fibers were almost unstained (Fig. 3). This means that, despite a high activity of oxidative enzymes, lipid droplets are scanty or absent in intrafusal gastrocnemius fibers of the cat. On the other hand, when the cat muscle spindle in Figs. 1 and 3 was stained for myofibrillar-bound adenosine triphosphatase, two types of fibers were observed (Fig. 4). The smaller fibers had a higher content of this enzyme.

Toad muscle spindles stained for fat always appeared almost unstained, just as cat spindles did, whereas in sections stained for myofibrillar-bound adenosine triphosphatase, spindles with one or two types of intrafusal muscle fibers were identified. In physiological investigations, two types have usually been reported (*1*).

It can be inferred that all intrafusal muscle fibers of cat spindles showed equal staining intensity when stained for $NADH_2$-tetrazolium reductase or fat, whereas morphological and physiological studies generally disclose two types of fibers. When the same spindles were stained for myofibrillar-bound adenosine triphosphatase, two types of fibers appeared. However, other staining reactions may reveal even more types (*4, 5*). Thus, it seems premature—at any rate with respect to intrafusal muscle fibers—to correlate physiological and histochemical results until those chemical processes specifically associated with the physiological properties are better known.

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Lysine Transport in Human Kidney: Evidence for Two Systems

Abstract. *Experiments with slices of human kidney cortex from two control subjects demonstrated two distinct transport systems for lysine (α and β) which differ greatly in affinity and capacity. Both systems were found in kidney from two patients with cystinuria. Studies with rat kidney confirmed these findings. These experiments defined only a single transport system for cystine in kidney from both control and cystinuric subjects.*

Intestinal absorption and renal tubular reabsorption of dibasic amino acids in man and other mammals is accomplished by specific, energy-dependent, active transport processes. Renal clearance (*1*) and stop-flow (*2*) analyses, oral-absorption studies (*3*), and uptake experiments with gut mucosa in vitro (*4*) indicate that the dibasic, monocarboxylic amino acids—lysine, arginine, and ornithine—are transported by a common system in the gut and kidney and that this system may be shared by cystine, a dibasic dicarboxylic amino acid. This transport system is defective in individuals with cystinuria, an inherited disorder leading to impaired intestinal absorption and marked urinary hyperexcretion of these dibasic amino acids. However, several important findings from experiments in vivo and in vitro have not been explained satisfactorily by the prevailing concept of a single renal and intestinal transport mechanism for this group of structurally related compounds. (i) Cystine clearance has equaled or even exceeded inulin clearance in all homozygous cystinuric subjects studied, but simultaneous clearances of lysine, arginine, and ornithine have been significantly less than the clearance of inulin (*5*). (ii) Lysine uptake was reduced by only 50 percent in kidney from homozygous cystinuric subjects in vitro and was further inhibited by arginine (*6*). (iii) Cystine uptake by kidney from cystinuric subjects was not significantly impaired, nor could cystine be shown to share a common transport mechanism with lysine, arginine, or ornithine (*6, 7*). (iv) In subjects homozygous for type I cystinuria (*8*), mediated intestinal transport of cystine, lysine, and arginine was completely abolished, in contrast to the partial transport defect in kidney tissue (*6*). The first two observations are consistent with the hypothesis that the mutations responsible for homozygous cystinuria alter the configuration, quantity, or activity of a single carrier protein or enzyme with different affinities for the several dibasic amino acids. The other two observations, however, strongly suggest an alternate hypothesis, namely, the presence of two transport systems in human kidney for dibasic amino acids, only one shared by the gut and mutant in cystinuria.

There is ample evidence for this alternative hypothesis in nonmammalian systems. Ames (*9*) reported that histidine enters *Salmonella typhimurium* by two distinct processes, one shared by the other aromatic amino acids and one transporting only histidine. This conclusion was based on the demonstration of two apparent affinity constants (K_m) for histidine uptake. Similar results were reported subsequently for galactose uptake in *Escherichia coli* (*10*) and for β-alanine and diaminobutyric acid transport in ascites tumor cells (*11*). Our studies tested the hypothesis that dibasic amino acid transport in human kidney also depends on more than a single mechanism.

Human kidney cortex from three healthy adults who showed no evidence of renal tubular dysfunction was obtained when these individuals were operated on for repair of benign renal cysts, and from two patients with homozygous cystinuria, who underwent surgery for removal of stones. Only normal kidney cortex was used. All tissues were prepared for incubation within 10 minutes of removal (*6*). Uptake of uniformly labeled L-lysine-C^{14} was studied over a 40-fold range of substrate concentrations, chosen to span the physiologic range (0.06 to 2.4 mmole/liter). Uptake of L-cystine-S^{35} was evaluated over a less wide range (0.04 to 0.9 mmole/liter) because of its limited solubility. A sharp break in the curve showing uptake of lysine was noted (Fig. 1) at substrate concentrations exceeding 0.5 mmole per liter, an indication of a second mode of mediated uptake of lysine (β-system), whose affinity was much less than that at lower substrate concentrations (α-system). Uptake of cystine, however, showed no such deviation from linearity (Fig. 1). Similar findings were noted in kidney tissue from a second control subject, (Table 1), but no break in the curve of lysine uptake was observed in kidney from a third control (F.B.). Sufficient tissue was not available for experiments in which true maximal velocity could be determined by using shorter incubation intervals. Such experiments with rat kidney cortex were made with a 10-minute incubation period and several substrate concentrations higher and lower than those reported in Fig. 1. The dual system for lysine was confirmed (Fig. 2).

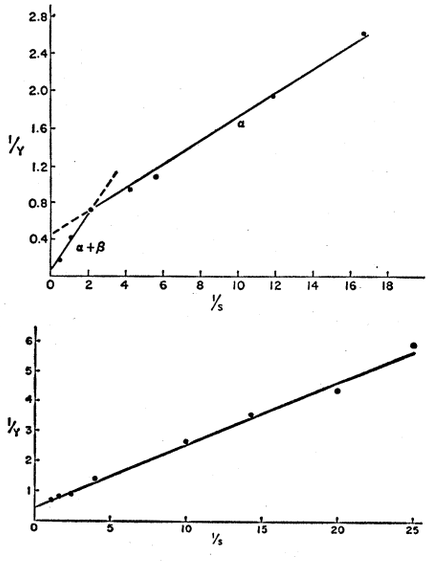

Fig. 1. Uptake of lysine (above) and cystine (below) by kidney cortex from control subject (V.R.) plotted by double reciprocal method. Slices of kidney (5 to 15 mg) were incubated in Krebs-Ringer bicarbonate buffer (*p*H 7.4) at 37°C for 30 minutes, and amino acid uptake was calculated (*6, 7*). *S*, substrate concentration (mmole/liter). *Y*, velocity of mediated uptake (mmole/liter for 30 minutes), after nonsaturable contribution is deducted (*7*). No correction was made, in calculating K_m and V_{max} of the system operating at high lysine concentration (β), for contribution of the second system (α). Qualitatively similar findings were noted in patients S.W., V.G., and C.L. (Table 1).

Table 1. Estimates of affinity (K_m) and capacity (V_{max}) for transport of lysine and cystine in human kidney cortex. Values for K_m (mmole/liter) and V_{max} (mmole/liter per 30 minutes) were calculated as described (*7*). Symbols: α, system at low substrate concentration; β, system identified at high substrate concentration (Fig. 1); −, study not performed; +, not defined by study. Roman numerals refer to genetic type of cystinuria (*8*).

Patient	Age (yr.)	Sex	Cystine		Lysine			
			K_m	V_{max}	$K_m\alpha$	$V_{max}\alpha$	$K_m\beta$	$V_{max}\beta$
Control patients								
V.R.	40	F	0.5	2.5	0.2	2.1	5.0	16.7
S.W.	25	M	.4	1.2	.4	2.5	2.6	7.1
F.B.	48	M	−	−	+	+	2.0	8.3
Cystinuric patients								
V.G.	24	F (III)	0.4	1.0	0.6	1.8	5.0	10.0
C.L.	27	F (II)	.3	0.6	.1	0.9	5.0	12.5

In kidney from V.G., who was homozygous for type III cystinuria (*8*), and from C.L., who was homozygous for type II cystinuria (*8*), the α- and β-systems for lysine and the single process for cystine were present (Table 1). The significance of the quantitative differences in the estimates of V_{max} of the α-lysine and cystine systems observed between controls and cystinurics must await additional data.

Our results show that lysine transport in human and rat kidney depends on two processes, distinguishable by appropriate kinetic analysis. Similar findings were noted for cysteine uptake in human kidney (*13*). The break in the lysine uptake curve may represent the participation of two different carrier proteins (or enzymes), but it could also result from a steric alteration in a single carrier molecule exposed to a wide range of substrate concentrations. The inability to demonstrate two lysine systems in kidney from one control subject may reflect either true genetic heterogeneity for the lysine transport system or differences in affinity and capacity of the transport processes too small to be detected by our analysis.

The differences between cystine and lysine clearance, the partial sparing of lysine transport in cystinuric kidney, the failure to demonstrate competition between lysine and cystine uptake in kidney tissue, and the distinct difference noted in lysine uptake between gut and kidney from cystinuric subjects are consistent with the thesis that the kidney contains at least two discrete mechanisms for lysine transport under separate genetic control and that only one of these systems, shared by cystine, arginine, and ornithine, also exists in the gut and is defective in cystinuria. Group-specific and substrate-specific transport processes for the dibasic amino acids may exist in the kidney, as demonstrated for the aromatic amino acids in *Salmonella* (*9*).

There remains a disparity between the observations in vivo in cystinuric subjects and the findings in vitro. Uptake of cystine by kidney slices may

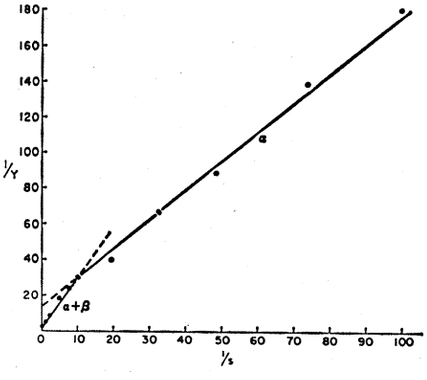

Fig. 2. Lysine uptake by kidney cortex from male Sprague-Dawley rats (140 to 160 g). Cortex slices (60 to 100 mg) were incubated for 10 minutes in bicarbonate buffer (*p*H 7.4) at 37°C. *S*, substrate concentration (mmole/liter); *Y* (mmole/liter per 10 minutes), velocity of mediated uptake calculated by correcting for nonsaturable contribution (*7, 12*). Data represent mean of at least six observations at each concentration. Estimates for K_m were obtained: $K_m\alpha = 0.17$ mmole/liter; $K_m\beta = 3.3$ mmole/liter. No correction for the α-system was applied in calculation of $K_m\beta$.

measure secretory as well as reabsorptive mechanisms. A common reabsorptive process for cystine, lysine, arginine, and ornithine could be obscured in vitro by a secretory mechanism specific for cystine (see *5, 14*).

The report by Scriver and Wilson (*15*) demonstrates more than a single renal reabsorptive mechanism for the imino acids and glycine. Additional experiments are needed to determine whether other substances reabsorbed in the proximal tubule also demonstrate multiple transport systems under specific genetic control.

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Amino Acid Transport: Evidence for Genetic Control of Two Types in Human Kidney

Abstract. *A mutation affecting renal transport of proline, hydroxyproline, and glycerine occurs in man. In the presumed homozygote there is still significant residual transport of these compounds; however, this remaining function is saturated at normal concentrations of substrate in the plasma and is not inhibited by* L*-proline in the expected way. The presumed heterozygote has partial loss of a transport system common to the three substrates, which becomes saturated at high concentrations of substrate and is inhibited by* L*-proline. Two different types of transport systems are proposed: a common system and systems with lower capacity and greater specificity. The two types of transport appear to be controlled by separate genes.*

Absorption of amino acids in the intestine and renal tubule of man and other mammals appears to be accomplished either wholly, or in part, by five transport systems, each of which is common to a particular group of amino acids, classified as follows: (i) dicarboxylic-monoamino (acidic) (*1*); (ii) diamino-monocarboxylic (basic) (*2*) —this group also has a complex relation to the transport of L-cystine, which is not a basic amino acid; (iii) glycine and the "imino acids" proline and hydroxyproline (*3*); (iv) a large group of neutral aliphatic, aromatic, and heterocyclic α-amino acids (*4*); and (v) the β-amino compounds (*5*). A common transport system exhibits certain characteristics, both in vivo and in vitro, which imply a mediated or carrier type of function (*1–3*). Specificity is expressed as preferential affinity for its own group of substrates; members of the substrate group can saturate the system and can competitively inhibit the uptake of the other members. The occurrence of mutant genes causing impairment of transport function in both kidney and intestine, as in cystinuria (*2*) where cystine and the basic amino acids lysine, arginine, and ornithine are affected, and in Hartnup disease (*4*) where the large group of neutral α-amino acids is involved, also implicates genetic control of some of these common transport systems.

An explanation for two intriguing observations in mutant transport phenotypes has long been wanting. First, at normal plasma concentrations of basic amino acids in cystinuric homozygotes and of the neutral amino acids in the Hartnup homozygote, the major portion of tubular transport activity is still retained. Secondly, Robson and Rose (*6*) observed during their investigations of the common transport system for basic amino acids, that intravenous infusions of lysine produced little or no further inhibition of tubular absorption of basic amino acids in some homozygous cystinurics, unlike the brisk inhibition produced by the same procedure in normal subjects. Recent investigation of another "experiment of nature" affecting renal tubular absorption of the imino acids and glycine, has provided an opportunity to interpret these earlier observations.

In the human infant selective impairment of tubular absorption of the imino acids and glycine is a normal occurrence for several weeks after birth (*7*). Occasionally imino-glycinuria persists into childhood (*8*), where it has been considered as another example of a selective, and probably inherited, defect in amino-acid transport. We have recently found an otherwise healthy 42-year-old man who has urinary hyperexcretion of proline, hydroxyproline, and glycine; partial impairment of net tubular absorption of these three compounds was documented in this subject (Table 1). The propositus is an Ashkenazic Jew, whose mother died of hepatic carcinoma in her 68th year. His father exhibits hyperglycinuria without iminoaciduria (*9*). One brother and one sister also have hyperglycinuria with diminished net tubular absorption of this amino acid (Table 1). An additional brother has normal aminoaciduria. The propositus is married to an unrelated woman, who has normal aminoaciduria; all three children of this marriage each have hyperglycinuria without iminoaciduria. The propositus is therefore presumed to be homozygous for a mutation affecting the common transport system for imino acids and glycine; his hyperglycinuric relatives are presumed to be heterozygous for the same mutation; four of them were available for estimation of endogenous renal clearance of amino acids (Table 1).