Localization of the Membrane Defect in Transepithelial Transport of Taurine by Parallel Studies In Vivo and In Vitro in Hypertaurinuric Mice

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ABSTRACT We investigated the mechanism of taurinuria in three inbred strains of mice: A/J, a normal taurine excretor (tau^{t+}); and two hypertaurinuric (tau^{t-}) strains, C57BL/6J and PRO/Re. Plasma taurine is comparable in the three strains (~0.5 mM), but taurinuria is 10-fold greater in tau^{t-} animals. Fractional reabsorption of taurine is 0.967±0.013 (mean±SD) in A/J); and 0.839±0.08 and 0.787±0.05 in C57BL/6J and PRO/Re, respectively. Taurine concentration in renal cortex intracellular fluid (free of urine contamination) is similar in the three strains. Taurine reabsorption is inhibited by β-alanine, in tau^{t+} and tau^{t-} strains. These in vivo findings reveal residual taurine transport activity in the tau^{t-} phenotype and no evidence for impaired efflux at basilar membranes as the cause of impaired taurine reabsorption.

Cortex slices provide information about uptake of amino acids at the antiluminal membrane. Taurine behaves as an inert metabolite in mouse kidney cortex slices. Taurine uptake by slices is active and, at < 1 mM, is greater than normal in tau^{t-} slices. Concentration-dependent uptake studies reveal more than one taurine carrier in tau^{t+} and tau^{t-} strains. The apparent K_m values for uptake below 1 mM are different in tau^{t-} and tau^{t+} slices (~0.2 mM and ~0.7 mM, respectively); the apparent K_m values above 1 mM taurine are similar in tau^{t+} and tau^{t-} slices. Efflux from slices in all strains is the same (0.0105–0.0113 $\mu mol \cdot min^{-1} \cdot g^{-1}$ wet wt), but tau^{t-} tissue retains about 10% more radioactivity over the period of efflux.

β-Alanine is actively metabolized in mouse kidney. Its uptake, in the presence of blocked transamination, is greater; its intracellular oxidation is attenuated; and its exchange with intracellular taurine is diminished in tau^{t-} slices.

These findings indicate impaired β-amino acid permeation on a low-K_m uptake system at the luminal membrane in the tau^{t-} phenotype. β-Amino acids are not reclaimed efficiently either from the innermost luminal pool in cortex slices or from the ultrafiltrate in the tubule lumen in vivo. The former leads to high uptake ratios in vitro, the latter to high clearance rates in vivo. In vitro and in vivo data are thus concordant. This is the first time that a hereditary defect in amino acid transport has been assigned to a specific membrane surface in mammalian kidney.

INTRODUCTION

Although numerous hereditary disorders of amino acid transport are known in man (1), very few equivalent mutations have been described in animals which would permit a detailed analysis of the membrane defect (2) in vitro. Harris and Searle (3) reported that the urine of certain strains of inbred mouse contained increased amounts of taurine (2-aminoethanesulfonic acid), a β-amino compound (4). Gilbert et al. (5) showed that intraperitoneal (i.p.)[1] injection of other β-amino compounds increased taurine excretion in the mouse. They postulated that a defect in a selective mechanism for β-amino acid transport might account for the taurinuria in mutant mice. Others (6-8) have confirmed the pres-

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[1] *Abbreviations used in this paper:* AIB, aminoisobutyric acid; AOA, (aminooxy)acetic acid hemihydrochloride; ECF, extracellular water; ICF, intracellular water; i.p., intraperitoneal; PAH, para-aminohippuric acid; tau^{t+}, normal in net tubular reabsorption of taurine; tau^{t-}, hypertaurinuric; TTW, total tissue water.

ence of a β-amino-preferring system for tubular reabsorption in mammalian kidney in vivo; and Christensen's group has delineated a β-amino-preferring uptake system in the Ehrlich cell as a representative of mammalian cell plasma membranes in general (9).

The present investigations began with studies in the PRO/Re mouse (10), a hypertaurinuric strain with hyperprolinemia (11, 12). In the course of these earlier studies, we discovered that the striking hyperprolinuria in PRO/Re was in part a function of an increased intracellular concentration of proline in renal cortex with backflux into urine. In the meantime, Blake et al. (13) reported that taurine excretion in PRO/Re was excessive for the genetic background of the strain; he attributed this to interaction between proline and taurine during tubular reabsorption. When Bartsocas (14) then reported that taurine uptake by kidney cortex slices is normal in hypertaurinuric mice, we initiated a study of the mechanism of taurinuria in these strains, and of the potential proline-taurine interaction during transport.

We have investigated the A/J mouse strain with normal net tubular reabsorption of taurine (tau^{t+}) and the hypertaurinuric C57BL/6J and PRO/Re strains (tau^{t-}). We have compared our data for in vivo reclamation of taurine with our findings in vitro, obtained from small kidney cortex slices. Occlusion of the luminal membranes and selective exposure of basilar membranes of absorbing epithelium to substrate in the medium, which are constraints imposed by the slice technique (15), have been put to advantage in this investigation. Moreover, we have found that taurine is virtually an inert substance with respect to its renal metabolism in kidney, so that isotopic tracer studies informed us specifically about uptake characteristics.

Our investigations lead us to believe that hypertaurinuria in mouse mutants is a selective defect in net reabsorption of taurine (when net reabsorption = [filtered load + tubular efflux] − excreted amount). The defect involves the partial loss of the β-amino-preferring system which is located on the urinary (luminal) surface of the brush border.

METHODS

Animals. Three inbred strains of mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. The A/J albino mouse has "normal" taurine excretion, and metabolizes proline actively; for purposes of this investigation its phenotype is tau^{t+}/Pro^{m+}, where the superscript "t" indicates transport and "m" indicates metabolism. The C57BL/6J black mouse has hypertaurinuria, and normal proline metabolism; its phenotype is thus tau^{t-}/Pro^{m+}. The PRO/Re white mouse has deficient proline oxidase activity and hypertaurinuria, and is designated "tau^{t-}/Pro^{m-}." The strains were caged and bred separately and fed on standard Purina rodent chow.

In vivo investigations. Urine collections were made in Gelman metabolic cages for 3–24-h periods. The animals (about 25 g body wt) were fasted during urine collections. The standard procedure was to collect pooled urine from 9 mice for a timed period (usually about 3 h) or from 3–5 mice for 24 h, at the end of which time the urine was placed in clean vials and frozen immediately at −20°C. Unclotted venous blood was collected by retro-orbital vein puncture into heparinized tubes; this technique will provide up to 1.8 ml per animal. The plasma was immediately separated, with care not to stir the buffy coat, since platelets and leukocytes contain high concentrations of taurine (16). The plasma was immediately diluted 4 to 1 with sulfosalicyclic acid (3% wt/vol) and centrifuged; the protein-free supernate was recovered and stored at −20°C. Recovery of taurine is complete under the proper conditions (16).

The timed urine collection and plasma collection allowed estimation of renal clearance of taurine. Estimation of simultaneous endogenous creatinine and taurine clearances permitted us to calculate filtered and excreted taurine and its reclaimed fraction; standard equations were used. We compared the endogenous creatinine clearance values with inulin clearance values determined by an inulin infusion method (10), and after serial intraperitoneal and subcutaneous injection of inulin to achieve a steady state in blood. Steady state was ascertained by measurement of isotopic inulin in blood at timed intervals. Isotopic ^{14}C-carboxy-labeled inulin was given as a 10% solution in 0.9% NaCl via the i.p. route (3 ml/100 g body wt) and as 5% solution i.p. (3 ml/100 g) at 15 min. The latter dose was given again s.c. at 35 min, and clearance determinations were begun at 60 min.

This protocol also served for the estimation of renal extracellular water spaces in vivo. Kidney cortex was removed, weighed, and homogenized manually in distilled H_2O with a Potter-Elvehjem glass homogenizer, fitted with a Teflon plunger. Slices were also prepared on a Stadie-Riggs microtome and placed on Whatman no. 1 filter paper dampened with 0.5 ml of 0.9% NaCl. The tissue: plasma inulin ratio was then determined on boiled homogenates and slices. The taurine content of outer renal cortex was determined in homogenates and slice preparations. The tissue was treated as described above except for the addition of a homogenization step for slices. The concentration of taurine was measured in protein-free supernates after precipitation with sulfosalicylic acid (3% wt/vol). Taurine in heart muscle was determined on homogenates and slices in a similar manner. All supernatant preparations were stored frozen at −20°C, and all tissue preparation was done within a few moments of sacrifice. We determined that it is not necessary to quick-freeze the kidney before processing it for purposes of these experiments.

In vitro investigations. Thin cortex slices were prepared from adult mice weighing 22–30 g and incubated as described previously (17, 18). Single slices weighing between 3 and 15 mg were placed in Erlenmeyer or Warburg flasks prepared for measurement of uptake and oxidation of substrate. Each slice was incubated individually in 2.0 ml Tris-Ringer glucose buffer (300 mosM, pH 7.4, at 37°C under 100% O_2). After incubation the slice was removed, blotted, weighed, and placed in 1.0 ml boiling distilled water for 10 min. The supernate was recovered, placed in a scintillation vial containing Aquasol, and shaken vigorously. Filter papers soaked in 1 N KOH were used to trap CO_2 (17, 19) and were counted in a system containing water and Aquasol.

Total tissue water (TTW) was determined in slices by drying to constant weight at 110°C for 48 h. Extracellular

water (ECF) was determined by the [^{14}C]carboxyinulin method (20). Intracellular water (ICF) was derived by subtracting ECF from TTW.

After incubation, an aliquot of the medium was placed on Eastman cellulose sheets (Eastman Kodak Co., Rochester, N. Y.), and the one-dimensional chromatogram developed in a mixture of acetone, butanol, acetic acid, and water (35:35:10:20). The sheets were cut into 1-cm strips and placed in Aquasol in scintillation vials. [^{14}C]-Inulin, [^{14}C]taurine, and [^{14}C]alanine were run as markers; amino acids were located by ninhydrin staining. The uptake of substrate was calculated as described previously (17, 18).

Efflux of accumulated substrate was measured by the method of Segal et al. (21). The tissue was incubated in 2.0 ml of buffer containing the substrate until a steady-state uptake ratio was achieved; it was then removed from the uptake medium, blotted, weighed, and transferred to the efflux medium (2–8 ml vol). The rate of appearance of taurine in the medium was measured; and at the termination of the period of efflux the isotope remaining in the tissue was also measured.

Inhibition studies were performed by incubation of slices in the presence of substrate and inhibitor; preincubation with inhibitor was not necessary. Other amino acids were present at 10 times the substrate concentration. Equimolar quantities of choline chloride were substituted for NaCl in studies of sodium-dependent uptake. (Aminooxy)-acetic acid hemihydrochloride (0.2 mM) (AOA) was added to incubation at zero time to block completely transamination of β-alanine to malonic acid semialdehyde in studies of β-alanine transport (22). AOA does not inhibit the uptake process, as determined by examining its effect on uptake of α-aminoisobutyricacid and taurine in slices.

Analysis. Partition chromatography on filter paper in two dimensions (23) was used to confirm the presence of hypertaurinuria. Quantitative measurement of taurine was performed in protein-free samples after treatment, on a 4 × 1.5-cm Dowex 50-X12 ion exchange resin column in the H^+ form from which taurine was eluted with 3–5 ml of 0.02 N HCl. The eluate was evaporated to dryness and resuspended in 0.2 M citrate buffer, pH 3.25, for quantitative analysis on a Beckman model-120 amino acid analyzer (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) modified for simultaneous analysis of paired samples (24). Since only taurine and urea appear in the first 5-ml fraction from the preparative Dowex column, the analytical column can be run without regeneration, and rapid sequential analysis of taurine is permitted. The mean recovery of taurine through the entire procedure is 91.2%.

Creatinine in plasma and urine was determined by the semiautomated method of von Pilsum (25).

Slice experiments were done in triplicate and comparisons of data were made among the three strains with Student's t test.

Corrected values for simultaneous uptake of taurine on more than one system were obtained by solving the equation:

$$u_{obs} = \frac{V_{max_1} \cdot [S]}{K_{m_1} + [S]} + \frac{V_{max_2} \cdot [S]}{K_{m_2} + [S]} + \cdots n$$

according to the methods of Scriver and Mohyuddin (26) and Neal (27). Analysis of uptake kinetics is not perturbed in kidney slices when measurements are performed under steady-state conditions instead of initial-rate conditions (26, 28), particularly when the substrate is metabolically inert, as in this case with taurine.

Materials. [1-^{14}C]Taurine (sp act 4.5 μCi/μmol), [^{14}C]α-aminoisobutyric acid (sp act 4 μCi/μmol), [^{14}C]β-alanine (uniformly labeled) (sp act 4.2 μCi/μmol), and [^{14}C]carboxyinulin were purchased from New England Nuclear, Boston, Mass.; radiochemical purity was confirmed by appropriate one-dimensional partition chromatography.

Unlabeled amino acids and AOA were purchased from Sigma Chemical Co., St. Louis, Mo.

RESULTS

In vivo studies

PRO/Re and C57BL/6J mice, both classified as tau^{t-}, were confirmed to be hypertaurinuric relative to the A/J strain (tau^{t+}). Urine taurine concentration is increased 5–10-fold in the tau^{t-} strains while plasma taurine is comparable in tau^{t+} and tau^{t-} strains (Table I). The endogenous creatinine clearance per mouse is 0.202 ±0.088 ml·min^{-1} (mean±SD, $n = 36$) without significant variation between mouse strains. Creatinine clearance compares well with estimates determined by an inulin infusion technique in our laboratory (10). Appropriate calculation of fractional reabsorption of taurine from the data for creatinine clearance and taurine in plasma and urine reveal that net tubular reclamation of taurine is diminished in tau^{t-} strains (Table I).

The taurine concentration in the outer renal cortex was examined in tau^{t-} and tau^{t+} strains (Table II). Taurine in whole cortex homogenate was compared with taurine in the equivalent slice homogenate. Our purpose was to determine the contribution of urine trapped in uncollapsed nephrons to the estimate of tissue taurine. This artefact has been shown to be of some significance (10), since tubular urine is squeezed from the tissue during preparation of slices (15), but not in the preparation of whole cortex homogenate. Further precaution was taken by permitting the slices to leach on filter paper soaked in 0.9% NaCl at 4°C for 5 min as the tubule lumen collapsed. Taurine content was reduced by about 30% by the leaching procedure. The inulin content of renal cortex after in vivo infusion was also reduced by approximately one-third in kidney cortex slices handled in the foregoing manner, when compared to outer-cortex homogenate. Slices preloaded in vitro with α-aminoisobutyric acid (AIB), an inert amino acid, lose less than 15% of the label during the leaching procedure, indicating that the 30% loss of taurine from fresh slices is more likely to be "run out" from the collapsing lumen than efflux from cellular pools.

Slices and homogenates of heart muscle yielded comparable inulin spaces. The findings suggest that the kidney slice methodology used in the present study permits valid determination of tissue taurine content, uncontaminated by taurine in the lumen.

The taurine content of in vivo renal cortex was similar in the three strains of mice. Accordingly the cortex: plasma distribution ratio in vivo was comparable in

TABLE I

Concentration in Plasma and Urine and Fractional Reabsorption by Kidney of Taurine in Tau^{t+} and Tau^{t-} Mice

	Mouse strain			*P* values	
	A/J	C57BL/6J	PRO/Re	C57BL/6J vs. A/J	PRO/Re vs. C57BL/6J
Plasma, *μmol·liter^{-1} plasma water*	478 ±47 (*n* = 6)	557 ±91 (*n* = 6)	492 ±54 (*n* = 10)	NS	NS
Urine, *μmol·mg^{-1} creatinine*	2.51 ±0.75 (*n* = 6)	25.55 ±4.27 (*n* = 9)	25.69 ±3.32 (*n* = 10)	<0.001	NS
Urine, *μmol·ml^{-1}*	2.98 ±0.53 (*n* = 9)	14.34 ±3.83 (*n* = 5)	16.39 ±2.28 (*n* = 8)	<0.001	NS
Fractional reabsorption of taurine	0.967 ±0.013 (*n* = 3)	0.829 ±0.008 (*n* = 4)	0.787 ±0.05 (*n* = 6)	<0.001	NS

Urine was collected under timed conditions from 3–5 fasted animals for 24-h collections and from 9 fasted animals for 3-h (approx.) collections. Blood and urine were pooled from the mice for individual experiments; *n* refers to the number of experiments performed per cell in the table. Fractional reabsorption was calculated from the formula: 1 − (taurine clearance/creatinine clearance), where clearance is amount excreted per minute ÷ amount per milliliter plasma.

tau^{t-} and tau^{t+} strains (Table II), implying that tau^{t-} mice do not retain taurine in epithelial cells during in vivo transepithelial reclamation from urine to peritubular blood, or during any taurine flux that may occur in the reverse direction.

In vitro studies

Tissue water content. Water spaces in mouse kidney slices were similar in the three strains. The extracellular space is 24.5±1.6% (mean±SD, $n = 30$); the corresponding TTW is 79.2±0.4% and the derived intracellular space is 54.7% of the weight of the slice.

TABLE II

Concentration of Taurine in Kidney Cortex

	A/J	PRO/Re	*P* value
Homogenate, *μmol·g^{-1}*	13.97 0.73 (*n* = 6)	15.03 1.22 (*n* = 10)	NS
Homogenate, (mean values) *μmol·ml^{-1} ICF*	24.73	26.62	
Slice, *μmol·g^{-1}*	9.62 0.86 (*n* = 6)	9.43 0.76 (*n* = 10)	NS
Slice, (mean values) *μmol·ml^{-1} ICF*	16.96	16.63	
Slice: homogenate ratio	0.693	0.623	NS
Taurine distribution ratio*:			
Homogenate	49.5	53.2	NS
Slice	35.5	33.8	NS

Homogenate of whole outer cortex and slices of outer cortex were prepared as described in text. Slices were allowed to leach at 4°C for 5 min before homogenization.

* Micromole per microliter ICF/micromole per microliter plasma H_2O.

Time-course of uptake. Taurine uptake by cortex slices from the three mouse strains achieves a steady state after 45 min at 0.11, 1.0, and 20 mM taurine concentration in the medium (Fig. 1). Less than 2% of [^{14}C]taurine is converted to CO_2 and non-taurine-soluble derivatives during a 90-min incubation of tau^{t-} and tau^{t+} cortex slices. Accordingly, taurine behaves as an inert substance in mouse renal cortex; therefore, the isotopic distribution ratio can be considered equivalent to the chemical distribution ratio.

Taurine is taken up against a chemical gradient in all three strains of mice. The steady-state distribution ratio is significantly higher ($P < 0.01$) at 0.11 and 1.0 mM taurine in the tau^{t-} C57BL/6J and PRO/Re strains than in the A/J strain; PRO/Re kidney slices accumulate even more taurine than C57BL/6J slices (Table III). The taurine distribution ratio for small slices (4.98±0.3 mg, mean±SEM) was compared with that for the large slices (10.1±0.7 mg) used for most of the studies depicted in Fig. 1. The distribution ratio was increased in smaller slices in all strains; the relationship between tau^{t-} and tau^{t+} tissue was, however, retained (Fig. 1). Greater uptake for smaller slices is an expected observation (15, 29). The apparent paradox of enhanced uptake of taurine by tau^{t-} tissue will be illuminated by experimental data to follow.

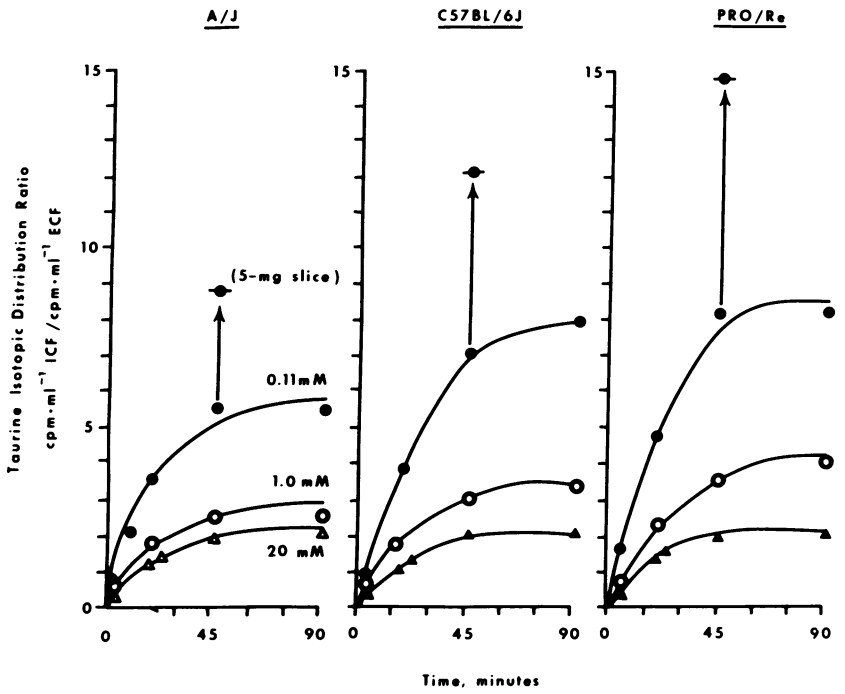

FIGURE 1 Time-course for uptake of [1-^{14}C]taurine by mouse kidney outer cortex slices at 37°C in pH 7.4 Tris buffer. The A/J strain is tau^{t+}; the C57BL/6J and PRO/Re strains are tau^{t-}. Slice weight affects uptake; small slices (approx. 5 mg) have higher uptake ratios than larger slices (approx. 10 mg). The effect of taurine concentration on uptake ratio is also shown. Conversion of taurine after uptake to CO_2 or other labeled material is $< 2\%$ of total uptake. Therefore, isotopic and chemical distribution ratios are comparable. Data are the mean of at least nine determinations at each point.

Concentration-dependent uptake. Taurine uptake at steady state is influenced by the extracellular concentration (Fig. 1); the uptake ratio falls as substrate concentration is increased in all three strains of mice. This finding suggests a saturable process for taurine uptake.

Uptake does not observe simple Michaelis kinetics over the concentration range 0.025–50 mM (Fig. 2). The appropriate Michaelis equation (26, 28) was solved by the method of Neal (27) to obtain corrected K_{m_1}, V_{max_1}, K_{m_2}, and V_{max_2} values (Table IV). The corresponding u vs. $u/[S]$ (Eadie-Augustinson) transformations of the uptake data yield biphasic regressions in each of the three species, which can be resolved into low-K_m and high-K_m components. Uptake of taurine on the low-K_m (high-affinity) system is apparently more avid in tau^{t-} strains, which have lower K_{m_1} values, than

TABLE III

*Distribution Ratio for Taurine (0.11 mM) in Mouse Kidney Cortex Slices**

Slice wt	A/J	C57BL/6J	PRO/Re	C57BL/6J vs. A/J	PRO/Re vs. C57BL/6J
mg					
4.98	8.90	12.22	14.87	$P < 0.001$	$P < 0.01$
±0.3 (SEM)	±0.62	±0.34	0.58		
	(n = 27)	(n = 24)	(n = 24)		
10.1	5.5	7.06	8.31	$P < 0.05$	NS
±0.7 (SEM)	±0.3	±1.17	±0.89		
	(n = 12)	(n = 12)	(n = 12)		

* Cortex slices were incubated at pH 7.4 for 45 min at 37°C and the distribution ratio determined as described in text.

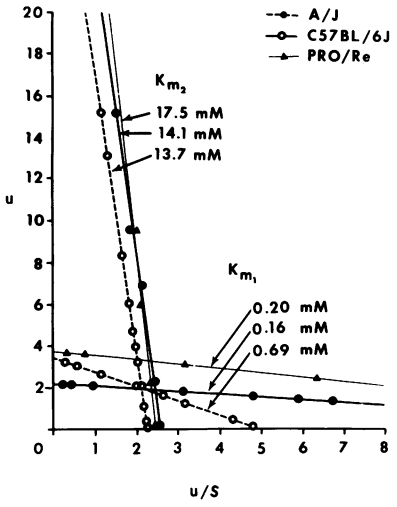

FIGURE 2 Concentration-dependent kinetics for taurine uptake at steady state by mouse kidney cortex slices examined over a 600-fold variation in substrate concentration. The Eadie-Augustinson transformations reveal more than one mode of uptake, both in tau^{t+} (A/J) and tau^{t-} (C57BL/6J and PRO/Re) strains. Uptake rates have been corrected for contribution of the "low-K_m" system to "high-K_m" uptake and visa versa at each substrate concentration, according to methods described in text. The apparently greater affinity for taurine uptake in tau^{t-} strains at low substrate concentrations is a function of the higher uptake observed in these strains (Fig. 1). Data are the composite of several hundred determinations over the total range of taurine concentration.

TABLE IV

Concentration-Dependent Uptake of Taurine at Steady State: K_m and V_{max} Values in Tau^{t+} and Tau^{t-} Strains

Parameter*	A/J	C57BL/6J	PRO/Re
K_{m_1} (low range), *mM*	0.69	0.16	0.20
V_{max_1} (low range) *mmol·45 min^{-1}·g^{-1}*	3.43	2.39	3.80
K_{m_2} (high range), *mM*	13.7	14.1	17.5
V_{max_2} (high range) *mmol·45 min^{-1}·g^{-1}*	31.3	36.7	43.2

* Evidence for more than one mode of taurine uptake was observed (Fig. 2). Total uptake is therefore characterized by the equation [16, 22]

$$u_{obs} = \frac{V_{max_1}\cdot[S]}{K_{m_1}+[S]} + \frac{V_{max_2}\cdot[S]}{K_{m_2}+[S]} + \cdots n.$$

Assuming only two uptake systems values for K_{m_1}, K_{m_2}, V_{max_1} and V_{max_2} can be obtained by inserting paired values for u and $[S]$ at various taurine concentrations and solving the equation by the method of Neal[27]. 14 pairs of data were used for each mouse strain.

in the A/J strain (Table IV). This finding is not apparent at the higher (> 1 mM) concentrations of taurine.

Taurine efflux. Slices were preloaded with taurine to comparable levels in ICF; efflux into taurine-free medium was then examined over 30 min at 37°C. At low levels of intracellular taurine (1 mM), substrate remaining in the slice was about 10% higher in tau^{t-} tissue compared to A/J cortex at 3 min and at 25 min (Fig. 3), but the efflux constant was comparable in all

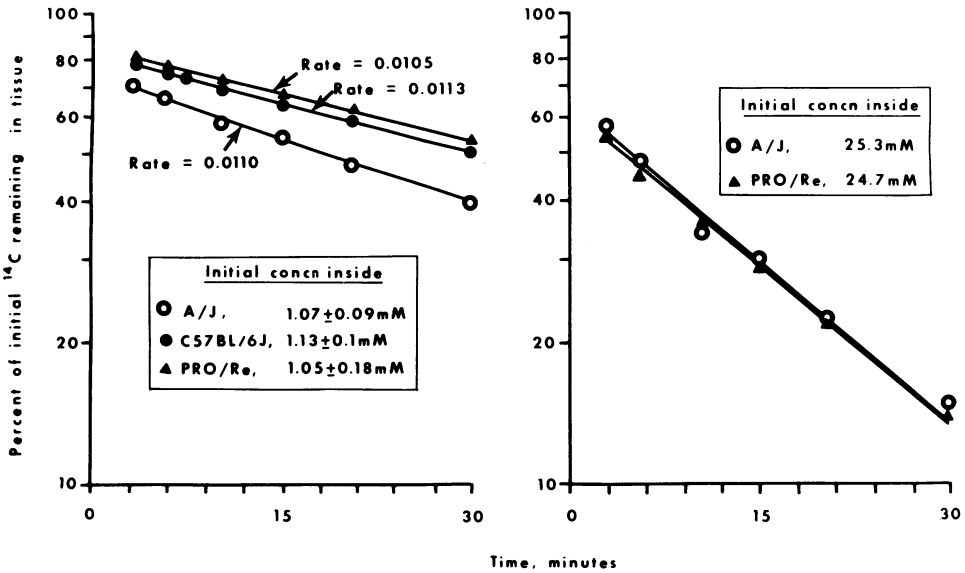

FIGURE 3 Efflux of [1-^{14}C]taurine at 37°C at low and high concentrations from mouse kidney cortex slices preloaded with the substrate so that the concentration in the slices (insets) is similar in the different strains. Efflux was performed into pH 7.4 Tris buffer free of taurine. Rates are calculated as micromoles/minute per gram wet weight. At lower taurine concentration, pool depletion is attenuated in tau^{t-} strains but efflux is similar in tau^{t+} and tau^{t-} strains. Data are the mean of at least 18 determinations at each point.

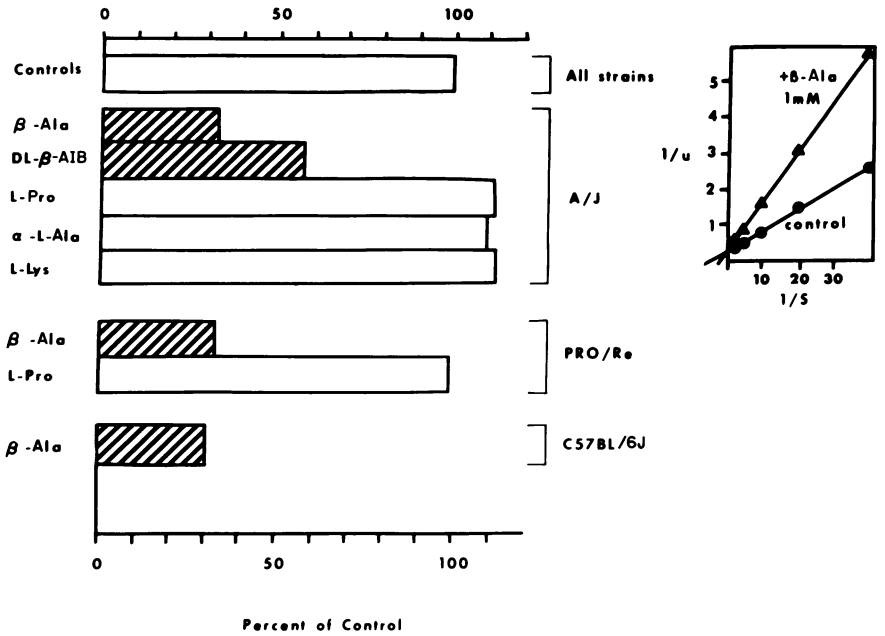

FIGURE 4 Effect of other amino acids (at 1.1 mM) on the initial 10-min uptake of taurine (0.11 mM) accommodated predominantly on the "low-K_m" system by tau^{t+} and tau^{t-} mouse kidney cortex slices. Uptake is significantly inhibited (hatched bars, $P < 0.001$) only by other β-amino acids. Conventional Lineweaver-Burk transformations of uptake data reveal competitive inhibition (inset). Similar observations are obtained when the taurine concentration is 5 mM so that the major fraction of taurine uptake takes place on the "high-K_m" system. Data are mean of quadruplicate determinations.

three strains (A/J, 0.0110 μmole·ml^{-1} ICF·min^{-1}; C57BL/6J, 0.0111; PRO/Re, 0.0105). At high internal taurine (about 25 mM) tau^{t-} and tau^{t+} tissue observed identical efflux (Fig. 3). Efflux performed at 17°C, was slower, but interstrain differences were retained as above. Simultaneous preloading with proline (10 mM) or efflux into 10 mM proline medium did not modify taurine efflux in the A/J strain.

These findings suggest that hyperexcretion of taurine in vivo and enhanced uptake in vitro are not the result of deficient taurine efflux at the basilar membrane of absorbing epithelium. These conclusions are supported by the comparable concentrations of taurine in renal cortex in tau^{t+} and tau^{t-} strains in vivo. The high intracellular proline concentration in PRO/Re epithelium (10) does not appear to interact with taurine during efflux to account for the higher taurine uptake ratios in this strain.

Inhibitors of taurine uptake. Concentrative uptake of taurine requires metabolic integrity. Boiling of the tissue, acid conditions (pH 5.0), and incubation under nitrogen or exposure to cyanide (10^{-3} M) abolish concentrative uptake of taurine by slices. Removal of Na^+ from the external medium also abolishes concentrative uptake at low and high taurine level.

Interaction between taurine and various α-amino acids and other β-amino compounds was examined. Only β-amino acids inhibit the uptake of taurine in all three strains (Fig. 4). The inhibition by β-amino acids was shown to be competitive by conventional methods (Fig. 4); the interaction pertained equally to the low-K_m and the high-K_m uptake systems.

β-Alanine uptake by tau^{t+} and tau^{t-} kidney cortex slices. Since β-alanine uptake by kidney is shared with taurine and other β-amino acids in vivo and in vitro (references 5-9 and the foregoing observations), we anticipated that β-alanine uptake would resemble that of taurine in tau^{t+} and tau^{t-} kidney. We found the isotopic distribution ratio for β-alanine to be higher in tau^{t-} slices (Table V). This is true when transamination of β-alanine and its subsequent oxidation to CO_2 are blocked in vitro by AOA (0.2 mM); it is also true when AOA (25 mg/kg, i.p.) is injected in vivo 90 min before sacrifice and preparation of kidney cortex slices. In the absence of AOA, ^{14}C derived from β-alanine is partitioned in the gaseous phase, as CO_2, and in the soluble pool, where chromatography shows it to appear as β-alanine alone. The fraction retained as β-alanine in the soluble pool is twice as large in the tau^{t-} slice when compared with the tau^{t+} slice (Table V). This is not be-

TABLE V

*Distribution of β-Alanine in Tau^{t+} and Tau^{t-} Mouse Kidney Cortex**

Isotopic distribution ratio for [^{14}C]β-alanine	Mouse strain‡ A/J	PRO/Re	*P* values
(*a*) Slice with AOA§			
In soluble pool‖	15.85±1.55	22.6±0.66	<0.01
(*b*) Slice without AOA			
In soluble pool, ‖ *cpm·g^{-1} wet wt slice*	3.8±0.21	9.54±0.71	<0.001
In CO_2 fraction, ¶ *cpm·g^{-1} wet wt slice*	2.9±0.098	2.56±0.193	<0.01
% of total uptake retained in soluble pool	28±0.5	54±3.9	<0.001
(*c*) Slice homogenate without AOA (0.01 mM β-Ala)**			
In CO_2 fraction, *cpm·g^{-1} wet wt homog.*	1.24±0.098	1.29±0.078	NS

* After exposure of tissue to 0.11 mM β-alanine (except *c*) for 60 min at 37°C at pH 7.4. Slices were 5 mg wet wt. All values are mean±SD for six or more determinations at steady state.

‡ A/J is tau^{t+}; PRO/Re is tau^{t-}.

§ AOA (0.2 mM) was added to medium at zero time to block oxidation of β-alanine.

‖ "Soluble pool" refers to ^{14}C recovered in supernate after boiling tissue for 5 min; recovered label is β-alanine exclusively as shown by chromatography.

¶ CO_2 collected in center well on KOH-soaked filter paper; cpm are for total time (60 min).

** Slices prepared as usual and then homogenized.

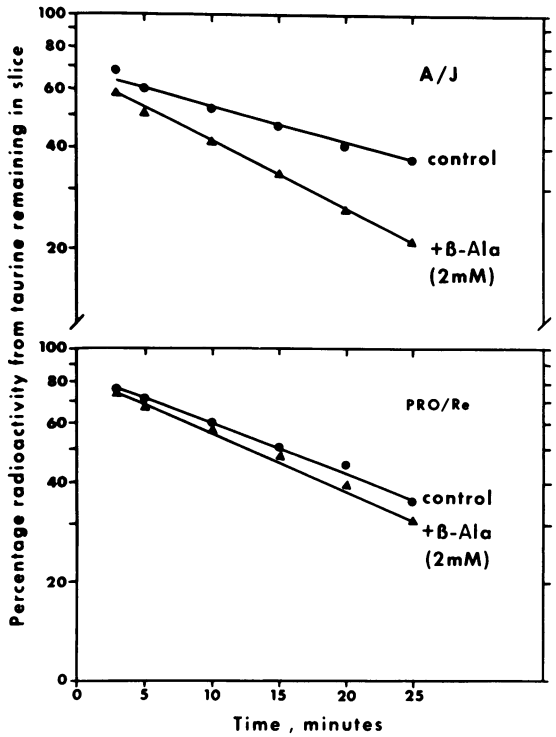

FIGURE 5 Effect of β-alanine (2 mM in external medium) upon taurine counterflow, when the internal concentration of the latter is 0.5 mM, in tau^{t+} (A/J) and tau^{t-} (PRO/Re) kidney cortex slices. Counterflow is significantly enhanced ($P < 0.01$) only in tau^{t+} slices. Data are the mean of six determinations at each point.

cause conversion of β-alanine to CO_2 is greatly impaired in tau^{t-} cortex, since we observed that CO_2 formed per gram wet weight is the same in homogenized slices from tau^{t+} and tau^{t-} animals. β-Alanine conversion to CO_2 is significantly diminished only in slices whose architectural integrity is retained (Table V).

Because β-alanine shares the taurine transport system, extracellular β-alanine can exchange with intracellular taurine by counterflow. We observed that taurine efflux from tau^{t+} slices is significantly enhanced by external β-alanine when compared with tau^{t-} tissue (Fig. 5).

TABLE VI

*Effect of β-Alanine Injection on Taurine Excretion in Urine by Tau^{t+} and Tau^{t-} Mice**

	A/J	C57BL/6J
(*a*) Control values		
μmol·ml^{-1}	1.35	10.95
Fractional reabsorption	0.94	0.84
(*b*) After β-alanine (25 mg i.p. per mouse)‡		
μmol·ml^{-1}	28.8	60.8
Fractional reabsorption	0.53	0.47
Change in fractional reabsorption (*a* minus *b*) §	−0.41	−0.37

* Studies performed as described in Table I. Results are the mean of duplicate observations on two sets of nine mice.

‡ Plasma level of β-alanine 1 h after injection was 0.58 mM in A/J and 1.43 mM in C57BL/6J; normal, <0.01 mM.

§ Sign indicates direction of change.

These findings imply that the β-alanine or taurine taken up by tau^{t-} slices is partially sequestered in a pool inaccessible to cytoplasmic oxidation or to exchange with solute in the medium.

β-Alanine interaction with taurine reabsorption in vivo. Taurine excretion in urine increases after intraperitoneal injection of β-alanine (Table VI); this observation corroborates earlier reports covering other strains of mice (5) and other mammalian species (6, 8). Taurine excretion is increased in the C57BL/6J strain (tau^{t-}), after exposure to β-alanine, indicating retention of a carrier with which β-alanine can interact in the mutant strain in vivo; the degree of inhibition by β-alanine is comparable in tau^{t+} and tau^{t-} strains when differences in plasma level of β-alanine achieved after i.p. injection are taken into account (Table VI).

DISCUSSION

Taurine, a β-amino acid produced by irreversible decarboxylation of cysteic acid or oxidation of hypotaurine (4), has virtually no further metabolism in mouse kidney. Consequently it is an ideal substrate for in vivo and in vitro investigation of the mechanism for the hereditary impairment of β-amino acid transport in mice.

Net renal tubular reclamation of taurine is decreased, at similar filtered loads of taurine, in both tau^{t-} strains (C57BL/6J and PRO/Re) when compared to the tau^{t+} strain (A/J) used in these investigations. The 10-fold increase in taurine excretion confirms earlier observations (3). Our studies also indicate that urinary loss of taurine in the tau^{t-} strains is not accompanied by excessive taurine storage in renal cortex in vivo. The latter finding indicates that accumulation of taurine in absorbing epithelium, with backflux into urine, is clearly not the mechanism for hypertaurinuria in tau^{t-} mice.

The in vitro steady-state distribution ratio for taurine, at physiological concentrations, is enhanced in kidney cortex slices prepared from tau^{t-} mice. β-Alanine, which shares the taurine (β-amino-preferring) transport system and is also excreted in excess in vivo in tau^{t-} mice, is also taken up by tau^{t-} slices more avidly than by tau^{t+} slices. β-Alanine, unlike taurine, is oxidized by kidney cortex. Its oxidation is less vigorous in tau^{t-} slices, but this anomaly is not the fault of a deficient oxidizing system. These findings suggest that both β-amino compounds are partially sequestered, after uptake by slices, in a tissue pool which does not equilibrate rapidly with the incubation medium and which is separated from the cytoplasmic pool where substrate oxidation occurs. We believe the kidney cortex slice method, in this case, is an effective means of delineating the topology of the transport defect in tau^{t-} kidney.

Wedeen and Weiner (15, 20) examined the distribution of tritium-labeled inert metabolites after incubation of mammalian kidney cortex slices in vitro. Inulin was confined to an extracellular space in contact only with basilar and lateral membranes; no inulin appeared in the tubular lumen. α-AIB was concentrated in proximal tubule cells, little being found in the lumen. Para-aminohippuric acid (PAH) was accumulated maximally in the lumen of the proximal tubule. These observations suggest a reinterpretation of earlier studies of slice pools (30) and lead to the following conclusion. Kidney cortex slices expose basilar (and lateral) membranes to solute uptake from the incubation medium. The punctate contacts (tight junctions) between epithelial cells (31) are essentially impervious to organic solutes; inulin cannot penetrate the tight junctions, and PAH does not readily leak back through them after being concentrated in the lumen. This implies that any AIB which passes from epithelial cells into the lumen is not necessarily removed by passage through tight junctions but is more likely to be reclaimed and concentrated by the epithelium. These relationships can exist only in the presence of normal architectural integrity of tubular epithelium and only if the extracellular (peritubular), intracellular, and luminal (urinary) pools in slices exist in series. It is then possible to explain why tau^{t-} kidney retains more taurine and β-alanine in slices and loses more of these substances into urine.

We believe the transport defect in tau^{t-} kidney occurs on the urinary surface of the luminal membrane. In vitro uptake by tau^{t-} slices exposes a defect in reclamation of taurine from the luminal pool, back across the luminal membrane of epithelial cells (designated flux "J_s" in Fig. 6). Under steady-state conditions, a higher taurine uptake ratio is observed because the luminal "lacuna" comes to retain more taurine with impaired reclamation from the lacuna.

The enhanced net uptake of taurine by the intact slice per unit time (u), caused by lacunar sequestration in the tau^{t-} slices, will cause the apparent K_m value to decrease, all other components being equal, as derived from the transformed Michaelis equation

$$K_m = \left(\frac{V_{max} \cdot [S]}{u}\right) - S.$$

The lowering of K_{m1} at low concentrations of taurine in C57BL/6J and PRO/Re tau^{t-} slices (Fig. 2) is therefore a reasonable finding and not a reflection of more avid binding and uptake at the basilar membrane. By similar reasoning β-alanine, trapped in the internal "lacuna," is prevented from being oxidized normally by cytoplasmic enzymes, hence the decrease in β-alanine oxidation by intact tau^{t-} slices, but not by slice homogenates.

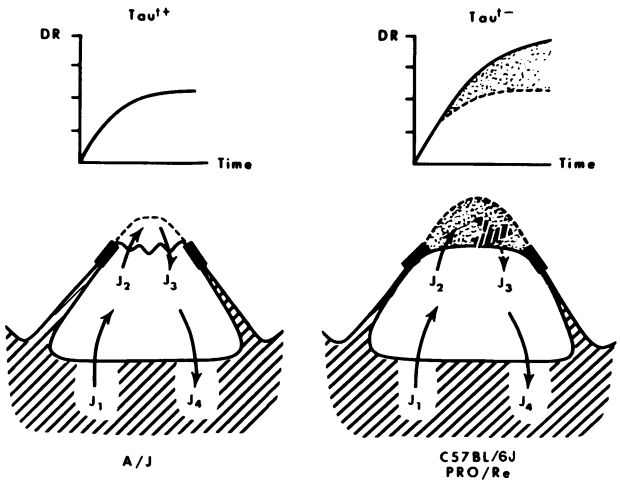

FIGURE 6 *Top half:* an interpretation of distribution ratio (DR) data obtained in tau^{t+} (A/J) and tau^{t-} (C57BL/6J and PRO/Re) slices. *Bottom diagrams:* hatched area is inulin space (extracellular pool); adjacent cell membranes are basilar and lateral membranes of epithelial cells. Luminal pool (bounded by brush border and dotted line) can be entered only serially via cytoplasmic pool because of tight junctions (heavy bars). Four fluxes (J_1–J_4) are shown: influx across basilar membrane (J_1), efflux into luminal pool (J_2), reclamation flux from luminal pool (J_3), and efflux across basilar membrane (J_4). Accumulation of taurine (and β-alanine) in luminal pool at steady state will reflect a decreased permeability component in flux J_3. Assuming $J_3 = [\text{tau}] \times \text{permeability}_{lc}$, where lc is the lumen-to-cell movement, the change in J_3 relationship in this case is the result of an impairment of transport at the β-amino-preferring site on the urinary surface of the luminal membrane.

Studies of taurine efflux from similar internal taurine pools in tau^{t+} and tau^{t-} slices reveal a retardation of total pool depletion but not a retarded rate in tau^{t-} slices. Counterflow between β-alanine and taurine from slice to medium is also attenuated in tau^{t-} slices. These findings are again compatible with sequestration of solute in the luminal pool in tau^{t-} kidney. The in vitro findings are thus concordant with the in vivo finding of taurine "sequestration" in urine. It is the topology of the transepithelial transport process, both in vivo and in vitro, that permits us to assign the site of the transport defect to the luminal membrane on its urinary surface. Direct evidence for our hypothesis must await the appropriate studies with soluble-label radioautography (32) and binding studies with isolated brush-border membranes.

A study of concentration-dependent uptake reveals more than one uptake system for taurine in the basilar membrane exposed by the slice preparation. We found a similar display of kidney sites for β-alanine uptake in earlier work in the rat (22). This finding is in keeping with the studies by Christensen (9) in Ehrlich ascites tumor cells with respect to β-alanine uptake. Our findings differ in that taurine is transported by both low- and high-K_m systems whereas it is not on the low-K_m, β-amino-preferring system in tumor cells; and β-amino acids interact on both systems in kidney whereas they do not on the high-K_m system in tumor cells. Our data suggest that tau^{t-} slices sequester taurine and β-alanine only at their lower concentration range. The finding suggests that a "low-K_m" system exists for β-amino acid uptake in the normal luminal membrane which is deficient in the tau^{t-} luminal membrane, but not in the basilar membrane. The ability of β-alanine to inhibit residual taurine reclamation in vivo in tau^{t-} implies retention of the "high-K_m" system alone or of a normal high-K_m system and a modified low-K_m system which permits transport at a reduced rate and reduced interaction with β-alanine. Our data do not permit us to choose between these alternatives. Our evidence for genetic independence of basilar and luminal β-amino acid transport systems is germane, however, in view of many current proposals that the two membranes possess different functional characteristics for biological transport (cited in reference 1).

Several details of our studies require comment. High serum taurine levels in tau^{t-} and tau^{t+} mice were reported by Blake (13); his data do not agree with our plasma findings. Different methodology is one explanation for the interlaboratory variation. Moreover, our use of plasma, instead of serum, avoids release of taurine from platelets during clot retraction (16).

Our handling of slice and whole-cortex homogenates before the chemical determination of taurine in kidney tissue has revealed that urinary solute trapped in the lumen apparently contributes to the tissue measurement. Attention to this detail in earlier studies allowed us to demonstrate true intracellular accumulation of proline in the transepithelial transport sequence in PRO/Re kidney (10).

We observed that small thin slices attain a higher solute uptake ratio than larger slices. The observation is not new (33, 34), and it is readily interpreted by the findings of Wedeen and Weiner (15, 29), who showed limited penetration of solute into the depths of the thicker slices. We believe this phenomenon may explain the failure of Bartsocas (14) to observe a statistically significant increase in the taurine uptake ratio in tau^{t-} kidney slices. However, in his study, the mean uptake ratio in 18 tau^{t-} slices was 11.6% higher than in 15 tau^{t+} slices, a finding in keeping with our own data based on several hundred slices.

The present and preceding (10) studies reveal how mutation in the mouse can serve the study of tubular transport of amino acids. In the present paper, we have also shown that the particular topology of the cortex slice, which in the opinion of some investigators is a detriment, can be put to use to define the component of transepithelial transport relationships.

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REFERENCES

1. Scriver, C. R., and M. Bergeron. 1974. Amino acid transport in kidney. The use of mutation to dissect membrane and transepithelial transport. *In* Heritable Disorders of Amino Acid Metabolism. W. L. Nyhan, editor. John Wiley & Sons, Inc., New York. 515–592.
2. Scriver, C. R., and P. Hechtman. 1970. Human genetics of membrane transport with emphasis on amino acids. *Adv. Hum. Genet.* **1**: 211–274.
3. Harris, H., and A. G. Searle. 1953. Urinary amino acids in mice of different genotypes. *Ann. Eugen. (Lond.).* **17**: 165–167.
4. Jacobsen, J. G., and L. H. Smith, Jr. 1968. Biochemistry and physiology of taurine and taurine derivatives. *Physiol. Rev.* **48**: 424–511.
5. Gilbert, J. B., Y. Ku, L. L. Rogers, and R. J. Williams. 1960. The increase in urinary taurine after intraperitoneal administration of amino acids to the mouse. *J. Biol. Chem.* **235**: 1055–1060.
6. Wilson, O. H., and C. R. Scriver. 1967. Specificity of transport of neutral and basic amino acids in rat kidney. *Am. J. Physiol.* **213**: 185–190.
7. Scriver, C. R., S. Pueschel, and E. Davies. 1966. Hyper-β-alaninemia associated with β-aminoaciduria and γ-aminobutyricaciduria, somnolence and seizures. *N. Engl. J. Med.* **274**: 636–643.
8. Goldman, H., and C. R. Scriver. 1967. A transport system in mammalian kidney with preference for β-amino compounds. *Pediatr. Res.* **1**: 212–213. (Abstr.)
9. Christensen, H. N. 1964. Relations in the transport of β-alanine and the α-amino acids in the Ehrlich cell. J. *Biol. Chem.* **239**: 3584–3589.
10. Scriver, C. R., R. R. McInnes, and F. Mohyuddin. 1975. Role of epithelial architecture and intracellular metabolism in proline uptake and transtubular reclamation in *PRO/Re* mouse kidney. *Proc. Natl. Acad. Sci. U. S. A.* **72**: 1431–1435.
11. Blake, R. L., and E. S. Russell. 1972. Hyperprolinemia and prolinuria in a new inbred strain of mice, PRO/Re. *Science (Wash. D. C.).* **176**: 809–811.
12. Blake, R. L. 1972. Animal model for hyperprolinaemia: deficiency of mouse proline oxidase activity. *Biochem. J.* **129**: 987–989.
13. Blake, R. L., R. V. Grillo, and E. S. Russell. 1974. Increased taurine excretion in hereditary hyperprolinemia of the mouse. *Life Sci.* **14**: 1285–1290.
14. Bartsocas, C. S. 1974. Nature of taurine transport defect in inbred mice: intracellular accumulation in vitro in kidney and intestine. *J. Int. Res. Comm. (IRCS/Med. Sci.).* **2**: 1290.
15. Wedeen, R. P., and B. Weiner. 1973. The distribution of *p*-aminohippuric acid in rat kidney slices. I. Tubular localization. *Kidney Int.* **3**: 205–213.
16. Scriver, C. R., C. L. Clow, and P. Lamm. 1971. Plasma amino acids: screening, quantitation and interpretation. *Am. J. Clin. Nutr.* **24**: 876–890.
17. Mohyuddin, F., and C. R. Scriver. 1970. Amino acid transport in mammalian kidney: multiple systems for imino acids and glycine in rat kidney. *Am. J. Physiol.* **219**: 1–8.
18. Rosenberg, L. E., A. Blair, and S. Segal. 1961. Transport of amino acids by slices of rat-kidney cortex. *Biochim. Biophys. Acta.* **54**: 479–488.
19. Buhler, D. R. 1962. A simple scintillation counting technique for assaying $C^{14}O_2$ in a Warburg flask. *Anal. Biochem.* **4**: 413–417.
20. Rosenberg, L. E., S. J. Downing, and S. Segal. 1962. Extracellular space estimation in rat kidney slices using C^{14} saccharides and phlorizin. *Am. J. Physiol.* **202**: 800–804.
21. Segal, S., C. Rea, and I. Smith. 1971. Separate transport systems for sugars and amino acids in developing rat kidney cortex. *Proc. Natl. Acad. Sci. U. S. A.* **68**: 372–376.
22. Nutzenadel, W., and C. R. Scriver. 1975. Transport and metabolism of β-alanine and β-alanyl-L-histidine (L-carnosine) by rat intestinal enterocytes, kidney cortex and striated muscle in vitro. Role in nutrition. *Am. J. Physiol.* In press.
23. Dent, C. E. 1948. A study of the behaviour of some sixty amino-acids and other ninhydrin-reacting substances on phenol-"collidine" filter-paper chromatograms, with notes as to the occurrence of some of them in biological fluids. *Biochem. J.* **43**: 169–180.
24. Scriver, C. R., E. Davies, and P. Lamm. 1968. Accelerated selective short column chromatography of neutral and acidic amino acids on a Beckman-Spinco analyzer, modified for simultaneous analysis of two samples. *Clin. Biochem.* **1**: 179–191.
25. Von Pilsum, J. F. 1959. Picric acid method: Lloyd's reagent modification for creatinine, in blood serum and urine. *Methods Biochem. Anal.* **7**: 200–201.
26. Scriver, C. R., and F. Mohyuddin. 1968. Amino acid transport in kidney: heterogeneity of α-aminoisobutyric uptake. *J. Biol Chem.* **243**: 3207–3213.
27. Neal, J. L. 1972. Analysis of Michaelis kinetics for two independent, saturable membrane transport functions. *J. Theor. Biol.* **35**: 113–118.
28. Tenenhouse, A., and J. H. Quastel. 1960. Amino acid accumulation in Ehrlich ascites carcinoma cells. *Can. J. Biochem. Physiol.* **38**: 1311–1326.
29. Wedeen, R. P., and B. Weiner. 1973. The distribution of *p*-aminohippuric acid in rat kidney slices. II. Depth of uptake. *Kidney Int.* **3**: 214–221.
30. Rosenberg, L. E., M. Berman, and S. Segal. 1963. Studies of the kinetics of amino acid transport, incorporation into protein and oxidation in kidney-cortex slices. *Biochim. Biophys. Acta.* **71**: 664–675.
31. Diamond, J. M. 1974. Tight and leaky junctions of epithelia. A perspective on kisses in the dark. *Fed. Proc.* **33**: 2220–2224.
32. Wedeen, R. P. 1972. Section freeze-dry autoradiography of *p*-aminohippuric acid and amino acid transport in the mammalian tubule. *In* Radionuclides in Nephrology. M. D. Blaufox and J-L. Funck-Brentano, editors. Chapter 8. Grune & Stratton, Inc., New York. 209.
33. Webber, W. A. 1970. The influence of size and shape of kidney tissue from newborn and mature rats on the uptake of amino acid. *Can. J. Physiol. Pharmacol.* **48**: 152–154.
34. Smith, I., and S. Segal. 1968. The influence of size of rat kidney cortex slices on the accumulation of amino acids. *Biochim. Biophys. Acta.* **163**: 281–283.