Proceedings of the National Academy of Sciences
Vol. 68, No. 2, pp. 372-376, February 1971

Separate Transport Systems for Sugars and Amino Acids in Developing Rat Kidney Cortex

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Communicated by W. M. Krogman, November 23, 1970

ABSTRACT **The ability of renal cortical slices of newborn and young rats to accumulate a nonmetabolizable sugar, α-methylglucoside, is slight and does not reach adult capacity until 25 days of age. However, a rudimentary sugar transport system is present, as indicated by a further decrease in accumulation in the presence of phlorizin or absence of sodium ion.**

Amino acid uptake in immature kidney tissue is not deficient; on the contrary, the tissue took up and concentrated more glycine and lysine than adult tissue. Decreased amino acid efflux from the immature cells appears to be the explanation. Concentration dependence of amino acid uptake was the same in 5-day-old and adult tissue.

These differences between the transport characteristics of a model sugar and representative amino acids during development indicate separate transport systems for the two types of substrate.

A relationship between renal transport of hexoses and amino acids has been well documented. Aminoaciduria exists with the mellituria of diabetes mellitus (1), galactosemia (2), fructose intolerance (3), and the Fanconi syndrome (4); galactose feeding to rats (5) and hexose infusion into humans produces aminoaciduria (6). Previous reports from this laboratory have substantiated an interaction of amino acids and sugars in the kidney by the demonstration *in vitro* that glucose, galactose, and fructose inhibit the accumulation of some amino acids by rat kidney cortex slices (7, 8). The inhibition was noncompetitive.

After the interplay between hexose and amino acid *in vitro* transport in kidney had been reported, a similar phenomenon was reported to occur in intestinal transport (9, 10). Among those investigating intestinal amino acid transport, a controversy has arisen in regard to the basic mechanism of the sugar inhibition (11). This has been well summarized by Alvarado (12). A major area of discussion has been whether the same transport mechanism or system is involved in transmembranous movement of both sugars and amino acids.

To further explore the basis of the hexose inhibition of amino acid transport in mammalian kidney cortex, we have compared *in vitro* the developmental pattern of the transport capability for a nonmetabolizable glucose analog, α-methylglucoside (α-MeG), with that for glycine, L-lysine, and L-proline. The transport differences observed during postnatal maturation of the rat kidney cortex indicate that the transport systems for sugars and amino acids are different in this tissue.

METHODS

The technique for assessing intracellular accumulation of radioactive amino acids and sugars by kidney cortex slices of adult, newborn, and immature Sprague-Dawley rats in Krebs-Ringer bicarbonate buffer, pH 7.35, has been described (13–15). Conditions for the determination of efflux (14) and for the demonstration of exchange diffusion have been published (15). The validity of the comparison of transport in small segments of renal cortex weighing 1 mg with larger slices or segments of slices has also been established (16).

[U-^{14}C]α-methylglucoside was used as a model sugar since it has been shown to share the characteristics of the glucose–galactose transport system in rabbit renal cortex but it is not metabolized by rat renal cortex (17, 18). [2-^{14}C]glycine and [U-^{14}C]L-lysine were used to represent the "neutral" and basic amino acid, and [U-^{14}C]L-proline the imino acid transport systems. While the intracellular radioactivity after incubation of glycine and lysine is essentially all in the form of the substrate, as shown by chromatographic methods (13), the ^{14}C after incubation of proline is, to a great extent, found in glutamic acid (11).

The uptake of the substrate by the renal cortical slices was calculated by the technique of Rosenberg, Blair, and Segal (13) in terms of tissue water spaces assessed previously (15), and is expressed as the distribution ratio, the ratio of the concentration of radioactivity (cpm/ml) in intracellular fluid to the corresponding concentration in the medium. This represents a true concentration gradient for α-MG, glycine, and L-lysine. For proline uptake this is a ratio of radioactivity only, but it has been shown to be representative of proline transport by Baerlocher, Scriver, and Mohyuddin (19).

MATERIALS

[U-^{14}C]α-methyl-D-glucoside, 73.4 μCi/mmol, was purchased from Calbiochem. [2-^{14}C] Glycine (5.13 mCi/mmol, [U-^{14}C]L-lysine (269 mCi/mmol), and [U-^{14}C]L-proline (209 mCi/mmol) were purchased from New England Nuclear Corp. Substrates were found to be pure by thin-layer or paper chromatography. Unlabeled α-MeG was purchased from Pfanstiehl Co. and was found to be free of glucose by gas–liquid chromatography. Unlabeled amino acids were purchased from Mann Research.

RESULTS

Age-dependent uptake of α-MeG and amino acids

The accumulation of α-MeG glycine, and lysine by kidney cortex slices from rats of various ages from birth to adulthood was assessed at both low and high concentrations because of the known differences in concentration dependence in adult tissues (21, 22). The results are shown in Fig. 1. αMeG

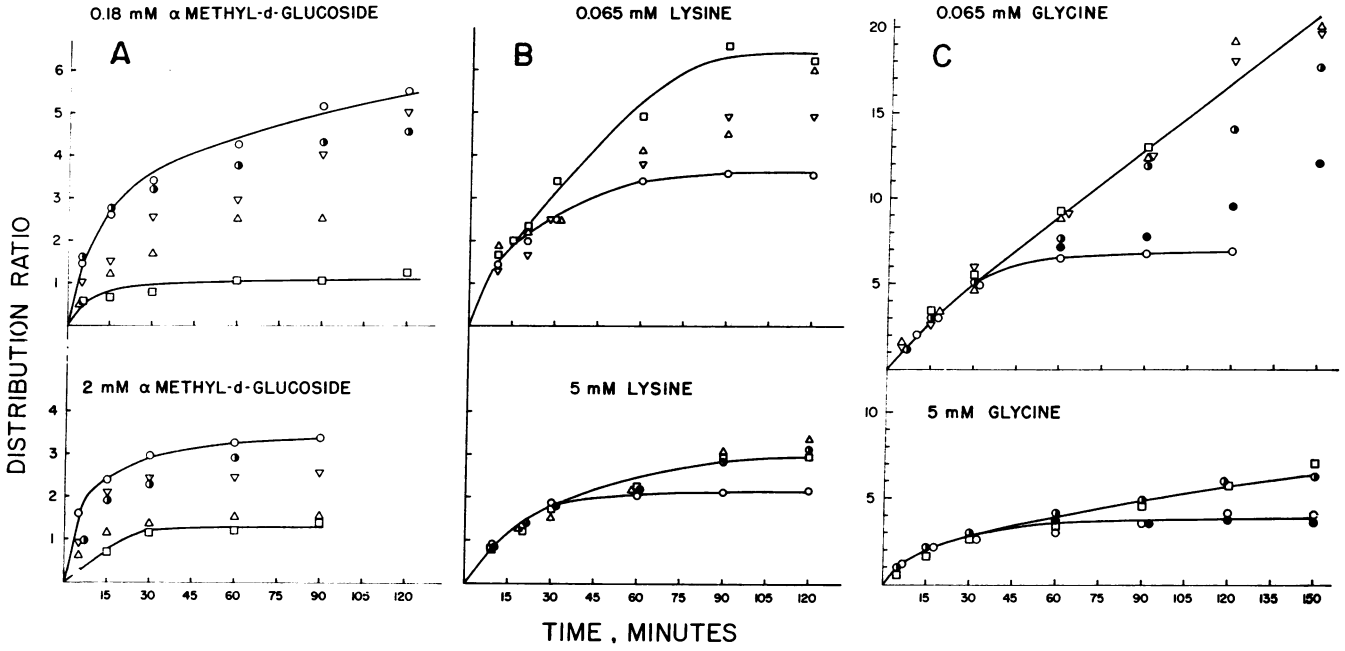

FIG. 1. Relationship of animal age to the uptake of [^{14}C]α-methylglucoside (*A*), [^{14}C]glycine (*B*), and [^{14}C]lysine (*C*) by rat kidney cortex slices. Incubations were performed in plastic flasks with 2 ml of Krebs-Ringer bicarbonate buffer, pH 7.35, containing the substrate at the concentrations shown and 0.25 μCi/ml of label at 37°C in a Dubnoff shaker. Freehand slices of cortex from young animals (made with a fine blade) were pooled and three slices were added per flask, to give a total weight of 3–7 mg wet weight. Three small segments of adult slices, one from each of three animals, made with a Stadie-Riggs microtome and weighing 10–20 mg, were employed in adult incubations. The uptake is designated by the distribution ratio, the ratio of cpm/ml intracellular fluid to cpm/ml medium, using tissue water and inulin spaces appropriate for various age tissues in making the calculation. Each point is representative of 6–20 incubations (the tissues representing 18–60 animals).

Rat ages in days: □ 1, △ 5, ▽ 10, ◑ 15, ● 23, ○ 42 (adult).

accumulation in the newborn was slight and a concentration gradient (defined here as a distribution ratio >1, a characteristic of active transport) was not achieved after 120 min of incubation. These results were obtained both at 0.2 mM, when the adult transport system is unsaturated, and at 2 mM, which is in the saturable range. Concentration gradients were established in cortex from 5-day-old animals incubated with low substrate levels and in tissue from 10-day-old animals incubated with high levels, but the normal adult gradient was not established until after 25 days of age. Although the newborn cortex could not establish a concentration gradient of α-MeG, sugar uptake into the cell was facilitated by a sodium-dependent mechanism (Table 1) and diminished both by phlorizin (a known competitive inhibitor of sugar transport) and by glucose, which shares the same transport system (Table 1). It thus appears that a rudimentary mechanism for sugar transport across the cell membrane is present in the newborn kidney cortex, but it is apparently unable to bring about adequate active transport. This rudimentary mechanism was unable to participate in the counterflow or exchange diffusion phenomenon that is characteristic of α-MeG in adult tissue.

TABLE 1. *Effect of sodium deprivation, phlorizin, and glucose on the accumulation of α-methylglucoside by rat kidney cortex slices*

Conditions	Distribution ratio	
	Adult	Newborn
Control	2.46 ± 0.14	0.74 ± 0.04
Sodium-free medium	0.75 ± 0.04	0.38 ± 0.04
Phlorizin (0.5 mM)	0.77 ± 0.02	0.34 ± 0.01
D-Glucose (10 mM)	0.89 ± 0.11	0.37 ± 0.05

α-MeG concentration, 2 mM; incubation time, 30 min. For sodium-free media, Tris buffer replaced Krebs-Ringer bicarbonate as described previously (20). Values are the mean ±SE of 3–12 determinations.

The accumulation of glycine and lysine by animals is also shown in Fig. 1. Amino acid uptake is not deficient in kidney cortex of newborns, either in the initial rate or the eventual concentration gradients. Indeed, at both high and low substrate concentrations the gradient after 60–120 min was higher in the newborn and decreased with older tissue.

Efflux of substrates

The steady-state concentration gradient of sugar or amino acid in the renal tubule cell is dependent on the rates of both entry and exit from the cell. In essence, this is a two-compartment system consisting of intracellular fluid and medium pools (23). The lack of establishment of a concentration gradient for sugar in newborn tubule cells could therefore be due to accelerated efflux, while the higher than adult concentration gradient for amino acids could be due to slow efflux. We studied this by permitting the tissues to accumulate the substrate, transferring the tissue to buffer, and measuring the rate of appearance of substrates in the buffer (Fig. 2). The efflux of α-MeG was *slower* in newborn than adult tissue, with a decrease of the efflux rate constant from 0.087 min^{-1}

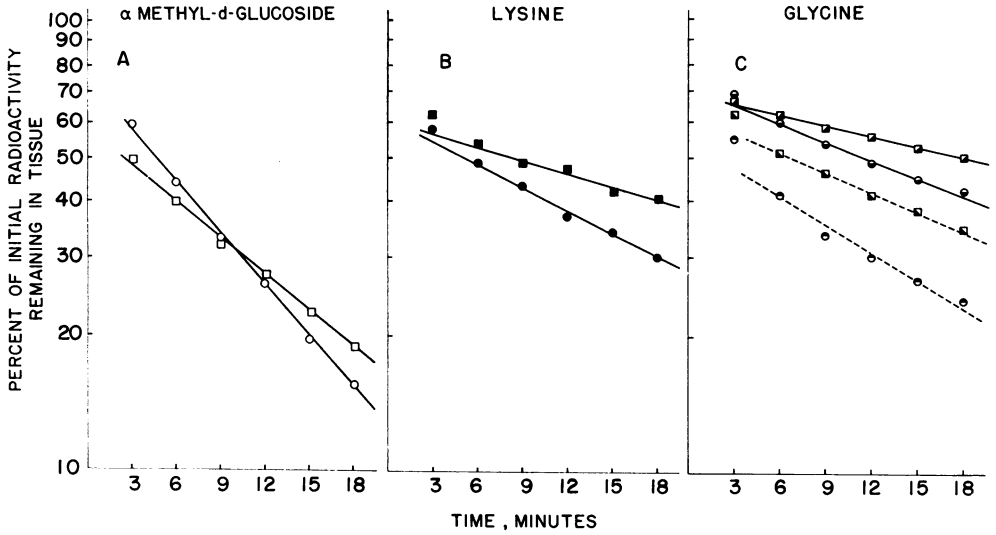

FIG. 2. Efflux of accumulated substrate from newborn and adult kidney cortex slices. *A*. Slices from newborn (-□-) and adult (-○-) rats were incubated for 60 min in 0.6 mM and 0.2 mM [U-^{14}C]α-methylglucoside, respectively. [These concentrations were chosen (14) so that the intracellular concentrations at the end of the incubation would be the same in the two tissues, taking into account that the concentrating ability of adult tissue is three times as high as that of newborn.] The tissues were quickly rinsed in saline, blotted, and transferred to flasks containing 3 ml of buffer. At 3-min intervals, the medium was sampled for radioactivity assay. After 20 min, the α-methylglucoside remaining in the tissue was determined.

B. Lysine efflux after 60-min incubation of newborn (-■-) and adult (-●-) slices in 0.07 mM and 0.035 mM substrate, respectively.

C. Glycine efflux after 30-min incubation of newborn slices in 0.065 mM -◪- and 5 mM -◧- and adult slices in 0.065 mM -◒- and 5 mM -◓- substrate.

in the adult to 0.063 min^{-1} in the newborn. Efflux of both glycine and lysine was also slower in newborn tissue (Fig. 2), which would be consistent with increased accumulation on prolonged incubation.

Concentration dependence of amino acid uptake

The adult renal tubule cell can take up and concentrate amino acids over a wide range of substrate concentration. Lineweaver-Burk or other analysis of the velocity of the concentration-dependent uptake indicates that two slopes are obtained, from which two apparent K_m values may be calculated for the transport process (21, 22). Though the exact nature of the transport mechanisms leading to these observations is unknown, it has been speculated that there may be two transport systems, one operative at low and the other at high substrate concentrations. Figs. 3 and 4 show the results of concentration-dependence studies in renal cortical slices from 5-day-old rats, which show that these young tissues also possess two transport systems. The calculated K_m and V_{max} values obtained in these experiments may be seen in Table 2.

TABLE 2. *Apparent K_m and V_{max} of glycine, lysine, and proline in 5-day-old and adult rat kidney cortex*

	K_{m1}		K_{m2}		V_{max1}		V_{max2}	
Amino acid	Adult	5-day	Adult	5-day	Adult	5-day	Adult	5-day
Lysine	23		0.43	0.76	27.3	—	0.60	0.60
	5	6.6	0.26	1.25	5.2	7.6	0.43	2.20
	21.5	21.5	0.25	0.25	22.0	22.0	0.40	0.40
			1.41	1.41			3.0	5.0
Glycine	12	12	0.22	0.77	12.2	12.2	0.68	2.5
	12	4	0.37	0.37	20.0	5.4	1.20	2.0
			0.25	0.25			1.00	1.4
			0.15	0.15			0.62	0.62
Proline	10	10	0.2	0.4	20.0	33.0	1.00	2.5
			0.3	0.3			0.15	0.83
			1.1	0.5			0.83	0.58

Incubations were for 30 min. Radioactive amino acid was diluted with unlabeled compound to give at least five appropriate concentrations between 1.3 and 15 mM to determine K_{m1} and between 0.022 and 0.26 mM to determine K_{m2}. In some experiments the entire range was examined, whereas in others only one range was determined. Adult and 5-day-old tissues were examined at the same time. K_m is expressed in millimoles per liter and V_{max} as millimoles per liter per 30 min.

Urinary sugar and amino acids

No glucose was found in bladder urine obtained from newborn rats as estimated by gas–liquid chromatography of silylated derivatives (17). On the other hand, the higher than adult urinary excretion of amino acids seen in a variety of newborn or young animals, including man and rat (19), was substantiated in the rats employed in this study. Glycine, lysine, and proline excretion determined by quantitative ion-exchange chromatography in urine of eight 5-day-old animals ranged from two to six times that of adult Sprague-Dawley rats.

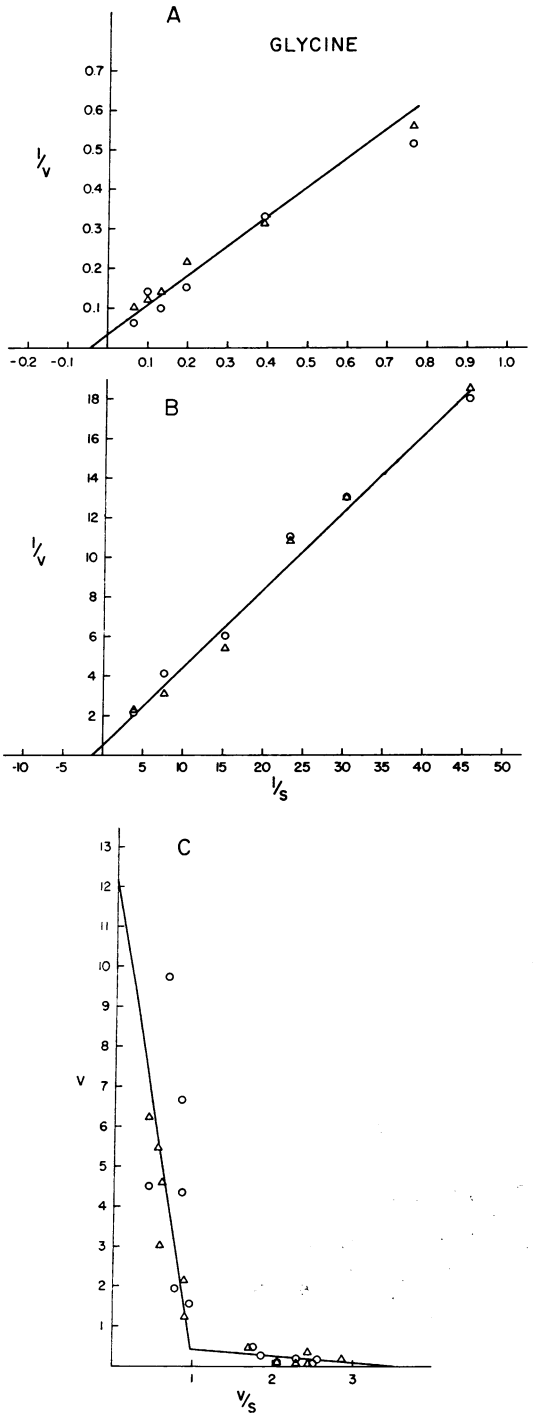

FIG. 3. Concentration dependence of glycine uptake in 5-day-old (△) and adult (○) kidney cortex slices after a 30-min incubation. *A*, Lineweaver-Burk plot (*V* in mmol/liter per 30 min, *S* in mM) over the substrate range 0.022–0.26 mM. *B*, substrate range 1.3–15 mM. *C*, Hofstee plot, *V* against *V/S*, of the data shown in *A* and *B*.

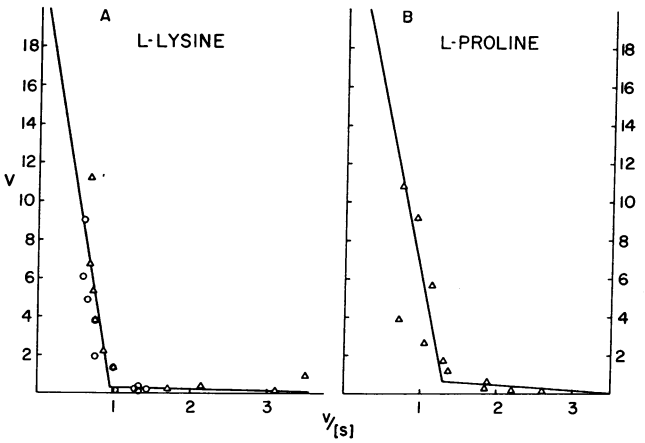

FIG. 4. Hofstee plots (as in Fig. 3) for the concentration dependence of lysine uptake in newborn (△) and adult (○) tissue (*A*), and of proline uptake in newborn tissue, (*B*).

DISCUSSION

The evidence is now considerable that, in the kidney cortex, sugars and amino acids have distinctive transport features. It was reported earlier (6, 7) that only metabolizable sugars cause inhibition of amino acid transport in kidney tubule cells, which implies a metabolic basis for the prescribed uptake. Recent (unpublished) studies in our laboratory, however, have shown that the nonmetabolizable models, α-MeG and α-aminoisobutyric acid, mutually inhibit each other's uptake in a noncompetitive fashion. These observations are consistent with a membrane transport interaction without involvement of a common binding site for the two classes of substrate. The findings presented here are perhaps the most cogent observations against a common transport process for sugars and amino acids. It is difficult to accept a single transport mechanism for sugar and amino acid influx which at birth is markedly impaired for the former but essentially normal for the latter. It seems likely that the maturation of the sugar transport system may be related to the development of the microvillus of the tubule cell, which occurs after birth (24).

If transmembranous movement of sugars and amino acids is indeed mediated by genetically determined binding carrier proteins (25, 26), our data imply that the protein involved in sugar transport is distinguished from that associated with amino acid transport by being synthesized or evolved into a completely functional unit at a different time in development. It seems logical, as pointed out by Alvarado (12), that these individual "carrier" proteins become intimately associated in the membrane and that there may be an operational relationship between sugar and amino acid transport. The observed mutual noncompetitive inhibition of transport by the two types of substrates may involve configurational changes of the membrane components. An alternative explanation, however, may involve the partition of energy coupled to transport processes.

The association of aminoaciduria with mellituria in certain clinical situations has suggested an interaction between the

transport systems of sugars and amino acids (1–5). On the other hand, there are also instances of inherited transport disorders in man that support the distinction of the transport processes. Thus, Hartnup disease, with its inherited defect in renal tubule reabsorption of neutral amino acids, has no associated sugar transport abnormality (27), and renal glycosuria with a defect in glucose absorption has no concomitant defect in amino acid transport (28).

Bailey, Fishman, and Pentchev (29) observed, while studying mutarotase activity in the rat, impaired galactose uptake in kidney of newborn rats. This suggests that our developmental data with methylglucoside are representative of the glucose–galactose transport system. Our developmental observations with amino acids, on the other hand, are in certain respects at variance with those of Weber and Cairns (30) and Baerlocher, Scriver, and Mohyuddin (19), who employed rats other than the Sprague-Dawley strain for their experiments. Although there is agreement that newborn and young rat renal tubule cells *in vitro* achieve concentration gradients higher than adult cells on long incubation, Baerlocher *et al.* found initial rates of uptake of amino acids by young tissue to be lower than in the adult. In addition, they observed that 7-day-old cortex showed only one concentration-dependence curve for glycine and proline; this changed to the adult pattern at about 14 days of age. The explanation of this difference from our results (Fig. 3) for 5-day-old rats may be methodological. Our slices were cut free-hand with a fine razor blade from the surface of newborn and young rat kidneys, whereas Baerlocher *et al.* employed a Stadie-Riggs microtome and its thicker blade; we have been unable to make satisfactory cortical slices from newborn and young rat kidney with this instrument (15). Further, Baerlocher *et al.* used one slice per flask, whereas we employed three slices derived from a pool made from an entire litter.

Despite the presence of a rudimentary sugar transport system in the newborn cortex, no mellituria was observed in bladder urine. Either (*a*) this transport system was adequate to handle the filtered glucose load in the newborn, (*b*) there may be a glucose transport component separate from the system studied, or (*c*) the very thin bladder epithelium participated in sugar reabsorption since urine stasis in the bladder is prolonged in the newborn. We believe that the first and third possibilities are more likely than the second. On the other hand, in the presence of apparently normal amino acid influx in tubule cells *in vitro* there is aminoaciduria in the newborn. The explanation for this may reside in the observed slow efflux of amino acids from these cells. The reabsorption of amino acids requires uptake of amino acid from the kidney tubule lumen and movement across the cell to the basal area, where it enters the peritubular capillaries. If *in vitro* efflux can be equated to the *in vivo* movement of amino acid out of the cell into the capillaries, a slowing of this process may cause higher intracellular amino acid pools (as is seen *in vitro*) and slower removal of amino acid from tubular urine. *In vivo* experiments are now under way to test this hypothesis.

This work was supported by grants from the USPHS (AM-10894) and the John A. Hartford Foundation.

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