,II,5 believed to be heterozygote with variant genotype.
‖ Six separate investigations of two homozygotes, A,II,2 and B,II,7 showing mean and range.
¶ Subject A,III,12 believed to be heteroallelic homozygote of two mutant alleles (common and variant).

less than usual in the homozygote (Table II). The atypical heterozygote (subject A,II,5) has a normal renal clearance of glycine.

The renal clearance of all other amino acids is normal in affected homozygotes and heterozygotes. The impairment of renal tubular transport is thus selective, implying that a specific structure–function relationship is altered by the mutation.

### Transport of L-proline

*In the homozygote.* Renal clearance of proline varied directly with the plasma–proline concentration (Fig. 2); the homozygote (B,II,7), who had a relatively low concentration of proline in plasma, had little hyperprolinuria; her homozygous sister (B,II,9) also excreted relatively little of the imino acids, presumably, for the same reason (Table I). This observation suggests that a system for proline transport is still operative, with a capacity of about 15–20 μmoles/min per 1.73 m².

The infusion of L-proline in the mutant homozygote, A,II,2, demonstrated that there was no further capacity for proline absorption in this subject beyond that operative at the endogenous concentration of proline in plasma (Fig. 3); the low Tm value (Fig. 3 and Table III) indicates the loss of the high capacity (Tm) system which is normally present (1). The Tm–proline value and the venous plasma threshold for prolinuria in the homozygote were both about one-tenth of that measured in normal subjects (1).

*In the heterozygote.* The Tm–proline values obtained in the heterozygotes, A,II,3 and B,I,2, were intermediate between those of the mutant homozygote and those of the normal subjects (Fig. 3 and Table III). There was no splay in the absorption rate at low filtered amounts of proline before achieving the Tm; this suggests that the affinity for substrate is probably not different from the normal affinity.

### Transport of hydroxy-L-proline

*In the homozygote.* i.v. infusion of hydroxyproline produced an effect analagous to that of an in-

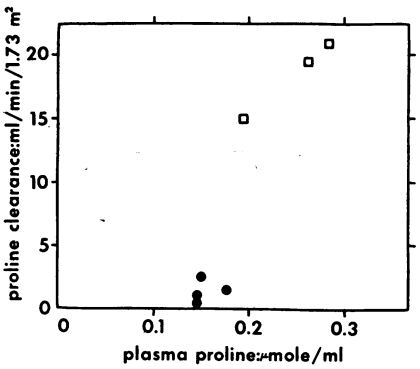


FIGURE 2 The endogenous renal clearance of proline is dependent on the concentration of proline in plasma in mutant homozygotes (circles, B,II,7; squares, A,II,2) as in normal subjects (1). The points of emphasis in this graph are: (*a*) the renal clearance of proline is negligible at low plasma levels, which is thus indicative of a persistent absorptive function in the homozygous phenotype; (*b*) the threshold for abnormal proline clearance is about one-fifth of that for normal subjects (1).

fusion of L-proline. The homozygote (A,II,2) exhibited no additional capacity to transport hydroxy-L-proline at filtered loads beyond the existing endogenous capacity (Fig. 4 and Table III).

*In the heterozygote.* The heterozygous subject A,II,3, had a reduced capacity to transport hydroxy-L-proline (Fig. 4 and Table III) when compared with the normal subjects (2), but the capacity was greater than in the homozygote. There was no splay in the absorption rate at low concentrations of substrate.

## Transport of glycine

The normal subject absorbs about 95% of the filtered load of glycine (8); the normal endogenous clearance rate averages 4.4 ml/min per 1.73 $m^2$, with a maximum rate not exceeding 8.6 ml/min per 1.73 $m^2$ (2, 8, 17). The average endogenous clearance rate calculated from 13 observations in 11 heterozygous subjects was 14.6 ml/min per 1.73 $m^2$ (range, 8.6–26.2) (Table II); there were no significant differences in the clearance rates between the sexes. The wide variation in glycine clearance rates reflects, in part, an equivalent variation in the concentration of glycine in plasma of the different subjects. The average clearance rate of glycine in six observations on two homozygotes (A,II,2 and B,II,7) was 25.7 ml/min per 1.73 $m^2$ (range, 17.0–34.9). The clearance of glycine by the homozygote did *not* equal the glomerular filtration rate, as expected if the agency for glycine transport were totally absent in this phenotype.

## Interaction between imino acids and glycine during tubular absorption

Either imino acid (proline or hydroxyproline) is a competitive inhibitor of the uptake of the other, and of glycine by normal mammalian kidney (1–4). By contrast, neither imino acid inhibits the endogenous transport of glycine in the mutant homozygote (Fig. 5 and Table IV); about 10–16 μmoles/min per 1.73 $m^2$ of glycine transport in human kidney is not subject to inhibition by either imino acid. Both imino acids can inhibit tubular transport of glycine in the heterozygous phenotype; however, the concentration of the inhibitor (imino acid) required to achieve inhibition of substrate (glycine) transport equivalent to that in normal subjects is much lower in the heterozygote

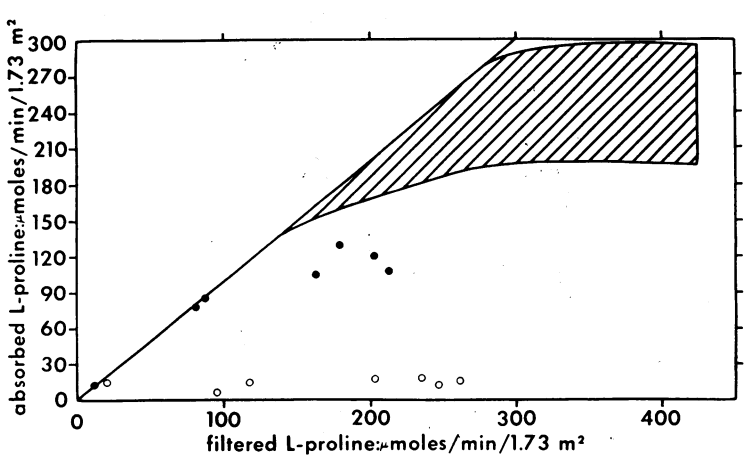


FIGURE 3 The maximum rate of tubular absorption of L-proline (Tm pro) for normal subjects (1) (shaded area), a heterozygote (A,II,3) (solid circles), and a homozygote (A,II,2) (open circles). The Tm pro in the heterozygote is intermediate between normal and homozygous subjects. The Tm pro in the latter subject is already saturated at the endogenous concentration of proline in the glomerular filtrate. There is no "splay" in the mutant absorption curves.

TABLE III

*Tubular Transport of Imino Acids*

| Imino Acid | Subject | Average $C_{In}$ at Tm | Maximum plasma imino acid conc. | Maximum filtered load:Tm | Venous plasma threshold | Tm |
|---|---|---|---|---|---|---|
| | | *ml/min per 1.73 m²* | *μmoles/ml* | | *μmoles/ml* | *μmoles/min per 1.73 m²* |
| L-proline | Normal | — | — | 2.4 | 1.0 | 170–260 |
| | Homozygote (A,II,2) | 97 | 2.7 | 14.5 | 0.15 | 18 |
| | Heterozygote (A,II,3) | 110 | 2.1 | 1.84 | 0.75 | 117 |
| | (B,I,4) | 100 | 4.3 | 11.6 | 1.0 | 35 |
| Hydroxy-L-proline | Normal | — | — | 4.4 | 0.4–0.7 | 60–135 |
| | Homozygote (A,II,2) | 125 | 1.8 | 36 | 0.01 | 6.0 |
| | Heterozygote (A,II,3) | 134 | 0.9 | 2.4 | 0.38 | 50 |

(Fig. 5 and Table IV). The portion of glycine transport in the heterozygote not inhibited by imino acid (8–16 μmoles/min per 1.73 m²) was about equivalent to that found in the homozygote (Fig. 5 and Table IV).

The interaction between the two imino acids was different. Infusion of either imino acid increased the urinary excretion of the other in normal, heterozygous, and homozygous subjects (Fig. 6). If an estimate is made of the endogenous renal clearance of hydroxyproline, assuming that the plasma concentration is about 0.010 mmole/liter [1] one finds that the clearance of hydroxyproline in homozygote and heterozygote as the filtered load of L-proline is increased, approaches the value for simultaneous clearance of inulin. Conversely, an equimolar infusion of hydroxy-L-proline only partially inhibits the tubular absorption of proline (Fig. 6) in homozygote and heterozygote. This suggests that hydroxyproline is the less effective inhibitor, and that proline is preferentially absorbed at the site where the two imino acids mutually interact in the mutant homozygote.

[1] Hydroxyproline cannot be measured accurately at normal endogenous concentrations in plasma by the chromatographic method used in this study. However, the amounts of hydroxyproline measured on the chromatograms of the subjects in this investigation were of an order not exceeding that of 0.010 μmole/ml.

### Intestinal transport

The fecal content of amino acids was examined in one homozygote (A,II,2) while on a normal diet, and again after 5 days on Neomycin (50 mg b.i.d.) by mouth and while receiving a high protein diet containing gelatin supplements. On neither occasion was the amount of proline, hy-

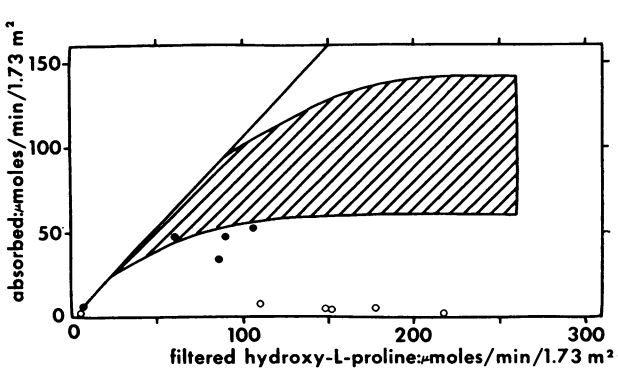


FIGURE 4 The maximum rate of tubular absorption of hydroxy-L-proline (Tm hypro) for normal subjects (2), and the same heterozygous and homozygous subjects depicted in Fig. 3. The Tm hypro of the heterozygote is intermediate between normal and mutant homozygote values; uptake of hydroxyproline is virtually saturated at the endogenous filtered load in the homozygote. There is no "splay" in the mutant absorption curves.

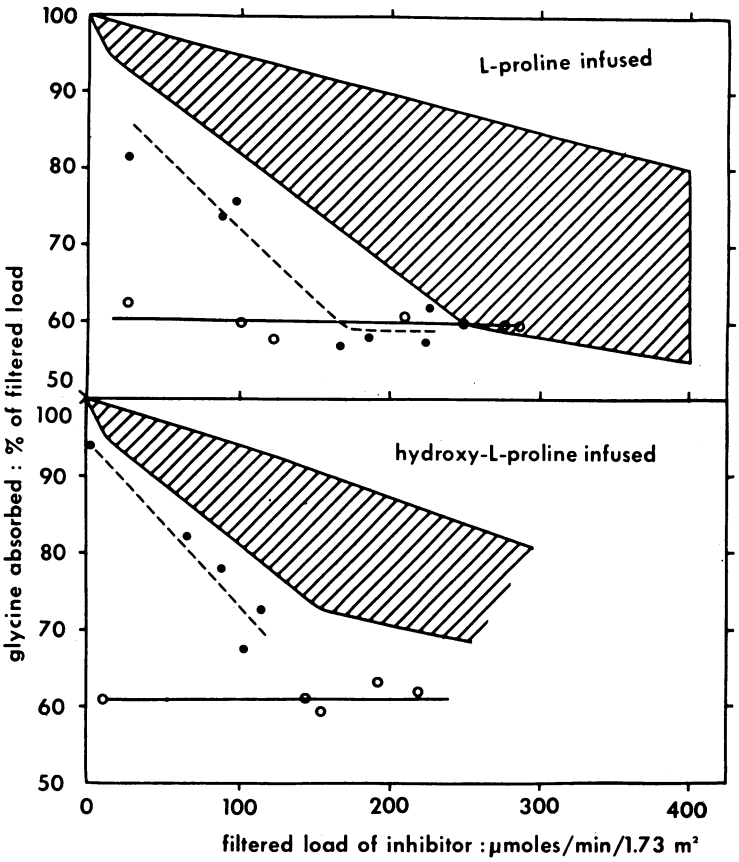


FIGURE 5 Tubular absorption of glycine at endogenous concentrations in the presence of an increasing exogenous load of imino acids in the glomerular filtrate. The normal subject (shaded area) manifests inhibition of glycine uptake in the presence of either imino acids (1, 2). The persistent glycine transport in the homozygote A,II,2 (open circles) is not inhibited by either imino acid even at inhibitor : substrate ratios of 10 : 1. Glycine transport in the heterozygote A,II,3 (solid circles) is inhibited equivalently at proportionately lower concentrations of inhibitor in comparison with normal subjects; a noninhibitable portion of glycine transport eventually becomes apparent during the proline infusion; the amount of this noninhibitable transport was equivalent to that measured in the homozygote.

droxyproline, or glycine noted in the feces in excess of that in normal feces.

The postabsorptive concentration of L-proline in plasma of the two mutant homozygous subjects, A,II,2 and B,II,7, after they had received L-proline (100 mg/kg) by mouth (Fig. 7), was equivalent to that observed in the normal subjects studied here and by other investigators (18, 19).

TABLE IV

*Effect of Imino Acids on Glycine Transport*

| | Preinfusion glycine transport | | Glycine absorbed at Tm pro | | Glycine absorbed at Tm hypro | |
|---|---|---|---|---|---|---|
| | *μmoles/min per 1.73 m²* | *% absorbed* | *μmoles/min per 1.73 m²* | *% absorbed* | *μmoles/min per 1.73 m²* | *% absorbed* |
| Normals (1, 2) | | 93–99 | | 60 | | 65 |
| Homozygote A,II,2 | 10 | 63 | 10* | 60 | | |
| | 14.5 | 60 | | | 16* | 60 |
| Heterozygote A,II,3 | 10.2 | 83 | 7.8* | 59 | | |
| | 14.7 | 95 | | | 16‡ | 60 |
| Heterozygote B,I,4 | 41.2 | 83 | 11.1* | 24 | | |

* Value represents noninhibitable portion of glycine transport.

‡ Maximum inhibition not clearly demonstrated (viz., Fig. 5); therefore capacity of noninhibitable component may be even lower.

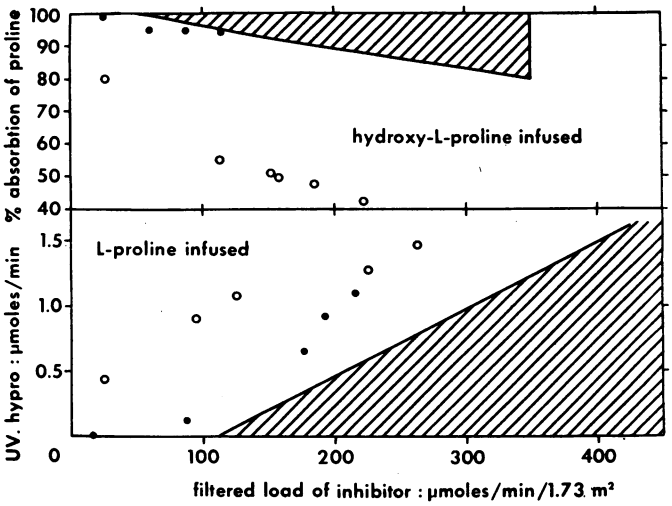


FIGURE 6 The effect of increasing the concentration of one imino acid in the glomerular filtrate upon the excretion rate or tubular absorption of the other. The imino acids interact with each other in all three phenotypes, in contrast to their effect upon glycine absorption. Symbols and subjects are as shown in Fig. 5. Shaded areas indicate responses observed in normal subjects (data derived from previous studies, references 1 and 2).

This suggests that intestinal transport of L-proline was normal in these particular homozygotes.

## DISCUSSION

Hyperiminoglycinuria was discovered incidentally in three unrelated, healthy adult subjects, because illness of one form or another in one of their relatives brought them to our attention. Mutation affecting renal transport of proline, hydroxyproline, and glycine only awaited such recognition once it was evident that cellular uptake of these solutes by many different tissues is mediated wholly, or in part, by a transport agency with preference for them (1–4, 20–23). Several investigators have now observed sibships or families (18, 19, 24, 25 and footnotes 2 and 3) in which iminoglycinuria persisted beyond the period of infancy when this is a normal phenomenon (23). In some of these families the imino-glycinuric phenotype was associated with mental retardation or convulsions (19, 24, 25). However, because serious illness does not necessarily accompany the mutant imino–glycinuric phenotype, it may be assumed, either that the two events are only incidentally associated, the primary illness leading to the detection of the other benign condition (aminoaciduria), or that the benign phenotype is associated with a genotype different from that responsible for the phenotypes associated with illness.

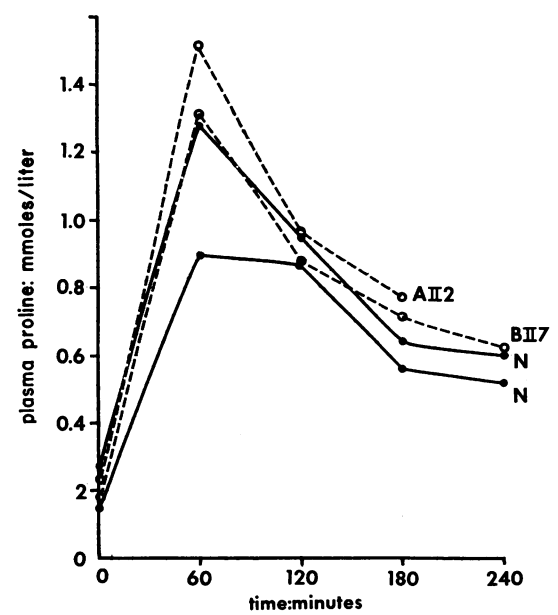


FIGURE 7 Postabsorptive concentration of proline in plasma after a load of L-proline (100 mg/kg) by mouth. The homozygous subjects (A,II,2 and B,II,7) and the normal subjects have the same response.

Autosomal recessive inheritance of the imino–glycinuric trait, and dominant inheritance of a simple hyperglycinuric trait is evident in the three pedigrees. On the basis of the inheritance pattern, it is assumed that the former are homozygous and the latter are heterozygous for a pair of mutant alleles at one locus controlling tubular transport of the imino acids and glycine. Thus "dominantly" inherited renal glycinuria, found by DeVries et al. (16) in an Ashkenazi-Jewish pedigree, has been reinterpreted as the heterozygous phenotype for the mutation which we have investigated.

[2] Jonxis, J. H. P. Personal communication (cited in reference 1).

[3] Rosenberg, L. E. Personal communication.

The possibility that the imino–glycinuric mutation is in fact quite common should be considered in view of its widespread occurence in several races and ethnic groups. Three subjects with the trait have been found among about 10,000 who were investigated in the past 6 yr in Montreal. Although this gives no indication of the true frequency of the trait, it would appear to be relatively common. Heterogeneity of the trait with the possibility that heteroallelic subjects do occur has been proposed to account for the phenotypic relationships of subject A,III,12 and his parents, A,II,4 and A,II,5. The phenomenon of heteroalleleism has already been carefully documented in cystinuria by Rosenberg (26) following the discovery of more than one genotype in classical cystinuria (27, 28). If mutations occur at multiple alleles within a single genetic locus, then one may also predict the discovery of genetic and phenotypic heterogeneity for other heritable disorders of amino acid transport (29).

The likelihood that more than one mode of transport participates in the tubular absorption of imino acids and glycine has been raised by the present investigation. The hyperimino-glycinuric trait observed in our pedigrees can be attributed to a genetic modification of a group–specific site at which the three solutes mutally interact during uptake into the tubular cell (1–4); other mechanisms of interference with transport, such as saturation with substrate and competition from inhibitors at the transport site (1–4, 29), do not apply in this instance. Despite the apparent deletion of this transport system, the mutant homozygote loses only a portion of his endogenous tubular transport function. When we became aware of this seeming paradox, we reviewed available data (30) on the other heritable disorders of renal transport of amino acids (29) to see if this phenomenon also occured in their homozygous phenotypes. In classical cystinuria, in hypercytinuria (31), and in Hartnup disease, the homozygote retains an important fraction of his endogenous transport function (30).

The explanation of this phenomenon now seems apparent, at least in the imino–glycinuric trait; presumably a similar interpretation could be extended to the other mutant transport phenotypes. The normal capacity, Tm, of the intact wild-type tubular transport system common to the imino acids and glycine is at least 10-fold greater than the normal endogenous load (1, 2), and the affinity of this system is greater for the imino acids than for glycine (1–4). Imino-glycinuria occurs in the mutant homozygote because this system is functionally deleted; partial deletion of the system in the heterozygote compromises glycine transport because of the heirarchy of substrate affinity for the system. The paradox raised by the homozygote that nonetheless still absorbs the majority of the filtered endogenous load of imino acids and glycine, can be resolved by proposing one of two alternatives: either the affinity of the common system for its substrates has been altered by the mutation–persistent transport thus reflects reduced efficiency for uptake by the modified common system; or, there may be complete functional deletion of the common system, with subsequent unmasking of an alternate mode of transport which has different kinetic characteristics. There is no evidence supporting the first alternative, and the second explanation can account for all of the observations reported herein.

Certain characteristics of the residual mode of transport are evident. It has a low capacity and high affinity for substrate. It is saturated at the endogenous concentrations (in plasma) of its substrate (Figs. 3 and 4) and, hence, the capacity for these substrates can be estimated (Table V). The ability of this second mode to transport substrate with high efficiency is evident in subjects B,II,7 (Table I) and B,II,9 (Fig. 2). Considerable substrate specificity is evident, first from the inability of imino acids to compete with glycine (Fig. 5), and second, from their interactions with each other, suggests that the imino acids share a low-capacity system distinct from the glycine system (Fig. 6). The suggestion that hydroxyproline shares a proline site (even in this alternate mode of transport) is in keeping with recent observations which indicate that hydroxyproline does not gain access to cells as diverse as osteoblasts (21) and micro organisms (32) by its own transport agency.

The evidence for uptake of the imino acids and glycine in human kidney by more than one system with widely differing characteristics is in keeping with recently published data indicating that other amino acids are also taken up by more than one agency in a variety of phyla, species, and tissues, including mouse–Ehrlich ascites tumor cells (33),

TABLE V

*Summary of Characteristics Accounting for Phenotypic and Genetic Heterogeneity of Imino Acids and Glycine in Man*

| Characteristic | A system | | B systems | |
|---|---|---|---|---|
| | Group specificity | | Substrate specificity | |
| Substrate preference | proline<br>hydroxyproline<br>glycine | | glycine | proline + hydroxyproline |
| Approximate capacity, μmoles/min per 1.73 m² | proline:<br>hydroxyproline:<br>glycine: | 180–300<br>60–150<br>* | 10–15 | 20–25 |
| | Kidney | Gut | | |
| Mutant Phenotypes in: | | | | |
| Homozygote | 1) Imino-glycinuria | Normal | None recognized at present | |
| | 2) Imino-glycinuria | Proline transport defect | | |
| Heterozygote | 1) Hyper-glycinuria | ? | | |
| | 2) Normal(?)‡ | | | |
| Inheritance | Autosomal recessive | | — | |
| Mutant genotypes | Probably two or more. | | Will be separate from A mutants. | |

* No available data in man; a value of 3000 is derived from canine species (43).

‡ Conclusion based only on evidence in Pedigree A, subject A, II,5.

mouse bone (21), human kidney (34), as well as micro organisms (32, 35), and yeasts (36). The present investigation is, however, the first opportunity to demonstrate the evidence in vivo in man.

If heterogeneity of transport is a phenomenon of more universal than particular significance, then mutations affecting the low-capacity, substrate–specific systems can be anticipated and sought in man. In this regard, human hypercystinuria (31) is probably the phenotype for a mutant cystine–specific transport system, in contrast to "classical" cystinuria in which a group–specific agency is involved. The recent discovery in our laboratory of hyperalaninuria and hyperlysinuria in different families [4] substantiates our belief that it is only a matter of time before most of the predictably specific transport defects will be recognized. The presumed disorders of methionine (37) and tryptophan (38) absorption could be claimed now as examples wherein the relevant substrate–specific transport agency in the intestine is affected.

Simultaneous manifestation of the mutant transport phenotype in kidney and in gut has been demonstrated in cystinuria (39) and in Hartnup disease (12, 40). However, it is also known that, whereas some mutant transport genotypes are accompanied by an intestinal transport defect, others were not (26, 28). Two of our imino–glycinuric homozygotes with a renal transport defect had no intestinal transport defect; Rosenberg [3] has made a similar observation in another imino–glycinuric pedigree. On the other hand, an intestinal transport defect has been found in other imino–glycinuric families (18, 19). On such preliminary evidence, one can plead for heterogeneity of the imino–glycinuric genotype.

The foregoing investigations provide a body of data which implicate a complex organization of membrane transport systems used by the imino acids and glycine (Table V); the present interpre-

[4] Scriver and Whelan. Unpublished observations.

tation may, however, change as more pedigrees carrying the mutant imino–glycinuric phenotype are discovered and investigated.

## ACKNOWLEDGMENTS

I am grateful to Miss Eluned Davies, Mr. Peter Lamm, Mrs. Carol Clow, and Miss Sandra Pilkington, R.N., for their technical assistance; and to Doctors Onslow H. Wilson and F. Clarke Fraser for helpful discussion. The continued interest of subjects A,II,2 and A,II,3 in this investigation is greatly appreciated.

This study was aided by research grants AM-05117-06 of the National Institutes of Health, and MT-1085 of the Medical Research Council of Canada.

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