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DEVELOPMENTAL PATTERN OF CYSTINE TRANSPORT IN ISOLATED RAT RENAL TUBULES

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Isolated renal cortical tubule fragments from rats ranging in age from less than 48 h to 15 weeks were used to examine the pattern of cystine uptake with development. Immature tubules took up cystine with a faster initial rate than mature tubules and did not reach a steady state by 60 min. By eight weeks of age, the timed uptake of cystine began to approach a steady state and between 8 and 11 weeks the uptake pattern achieved its adult form of reaching a steady state by 30 min of incubation. Analysis of the intracellular metabolism of the cystine taken up by the newborn tubules revealed that the majority had been reduced to cysteine with the formation of small amounts of reduced glutathione. Cystine entered the renal cortical tubule cell from the newborn via two saturable transport systems similar to the mature animal. The kinetic parameters of initial uptake of these two transport systems were similar in the mature and newborn animal except for a higher maximum transport velocity for the low K_m, low capacity system in the newborn. Lysine inhibited cystine uptake by newborn tubules and this inhibition appeared to occur on the low K_m, low capacity transport system similar to the adult. Cystine uptake was sodium dependent with an apparent affinity for sodium of 36 mequiv./l. From this data, the physiologic cystinuria of the immature animal does not appear to be referrable to a lower rate of influx as previously observed with the cortical slice. Other mechanisms should be sought to explain this phenomenon of immaturity.

Introduction

It appears clearly established that renal function in human infants [1] and in the very young animal [2] is characterized by increased clearance of amino acids and hyperaminoaciduria. This has fostered numerous in vitro investigations of the ontogeny of amino acid transport from two separate but related points of view. The first has sought an explanation of the changes in renal tubule cell transport mechanisms that could underlie the progressive increase with age in renal reabsorptive capacity. The most extensive studies of this type have focused on glycine uptake, initially by renal cortical slices [3–7] and more recently by isolated tubule fragments [8]. The use of the latter has eliminated difficulties inherent in making cortical slices of newborn kidney as well as obviated the effect of thickness of a slice on initial rate kinetics. The second has utilized differences in the developmental pattern of transport to distinguish transport mechanisms. Cortical slices from newborn and older animals have been employed to delineate the independent nature of sugar and amino acid transport [7] and to indicate that the mechanisms of cystine and dibasic amino acid uptake are unrelated [9].

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In this regard, the studies of Segal and Smith [9] revealed that cystine uptake by renal cortical slices of newborn rats was slower than that of adult slices while lysine uptake was not. Recent observations indicate that findings pertaining to cystine uptake by renal tubule cells based on in vitro use of cortical slices provide an inadequate representation of the cystine transport mechanism. Cystine uptake by adult cortical slices had been shown to occur by a single transport system [10] which did not interact with dibasic amino acids either in inhibition [11] or exchange diffusion experiments [12]. On the other hand, in isolated adult rat renal tubule fragments [13] and brush border membrane vesicles [14] two transport systems for cystine have been observed. One is a low K_m system which interacts with dibasic amino acids in inhibition [13,14] and exchange diffusion [15] experiments while the other is a high K_m system which corresponds to the single system observed in cortical slices and does not interact with dibasic amino acids. Because of these facts we have undertaken a reassessment of the developmental aspects of cystine transport by use of newborn and young rat renal tubule fragments. With this preparation we have examined the change of the kinetics of cystine uptake with age and explored the interrelationship of cystine transport to that of the dibasic amino acid, lysine. The results form the basis of this report.

Materials

L-[^{35}S]Cystine (760 mCi/mmol), 95% pure by thin-layer chromatography, and inulin[^{14}C]carboxylic acid (2.5 mCi/g) were obtained from Amersham Corporation, Arlington Heights, IL. Unlabeled cystine and *N*-ethylmaleimide were purchased from Calbiochem, San Diego, CA. OCS liquid scintillation cocktail was obtained from Amersham Corporation. Collagenase grade II (125–250 units/mg) was obtained from Worthington Biochemical Corporation, Freehold, NJ. Mesh with a 104 μm pore size was obtained from Advance Process Supply Co., Pennsauken, NJ. The dounce homogenizer with pestle A were obtained from Kontes, Vineland, NJ.

Methods

Sprague-Dawley 15-day-pregnant female and 7-week-old male rats were obtained from Charles River Breeding Laboratories (Wilmington, MA). Pregnant females were housed separately until parturition. The pups were weaned at 3 weeks of age. The animals were killed by decapitation at the appropriate age for developmental studies.

The kidneys were removed, decapsulated, and placed in ice-cold Krebs-Ringer bicarbonate buffer, pH 7.4. Cortical slices were cut with a sharp blade in a freehand manner for the rats younger than 2-weeks old and with a Stadie-Riggs microtome for older rats. For the preparation of tubule fragments, which has been reported previously [16–18], the slices were homogenized gently with four strokes of a pestle in a 15 ml loose dounce homogenizer and the suspension (1 g per 3 ml of buffer) was centrifuged for 1 min at $40 \times g$. The pellet was suspended in 3 ml of Krebs-Ringer bicarbonate buffer containing 0.375% collagenase (w/v) for each g of original kidney cortex. After a 45 min digestion at room temperature, three times the incubation volume of iced Krebs-Ringer bicarbonate buffer was added to the suspension and this was centrifuged at $40 \times g$ for 1 min. The supernate was discarded and the tubules were resuspended in the same volume of iced Krebs-Ringer bicarbonate buffer. This was repeated twice. After the final wash, the tubules were resuspended in Krebs-Ringer bicarbonate buffer to a concentration of approx. 3–5 mg weight per ml for transport studies and filtered through a 104 μm mesh. Fetal calf serum was added such that the final concentration was 5% (v/v).

Uptake studies were performed in newborn and adult tubule preparations simultaneously in Burg and Orloff flasks with continuous bubbling of a 95% O_2–5% CO_2 mixture as described previously [16–19]. The uptake was initiated by addition of [^{35}S]cystine and terminated by removing 2-ml samples into tared tubes which were placed in an ice water bath. The tubes were then centrifuged at 4°C for 10 min at $33000 \times g$ and the supernates removed for counting. The pellet surface and the test tube wall were washed once with ice-cold buffer and dried by suction. After weighing the tubes, the pellets were resuspended in 1 ml of

distilled water and the tubes were placed in a boiling water bath for 3 min. The tubes were then centrifuged and an aliquot (0.1 ml) of the water extract of the pellet and the original incubation supernates were added to 10 ml of a scintillation cocktail (2.8 ml absolute alcohol: 7 ml OCS) and counted in a liquid scintillation counter.

Distribution ratios of radioactivity, the ratio of cpm/ml of intracellular fluid to cpm/ml of incubation medium, were calculated as described previously [20]. The intracellular fluid volume was calculated as the difference between the total tissue fluid (wet weight − dry weight after overnight desiccation) and the volume of 'trapped fluid'. The trapped fluid volume was determined using inulin[^{14}C]carboxylic acid as previously described [21].

The incubation of slices, which were approx. 5 mg in weight, was also carried out in Burg and Orloff flasks in the same manner as the incubation of tubules. At the end of incubation, slices were separated from supernate by collecting them on a 104 μm mesh filter and rinsed once with ice-cold buffer. Five to ten slices were weighed as a group and the radioactivity distribution ratio was determined as described for the tubule pellets.

Analysis of the intracellular pool of radioactivity was performed using the methods described by States and Segal [22] to form stable *N*-ethylmaleimide adducts with cysteine and reduced glutathione. Isolated tubules from adult and newborn animals were incubated for 5 and 30 min with [^{35}S]cystine. Aliquots of the incubation mixture were placed in tared test tubes and centrifuged at 4°C and $33000 \times g$ for 10 min. The supernate was removed for chromatography. The top of the pellets and the inside of the test tube walls were washed once with ice-cold Krebs-Ringer bicarbonate buffer and dried by suction. The tubes were weighed and then the pellet was resuspended in 0.4 ml of 20 mM *N*-ethylmaleimide in 10 mM phosphate buffer (pH 7.4) at room temperature. After 5 min, 0.1 ml of 50% (w/v) trichloroacetic acid was added. After sitting in an ice-water bath for 10 min, the denatured tubule suspension was centrifuged for 20 min at 4°C and $33000 \times g$ and this supernate was removed for chromatography. The precipitate was resuspended and washed once with 10% (w/v) trichloroacetic acid to remove any radioactivity from the supernate trapped in the precipitate. After solubilizing the precipitate with 0.5 ml of Soluene 100, it was assayed for radioactivity in 10 ml of OCS using liquid scintillation techniques. The supernate was extracted three times with ether twice the aqueous volume to remove any unreacted *N*-ethylmaleimide and trichloroacetic acid. 10 μl of the supernate were chromatographed on cellulose thin-layer sheets using *n*-butanol/pyridine/acetic acid/water (3:2:0.6:1.5, v/v) for development according to the method of States and Segal [22]. Appropriate cystine, cysteine-*N*-ethylmaleimide and glutathione-*N*-ethylmaleimide standards were prepared by the method of Smyth et al. [23]. After developing and cutting the chromatography into 1/2 cm segments, each segment was assayed for radioactivity in 2 ml of OCS using liquid scintillation techniques. The segments corresponding to cystine, cysteine-*N*-ethylmaleimide, and glutathione-*N*-ethylmaleimide were identified by comparison to the location of simultaneously chromatographed standards stained with ninhydrin.

Concentration-dependent experiments were analyzed by Hofstee plots drawn by the least-squares method with the aid of a Monroe model 1775 programmable calculator to obtain the parameters of the equation:

$$V = \frac{V_{max1}[S]}{K_{m1} + [S]} + \frac{V_{max2}[S]}{K_{m2} + [S]}$$

In the studies of the dependence of uptake on the medium sodium concentration, the uptake of 0.025 mM [^{35}S]cystine in Krebs-Ringer bicarbonate buffer was compared to uptake in modified Krebs-Ringer bicarbonate buffers containing varied amounts of choline chloride substituted for sodium chloride.

Urinary excretion of cystine

Three 14-week-old animals were placed in individual metabolic cages with access to water. After overnight fasting, urine was collected for the following 24 h. Pooled urine was also collected for 24 h from two cages containing three 3-week-old animals after an overnight fast. A pooled urine was collected by bladder puncture from 30 newborn rats within 48 h of birth. These animals were not fasted because of their immaturity.

0.1 ml of 30% sulfosalicyclic acid (w/v) was added to 0.9 ml of urine and the treated urine was centrifuged at 33000 × *g* and 4°C for 20 min. The supernate was removed and the cystine concentration was determined on 0.5 ml of this by ion-exchange chromatography using a Beckman Amino Acid Analyzer with W-2 resin bed employing a lithium citrate buffer system. Urinary creatinine was determined using a modification of the Jaffe reaction [24] and the results were expressed as nmol of half cystine per mg of creatinine.

Results

Urinary excretion of cystine with age

Since the available data on cystine excretion with age is for Wistar rats [2], analysis of cystine excretion in our Sprague-Dawley rats was performed. Cystine excretion in newborn rats was 690 nmol half cystine/mg creatinine which fell to 144 nmol half cystine/mg creatinine in the 3-week-old rats. In the mature animals (14 weeks old), this excretion was below the sensitivity of the method being less than 5 nmol half cystine/mg creatinine. This trend of decreasing urinary excretion of cystine with maturity in Sprague-Dawley rats parallels the pattern described by Brodehl [1] for humans.

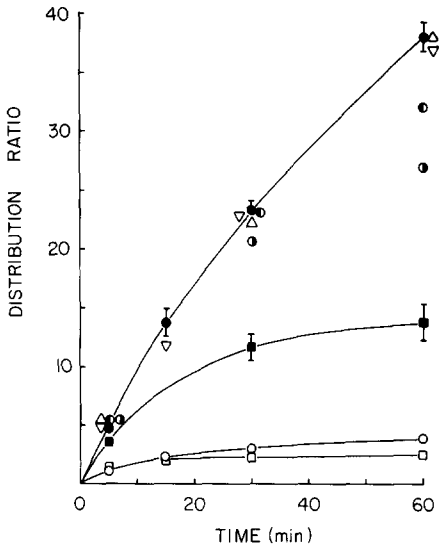

Fig. 1. The uptake of cystine by rat kidney cortical tubules. The tubule suspension of 3–5 mg per ml was incubated at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4, with [^{35}S]cystine, 0.1 μCi/ml. For 0.025 mM uptake: ●, represents newborn (12 determinations); ▽, 1-week old (8 determinations); △, 2-week old (8 determinations); ◐, 6-week old (4 determinations); ◑, 8-week old (4 determinations) and ■, 11–15-week old animals (26 determinations). For 0.5 mM uptake: ○, represents newborn (8 determinations) and □, 11–15-week old animals (8 determinations). Each value is the mean ± S.E. Where there is no line, the S.E. is within the size of the point.

Age dependence of cystine uptake

The uptake of [^{35}S]cystine by renal cortical tubules isolated from newborn and older rats is shown in Fig. 1. At a substrate concentration of 0.025 mM, the distribution ratio at 5 min was 4.8 ± 0.2 (mean ± S.E., $n = 12$) for the newborn and continued to increase over the 60-min period to a distribution ratio of 37.9 ± 1.3 ($n = 12$) without achieving a steady state. At 1, 2, 6 and 8 weeks of age the initial rate was similar to the newborn but there was an approach toward a steady state at 60 min by 8 weeks with a distribution ratio of 27.1 ± 1.8 ($n = 4$). By 11 weeks the uptake achieved its adult form since the curves at 11 and 15 weeks did not differ. The 5-min distribution ratio was 3.7 ± 0.3 ($n = 8$, p vs. young < 0.01) with a steady-state value of 13.8 ± 1.5. From these studies it appears that our previous report where cystine uptake was examined in 5–7-week-old animals, did not truly reflect the characteristics of the fully mature cystine transport systems [13].

Fig. 1 also shows the uptake of 0.5 mM [^{35}S]cystine, a relatively high concentration well beyond the physiological range and almost at the limit of cystine solubility, by tubules from newborn and 11–15-week-old animals. The distribution ratios obtained with tissue from both ages were much lower than those when the substrate level was 0.025 mM, suggesting that the uptake process is saturable. There was no difference in the uptake of 0.5 mM [^{35}S]cystine between newborn and mature tubules for the first 15 min of incubation. Thereafter the newborn tissue accumulated labeled material to a slightly greater level, the 60 min distribution ratio being 3.9 ± 0.2 for the newborn and 2.5 ± 0.1 for the 11–15-week-old tissue. This difference was statistically significant ($p < 0.001$, $n = 8$ for each age).

Nature of intracellular radioactivity in newborn tubules

Thin-layer chromatography of the trichloro-

acetic acid-soluble pool of intracellular radioactivity from newborn tubules, after treatment with *N*-ethylmaleimide to form stable adducts of cysteine and reduced glutathione, revealed that after 5 and 30 min of incubation with 0.025 mM cystine 87% of the label was in the cysteine-*N*-ethylmaleimide area, 10% in the cystine area and 3% in the glutathione-*N*-ethylmaleimide area. Cystine was the only detectable radioactive compound in the medium throughout the 30 min of incubation. After 5 min of incubation with 0.5 mM cystine, cystine accounted for 31% of the trichloroacetic acid-soluble intracellular radioactivity while cysteine accounted for 60% and reduced glutathione 9%. This changed somewhat after 30 min of incubation where cystine accounted for 25%, cysteine 70% and reduced glutathione 5% of the trichloroacetic acid-soluble intracellular radioactivity. After 5 and 30 min of incubation of newborn tubules with 0.025 mM [^{35}S]cystine, 10.4 ± 0.9% of the total tissue radioactivity was found in the trichloroacetic acid-precipitable fraction with 0.5 mM cystine, this was 12.6 ± 0.9%.

The predominant intracellular labeled compound in the newborn tubule was cysteine which was similar to results obtained previously with renal cortical slices from adult and newborn [9] animals and isolated cortical tubules from young adult rats [13]. Since the largest fraction of the intracellular pool of radioactivity was cysteine, the distribution ratios shown in Fig. 1 do not represent true concentration gradients for cystine. However, after 30 min of incubation with 0.025 mM cystine, the distribution ratios were over 20 and 10% of this was cystine. Therefore, after 30 min of incubation a concentration gradient of cystine was present in the immature animal.

Substrate concentration dependence of uptake

The substrate concentration dependence of cystine uptake was studied over the concentration range of 0.01 to 0.75 mM for newborn tubules and 0.005 to 0.5 mM for 11–15-week-old tissue at 5 min of incubation. As can be seen in Fig. 2A, a Hofstee plot reveals a two-limbed curve that is consistent with multiple transport systems for both the young and mature tissue. At substrate concentrations higher than 0.1 mM both the newborn tubules and 11–15-week-old tubules show the same

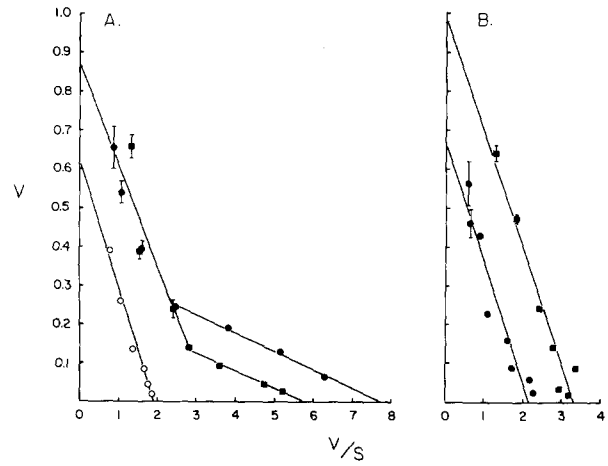

Fig. 2. Hofstee plot of concentration dependence of cystine uptake. (A) isolated cortical tubules, 5 mg/ml, were incubated for 5 min at 37°C in Krebs-Ringer bicarbonate buffer (pH 7.4) with 0.1 μCi/ml [^{35}S]cystine and sufficient unlabeled cystine to give the desired concentrations: ●———●, newborn; ○———○, newborn with addition of 3 mM lysine and ■———■, adult. *V* is expressed as mmol/l per 5 min and *S* as mM. (B) Cortical slices, 5–7 mg sections were incubated for 30 min under the same conditions as described for isolated cortical tubules. Circles represent newborn values and squares adult values. Each value is the mean±S.E. Where there is no line, the S.E. is within the size of the point.

substrate velocity relationship whereas at concentrations below 0.1 mM the two tubule preparations have different curves, that of newborn tubules being above and parallel to that of the adult tubules. These curves at low substrate concentration represent a high affinity system which in the newborn has the observed parameters of K_{m1} = 0.047 ± 0.002 mM and V_{max1} = 0.364 ± 0.005 mmol/l intracellular fluid per 5 min (n = 8) and in adult tissue, K_{m1} = 0.042 ± 0.008 mM and V_{max1} = 0.250 ± 0.048 mmol/l per 5 min (n = 8). The major difference between this system in the tubules of newborn and older rats is in the maximum velocity which is higher in the younger tissue ($p < 0.05$) and is reflected in the extrapolation of the parallel curves at V/S values above 2 in Fig. 2A to the V on the ordinate. The parameters for the low affinity transport system in the newborn are K_{m2} = 0.24 ± 0.04 mM and V_{max2} = 0.83 ± 0.10 mmol/l per 5 min while that in the adult is essentially the same with K_{m2} = 0.31 ± 0.04 mM and V_{max2} = 0.96 ± 0.04 mmol/l per 5 min.

Effect of lysine on cystine uptake

Since our previous studies with rat tubules from older animals showed that lysine inhibited cystine uptake by inhibition of the low-K_m uptake system, the concentration dependence of uptake of cystine by newborn tubules was examined over the range of 0.01 to 0.75 mM in the presence of 3 mM lysine. Lysine, indeed, inhibited cystine uptake. With 0.025 mM cystine, the distribution ratio at 5 min in the presence of lysine was 1.7 ± 0.1 with a control value of 4.8 ± 0.2 ($p < 0.001$) while with 0.5 mM cystine, it was 0.8 ± 0.1 in the presence of 3 mM lysine and 1.1 ± 0.1 without lysine ($p < 0.01$). The Hofstee plot shown in Fig. 2A of the substrate-velocity relationship in the presence of lysine reveals a single line indicating only one component of transport corresponding to the high-K_m system. The observed kinetic parameters for cystine uptake in the presence of lysine were $K_m = 0.33 \pm 0.02$ mM and $V_{max} = 0.62 \pm 0.01$ mmol/l of intracellular fluid per 5 min. Lysine at 3 mM, therefore, completely inhibited the low-K_m system for uptake as it did in older tubules [13].

Concentration dependence of uptake by cortical slices

In previous studies comparing cystine uptake by newborn and adult cortical slices only the pattern of uptake with time was examined [9]. In order to evaluate the reason for the different velocity of uptake by slices when compared to that by tubule fragments, the concentration-velocity relationship was determined in cortical slices. In Fig. 2B is shown the Hofstee plots of the results of these studies of cystine uptake over the 0.005 to 0.75 mM range by renal cortical slices of newborn and 11-week-old adult rats. Only a single line is obtained with each of the tissues corresponding to the presence of only one system for cystine uptake. The K_m and V_{max} for the adult tissue was 0.30 ± 0.03 mM and 0.95 ± 0.06 mmol/l per 30 min, respectively, and for newborn tissue they were 0.30 mM ± 0.02 and 0.65 ± 0.03 mmol/l per 30 min. The lower velocity of cystine uptake by newborn slices corresponds to the slower uptake of cystine with time by newborn slices previously reported [9].

Effect of medium Na^+ on uptake by newborn tubules

Medium sodium concentration has been shown to be important for cystine uptake by both rat renal cortical slices [9,25] and isolated cortical tubules [13] from older animals. The effect of medium sodium concentration on the uptake of 0.025 mM cystine by newborn tubules was, therefore, examined by replacing sodium chloride with choline chloride to give a final sodium concentration of 35 mequiv./l. This replacement of sodium with choline resulted in a distribution ratio of 3.2 ± 0.2 after 5 min of incubation which is significantly lower than the control value of 4.8 ± 0.2 obtained in standard Krebs-Ringer bicarbonate buffer ($n = 8$, $p > 0.01$). The uptake of 0.025 mM cystine was also studied in buffer where the sodium concentration was varied over the range of 35–130 mequiv./l. The apparent K_m for sodium was 36.5 mequiv./l, as determined from a Lineweaver-Burk plot, which is the same as that observed in older tubules [13].

Discussion

The newborn and young rat kidney tubule fragments take up cystine at physiologic concentrations with an initial rate that is about 30% higher than mature tubules while at higher substrate concentrations the rate of entry does not differ. The striking difference in cystine uptake between immature and mature tissue was observed at later time points. Cystine uptake had not come to steady state after 60 min of incubation in immature tubules while it had after 30 min in adult tubules which resulted in large differences in the distribution ratios observed at these later time points, those from immature tissue being higher than those from mature tissue. By 8 to 11 weeks of age the adult pattern of cystine uptake was reached which was characterized by achieving a steady-state after 30 min of incubation.

The cellular entry of cystine occurs by saturable processes in both newborn and adult tubules. An examination of the concentration dependence of uptake reveals that in both young and old tubule fragments two systems for cystine uptake are present, a high K_m, high capacity process and a low K_m, low capacity mechanism, both of which have

been reported in tubules [13] and isolated brush border membrane vesicles [14] from older rats. The only discernable difference between newborn and adult systems is that the low K_m component in the young has an observed V_{max} that is 30% higher than that of the adult tubules. This increase would account for the more rapid uptake at 0.025 mM cystine (Fig. 1) since at this low substrate concentration the majority of the uptake is via the low-K_m system. Although the high-K_m system appears to have the same observed V_{max} in old and young tissues, there is a small contribution to the total uptake which is due to the low-K_m system. Since this contribution is proportionately less in adult tubules, the suggestion can be made that the velocity of the high-K_m system in the mature tubule is actually somewhat higher than in the newborn tissue. The magnitude of the difference was not discernable, however, when the uptake by tubules at 0.5 mM cystine was evaluated (Fig. 1). It appears that the maturation taking place in the mechanism for cystine entry into the isolated tubule preparation may be a composite of two factors, a decreasing efficiency of the low-K_m system and increasing efficiency of the high-K_m component.

The presence of similar systems for cystine uptake by cortical tubules [13] and isolated brush border membrane vesicles [14], an observation also made for glycine [8,26], leads us to believe that the uptake by tubule fragments primarily reflects brush border transport activity. We cannot, however, separate luminal and antiluminal membrane transport in the tubule preparation and it may be possible that the increased early uptake at low substrate concentrations in the young may reflect a change in uptake at the basolateral membrane.

As in adult tissue, lysine inhibits cystine uptake by newborn renal tubules. This has been observed previously using both adult cortical tubules [13] and isolated brush border vesicles [14]. Also similar to adult tissue, this inhibition by lysine appears to occur on the low K_m system in the newborn. This is shown in Fig. 2A where the segment representing the low-K_m system is absent. The K_m and V_{max} for the residual system may be thought to be parameters of the entry process without the low-K_m component. The V_{max} is about 25% less than the observed V_{max} for the high-K_m component with uninhibited tubules.

The explanation for the difference in the rate of cystine uptake between slices and tubule fragments at low, physiological concentrations is that in slices only one high-K_m mechanism for cystine uptake is observed (Fig. 2 and Ref. 10). This mechanism is also the only one detectable in slices from newborn renal cortex; except in the young tissue the V_{max} is lower than that of adult tissue (Fig. 2). Thus, the slower uptake of cystine with time of newborn compared to adult slices, which we reported previously [9], would appear due to the presence of a less efficient high-K_m transport system in the newborn. Whereas, the high rate of entry in newborn tubules is due to the presence of a very efficient low-K_m transport system.

Why slices give no evidence of the low-K_m component of cystine transport is enigmatic. The fact that slices are incubated for 30 min to study concentration dependence because of low initial velocity and tubules are incubated for 5 min is not an explanation since tubules incubated for 30 min also show the two-limbed concentration dependence curves. Isolated tubules are prepared by collagenase digestion and this could suggest the low-K_m component appears as a result of the treatment. However, this is not the case since brush border membrane vesicles, prepared from adult renal cortex without exposure to collagenase, exhibit two transport systems for cystine [14]. These two-transport systems for cystine in brush border vesicles have similar K_m values to those of the isolated tubules and exhibit inhibition of the low-K_m system by lysine as does the low-K_m system of the isolated tubules. Moreover, the physiological data from experiments in which lysine infused into man [27,28] and dog [29] caused increased cystine excretion are consistent with the lysine inhibition of cystine uptake in the isolated tubule and brush border membrane vesicle and inconsistent with the lack of lysine inhibition of cystine uptake observed in the slice. Differences in transport characteristics of α-methyl-D-glucoside [7,18,30,31] and glycine [7,8] have also been observed between renal cortical slices and isolated tubule preparations.

As in adult tubule cells [13], the principal intracellular form of the transported cystine in newborn tubules is cysteine. Reduction of cystine to cysteine is not a necessary step for the transport of cystine since it is taken up by brush border mem-

brane vesicles which lack the ability to reduce cystine [15]. However, cystine reduction is necessary to prevent intracellular binding since much of the transported cystine by brush border vesicles is bound to the membrane [15]. In the intact cells, the reduction to cysteine occurs rapidly after cystine entry since the majority of the transported label is in the form of cysteine after 5 min of incubation. Indeed, the rate of this reduction could have an influence on the overall rate of cystine uptake, but it is impossible at this time to delineate clearly whether the increase in uptake at low cystine concentration in newborns can be attributed to a more rapid reductive process.

In adult tissue the distribution ratio approaches a steady state by 30 min of incubation where the influx of cystine and efflux of cysteine are balanced. In newborn tubules the lack of a steady-state and a higher distribution ratio may be explained by a relatively slow efflux process. Such has been the case in the study of transport of other amino acids by newborn tissue in vitro where the amount accumulated is higher than in adult tissue [7]. The approach to a steady state in 8 week old tubules even when the influx rate has not altered implies that the efflux process has become more rapid. It could be that this decreased efflux in the newborn is related to diminished activity across the basolateral membrane of the proximal tubule cell since this is the primary site for movement out of the cell for net transepithelial transport.

A characteristic of immature animals is a lower fractional reabsorption and increased urinary excretion of most amino acids. The extensive studies of Brodehl in humans clearly shows this to be the case for cystine [1]. Our own measurements of urinary cystine excretion is indicative of a similar pattern in Sprague-Dawley rats. Studies of clearance and urinary excretion are, however, essentially based on 'black box' concepts in which mechanisms are inferred from input and output measurements. We have sought an explanation for this 'physiological' cystinuria of young animals by examining the transport characteristics of isolated proximal tubules. We feel the data indicate that brush border uptake in the newborn occurs by the same modalities as the adult, but at physiological concentrations functions more efficiently. If, indeed, our data reflect the in vivo state then mechanisms for the aminoaciduria must be sought other than impaired luminal uptake by proximal tubule cells. The slowed efflux noted in the immature tubules raises the possibility that this 'physiological' cystinuria may be related to decreased transport out of the cell across the basolateral membrane. Such a mechanism appears to explain impaired intestinal transport of lysine in the inherited disease lysinuric protein intolerance [32]. Another possibility is suggested by the observation that patients with cystinuria 'secrete' cystine [33], although the nephron segment responsible for this secretion is unknown. Such a process may be more active in the normal neonatal kidney leading to increased urinary cystine. Studies of the proximal tubule may not yield a full explanation of the aminoaciduria of the young and exploration of the handling of amino acids by other segments of the nephron may be required.

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