Pediat Res. *16:* 684–687 (1982)

NEONATAL IMINOGLYCINURIA: EVIDENCE THAT THE PROLINURIA ORIGINATES IN SELECTIVE DEFICIENCY OF TRANSPORT ACTIVITY IN THE PROXIMAL NEPHRON

by

Charles R. Scriver, Marie-Françoise Arthus and Michel Bergeron

from

Le Département de Physiologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada

ABSTRACT

We investigated the process of neonatal hyperprolinuria in dog and rat. Plasma proline varied only 2-fold in the puppy whereas prolinuria increased up to 12-fold from birth to the 10th day declining thereafter to reach adult values (<0.1 µmole/mg creatinine) by the third wk. Stop-flow analysis in puppies (<17 days old, n=3) and one adult dog revealed that backflux of proline in distal nephron is not the source of neonatal hyperprolinuria. Prolinuria occurs in the Long-Evans rat pup during the first postnatal wk. We measured net uptake of L-proline at low (0.2 mM) and high (5 mM) concentrations by tubule fragments prepared from newborn and adult kidneys. At both concentrations and at initial rates, uptake was similar in newborn and mature tubules; at or near steady-state, tubules of newborn kidney had greater net uptake relative to mature kidney, apparently because efflux is attenuated. A difference in metabolic runout did not explain the difference in proline uptake by newborn kidney. Tubules from pups less than 7 days old did not exclude the competitive inhibitor AIB from interacting with proline during uptake at 0.2 mM when compared with mature kidney, ($p = 0.005$). These findings imply that transport of proline on the previously described proline-preferring high-affinity system is deficient in proximal nephron of newborn kidney.

SPECULATION

Maturation of proline reabsorption after birth requires 1 wk in the rat, about 1 month in the dog and 3 months or more in man. Deficient activity during these intervals on one among several carriers in brush-border and basal-lateral membranes in proximal tubule segments is sufficient to explain neonatal hyperprolinuria.

INTRODUCTION

Homeostasis of physiologic function is regulated by means of controlling (intrinsic) signals in response to disturbing (extrinsic) signals. The transition from intrauterine to postnatal life is a major disturbing signal. Accordingly, one expects the process of perinatal adaptation to be reflected by changes in cellular functions. The kidney is an organ of homeostasis that undergoes perinatal adaptation (29) and amino acid reabsorption reflects the processes of renal ontogeny.

In mature mammals, amino acids are reabsorbed from filtrate by saturable, Na^+-dependent cotransport systems located in the luminal membrane of the proximal nephron (6,33,49,50); the distal nephron does not participate in this process (6,36). Whereas the luminal membrane controls net reabsorption of amino acid (33,38,42,49), mediated systems in the basal-lateral membrane also serve cellular uptake of amino acids from peritubular fluid to meet metabolic needs along the nephron *in situ* (5,7,11,30,33,38,42).

Neonatal iminoglycinuria is a manifestation of renal ontogeny (35,37). Proline is transported by multiple systems in the mature nephron (10,34,35,51,52). Several investigators (2-4,12,32) have identified *in vivo* and *in vitro* differences between the newborn and adult rat in renal handling of imino acids and glycine that must involve one or more of the carriers. Possibilities to explain the neonatal prolinuria include diminished influx or increased backflux of proline at the luminal membrane, and diminished efflux at the antiluminal membrane.

We used isotopically labeled proline to investigate: (1) by stop-flow analysis *in vivo*, whether the distal tubule is a site of backflux of proline during ontogeny; (2) by collagenase-treated isolated renal cortical tubule fragments *in vitro*, whether net uptake of proline is different in newborn and adult kidney. Our findings imply that activity of the high-affinity proline transport system is attenuated in the proximal nephron of newborn kidney; they exclude the distal tubule as the site of neonatal prolinuria.

METHODS AND MATERIALS

Animals

Mongrel dogs were used for stop-flow studies. Collagenase-treated tubule fragments were obtained from Sprague-Dawley rats. Animals were raised in our facilities, housed under approved conditions, and studied at known postnatal age.

Stop-flow technique

Experiments were performed on 4 adult female dogs (11-20 kg) and 13 puppies (7-22 days after birth; 0.45-1.23 kg). The stop-flow method of Malvin et al. (25), modified by Lambert et al. (22), was used. Animals were fasted, anaesthetized with Nembutal (30 mg/kg), tracheotomized, and cannulated in the left jugular vein. An abdominal incision was made to expose and aspirate the urinary bladder and cannulate both ureters. Control samples of blood and urine were obtained and mannitol (5% in 0.3% NaCl) was then infused at 0.3 ml/min. When urine flow had stabilized, one ureteral catheter was clamped. After an interval (9 min), [^{3}H] L-proline (15 µCi) and [^{14}C] inulin (0.25 µCi) were injected together in 200 µl through the jugular catheter. The ureteral clamp was released after 3 min and urine collected in consecutive 40 µl aliquots for a further 6 min. When the urine collection terminated, a blood sample was drawn from the left femoral vein.

Isolation and incubation of renal tubules

Adult rats (250-400 g) and pups (2-14 d) were decapitated and the kidneys removed immediately, chilled, decapsulated, and hemisectioned. Thick cortical slices (>200 µm) were cut rapidly with a chilled Stadie-Riggs microtome, and incubated for 45 min at 37°C in collagenase (0.4%) as described by Burg and Orloff (8) and modified by Chesney and Jax (9) for newborn tissues. Krebs-Henseleit buffer (containing 5 mM $CaCl_2$ to enhance collagenase activity) was used for incubation of tubules at pH 7.4. Collagenase digestion was stopped by immersing the vessel in crushed ice. After centrifugation (40 x g for 30 sec at 4°C), the pellet was washed three times with cold buffer and filtered through a layer of nylon. Light centrifugation of the filtrate gave a pellet which was diluted to yield tubules at a final concentration of 70-100 µg wet wt/ml buffer.

Suspensions of tubules were incubated in special flasks (8) containing Krebs-Henseleit buffer at the usual 1 mM $CaCl_2$. At zero time, [^{14}C]-L-proline, was added to the solution which was gassed continuously with 5% CO_2 in O_2. [^{3}H]- or [^{14}C]-inulin was used to measure the extracellular space. Incubations were terminated at specified times by centrifugation of the tubule suspension (10,000 x g for 10 min) at 4°C, followed by washing with 0.5 ml NaCl (0.9%) and resuspension in double-distilled H_2O (0.5 ml). The contents were boiled for 5 min, centrifuged again, the pellet discarded, and an aliquot of supernatant added to Aquasol-II. In other experiments, α-aminoisobutyrate (AIB), was added to the incubation mixture at 10 times the substrate concentration to perform a constant-ratio inhibition test (28) at various concentrations of L-proline (0.02-10 mM); osmolarity of the buffer was adjusted to compensate for AIB content.

Analytical

Proline content of serum and urine was measured on a modified Beckman-Spinco amino acid analyzer (39). Radioactivity in urine (stop-flow studies) and supernatant (tubule fragment experiments) was measured by liquid scintillation counting in Aquasol-II with a Beckman L-200 counter. Net uptake by tubules was expressed as the soluble isotopic distribution ratio (17). Statistical analysis was performed by standard methods (44).

Materials

Uniformly-labeled [^{3}H]-L-proline (27.2 Ci/mmole), [^{14}C]-L-proline (2.5 Ci/mmole), inulin-[^{3}H] (127 mCi/g), inulin carboxy-[^{14}C] (1.91 mCi/g) and Aquasol-II were obtained from New England Nuclear, Boston, MA. Reagent grade L-proline and AIB were purchased from Mann Res. Lab. (New York). Collagenase (grade II) was obtained from Worthington, Freehold, N.J.

RESULTS

Proline excretion in vivo in the dog. Prolinuria increased 5-12 fold during the first 10 days of postnatal life (Fig. 1). It then decreased during the following 10 days until it was virtually extinguished, as in the mature animal. Plasma proline was approximately 0.2 mM; it varied less than 2-fold in the postnatal period (Fig. 1). These findings imply a renal origin of postnatal prolinuria in the dog.

Stop-flow analysis. Experiments were performed on puppies aged 8, 9, and 17 days with neonatal prolinuria and an adult dog without prolinuria. Proline excretion curves were alike in young and adult animals (Fig. 2). Proline excretion did not precede inulin excretion (no significant precession) after release of the ureteral clamp in either newborn or mature animals. This finding indicates that postnatal prolinuria does not originate in the distal nephron.

Proline uptake by isolated rat renal tubule

Net uptake of L-proline (at 0.2 mM and 5 mM) was measured as the soluble isotopic distribution ratio. Uptake was greater at steady-state in tubules from newborn rats (<7 days old) compared to those from the adult animal (Fig. 3). There was an inverse relationship between distribution ratio and extracellular proline concentration, indicating saturability of the uptake process in newborn and mature tubules. Initial-rate measurements indicated slightly slower uptake of 5 mM L-proline by newborn rat tubules compared with tubules from adult rats. This finding was apparent only at the higher proline concentration.

We measured accumulation of [^{14}C] in CO_2 during uptake by tubules to determine whether loss of isotope from the soluble intracellular pool into the gaseous phase could explain the differences in net uptake of proline by newborn and adult rat tubules. During 10-min incubation, at 0.02 mM proline, 81% of label entering tubule cells was converted to CO_2 in the newborn rat; the corresponding value in the adult rat was 87%; at 2 mM proline the conversions were 76% and 61% respectively. These findings imply that ontogeny of renal proline oxidation does not account for the observed differences in net uptake of proline into the soluble pool of isolated tubules from newborn and adult rat kidney.

AIB is a competitive inhibitor of proline uptake by the rat renal cortex slice at substrate concentrations above 1 mM (28,41). We confirmed that it is a competitive inhibitor of proline uptake by the isolated tubule of the mature rat (data not shown). We used the constant-ratio inhibition test (2,28) to show that tubules from adult rats exclude AIB from interaction with proline uptake at low concentrations (0.2 mM) of substrate (Fig. 4). On the other hand, AIB significantly inhibited proline uptake (p=0.005) at the low substrate concentration in tubules from rat pups less than 1-week-old. This finding indicates that the uptake system used by low concentrations of proline does not exclude AIB in very immature tubules. It is compatible with deficient activity of the low-Km transport system in the very young rat pup.

DISCUSSION

Various methods have been used to describe the process of L-proline transport in mammalian kidney. Whole-kidney and single-nephron clearance methods reveal the presence of more than one saturable system in the proximal nephron for net reabsorption of proline (10,34,51,52). Reabsorption from filtrate is achieved by Na^+-dependent and Na^+- independent systems with low and high capacity respectively (6,10,51,52). Studies with renal cortex brush-border membrane vesicles also reveal mediated proline transport (15,26,27) with heterogeneity of the uptake process (26,27), the characteristics of which are essentially compatible with those defined in the perfused tubule (51,52). Intrarenal oxidation (metabolic runout) of substrate influences net renal uptake of proline both *in vitro* (3,18,28), and *in vivo* (14,40). Decreased oxidation inhibits net reabsorption and increases the cellular proline content (40). Proline efflux at the basal-lateral membrane of renal epithelium is mediated by a Na^+-independent carrier (40). This carrier modulates permeability runout from cytoplasm to peritubular space.

These findings imply that net reabsorption of proline by the mammalian nephron is achieved by multiple carriers deployed in the brush-border membrane of proximal nephron and is influenced by events that sustain metabolic runout in cytoplasm and permeability runout at the basal-lateral membrane.

Renal cortex slices, in which the basal-lateral membrane appears to be predominantly exposed (1,53), display multiple, saturable proline transport systems that presumably serve epithelial nutrition. These systems have low and high capacities and higher or lower selectivity for proline respectively (2-4,28). Isolated renal tubules, which apparently expose both the brush-border and basal-lateral membrane (1), also possess multiple uptake systems for transport of proline (17).

Mutant phenotypes of renal proline carriers are largely confined to man, but they too indicate the presence of multiple carriers for proline in the nephron (24,34,35). Hereditary iminoglycinuria in the mature subject causes partial

loss of proline transport at normal levels of plasma proline. The phenotype is best explained by deficiency of a high-capacity carrier shared by proline and glycine (34). The normal newborn infant also has a partial loss of renal proline transport. The infant with hereditary iminoglycinuria, has little or no net reabsorption of proline (24) implying deficient activity at the shared carrier plus another carrier. By inference, the selective, high-affinity process of proline reabsorption is involved in neonatal prolinuria.

In the present study, we examined the mechanism of postnatal hyperprolinuria in dog and rat. In the presence of relatively constant plasma proline, increasing prolinuria in puppies during the initial postnatal period can be largely attributed to the increase in single-nephron filtration rate that occurs during ontogeny (29,48). Hyperprolinuria in the puppy after 10 days of age indicates elevated fractional proline excretion, as it does in the human infant (24). Stop-flow analysis confirmed that backflux from cell to lumen in distal nephron was not the cause of postnatal prolinuria in the dog. It follows that impaired transport at the principal sites of net reabsorption in the proximal nephron is the likely origin of neonatal prolinuria in mammalian kidney.

We used the isolated renal cortex tubule preparation to study proline uptake in newborn and mature rat nephrons. Net proline uptake was greater in tubules from newborn kidney. Attenuation of renal proline oxidation (runout) does not explain this finding. Slices prepared from newborn kidney also have enhanced uptake of proline after prolonged incubation (2-4); decreased cellular efflux of proline explains this finding (2-4). We did not measure efflux in the present work with the tubule preparation but we assume efflux was attenuated to account for the observations shown in Fig. 3.

AIB is a competitive inhibitor of proline transport at high concentrations (>0.2 mM) in renal cortex slices when the major portion of uptake can be assigned to the low-affinity, high-capacity system (41). AIB is also a competitive inhibitor of proline transport in isolated tubules (unpublished data). AIB is excluded from interaction with proline when the imino acid is transported at low concentrations in its high-affinity, proline-selective system in slices from adult rat kidney (2,28). Tubules from rat pups less than 1 wk old allowed AIB to inhibit proline uptake at the low concentration of substrate; AIB was excluded from the inhibitory action in tubules from adult rats and more mature rat pups. Accordingly, the findings in slices and tubules are concordant. Although we did not study the kinetics of concentration-dependent proline uptake by tubules, from the constant-ratio inhibition test we deduce that activity of the high-affinity, proline-specific carrier is diminished in tubules of the very young rat. This conclusion is compatible both with the finding of Goldmann et al. (12) who observed deficient activity of the carrier serving uptake of 0.06 mM proline in renal brush-border membranes of the newborn rat, and with the findings of Baerlocher et al. (2-4) who identified deficient high-affinity transport activity in the membrane exposed in renal cortex slices from rats less than 1 wk old. The fact that net uptake is ultimately greater at or near steady-state in the newborn tubule *in vitro* can be explained if efflux from the cell is also attenuated. The balance between influx and efflux determines the distribution ratio in this preparation; the conditions are not analogous to the circumstances of net reabsorption *in vivo*. The observed deficiency of net uptake under initial rate conditions in the newborn tubule exposed to the high concentration of proline can be explained by the reduced brush-border mass (total carrier activity) in the immature kidney (47).

Functional maturity of kidney during ontogeny is the sum of the different quantitative contributions from generations of nephrons at various levels of morphologic maturity (13); anatomical and biochemical evidence for maturational heterogeneity is considerable (16,19,20,21,23,31,46,47). Accordingly, newborn and adult kidneys are likely to be digested differently by collagenase thus releasing different forms of tubular segments; "dust" and disintegrating fragments were more commonly observed with newborn kidney in our experiments. This feature could explain the greater variance observed in the constant-ratio inhibition test with tubules from newborn kidney when compared with tubules from mature rat kidney (Fig. 4). Variance due to postnatal maturation of tubule transport activity is accomodated by studying tubule fragments from pups at specific ages, as described in the present study.

We propose that neonatal prolinuria reflects attenuated activity of the high-affinity proline transport system in brush-border membrane of proximal nephron; and perhaps in the basal-lateral membrane as well (2-4). The glycine component of neonatal iminoglycinuria can be attributed to a similar dearth of activity in the high-affinity glycine transporter; the latter is located in pars recta (5). On the other hand, because the diffusional mode of glycine transport is significant (43), ontogeny of glycine reabsorption may also reflect anatomical lengthening of the proximal nephron and pars recta during maturation (47).

REFERENCES

1. Arthus, M.-F., Scriver, C. R. and Bergeron, M.: Restriction of exchanges between medium and luminal membrane of nephron during incubation of renal cortex slices. Clin. Res. 28: 695A (1980).

2. Baerlocher, K. E., Scriver, C. R. and Mohyuddin, F.: Ontogeny of iminoglycine transport in mammalian kidney. Proc. Natl. Acad. Sci. 65: 1009 (1970).

3. Baerlocher, K. E., Scriver, C. R. and Mohyuddin, F.: The ontogeny of amino acid transport in rat kidney. I. Effect on distribution ratios and intracellular metabolism of proline and glycine. Biochim. Biophys. Acta 249: 353 (1971).

4. Baerlocher, K. E., Scriver, C. R. and Mohyuddin, F.: The ontogeny of amino acid transport in rat kidney. II. Kinetics of uptake and effect of anoxia. Biochim. Biophys. Acta 249: 364 (1971).

5. Barfuss, D. W., Mays, J. M. and Schafer, J. A.: Peritubular uptake and transepithelial transport of glycine in isolated proximal tubules. Am. J. Physiol. 238: F324 (1980).

6. Bergeron, M. and Morel, F.: Amino acid transport in rat renal tubules. Am. J. Physiol. 216: 1139 (1969).

7. Bergeron, M. and Vadeboncoeur, M.: Antiluminal transport of L-arginine and L-leucine following microinjections in peritubular capillaries of the rat. Nephron 8: 355 (1971).

8. Burg, M. B. and Orloff, J.: Oxygen consumption and active transport in separated renal tubules. Am. J. Physiol. 203: 327 (1962).

9. Chesney, R. W. and Jax, D. K.: Personal Communication (1979).

10. Dubord, L. and Bergeron, M.: Multiplicité des systèmes transporteurs à la membrane luminale du néphron chez le rat normal. Rev. Can. Biol. 33: 99 (1974).

11. Foulkes, E. C. and Gieske, T.: Specificity and metal sensitivity of renal amino acid transport. Biochim. Biophys. Acta 318: 439 (1973).

12. Goldmann, D. R., Roth, K. S., Langfitt, Jr, T. W. and Segal, S.: L-proline transport by newborn rat kidney brush-border membrane vesicles. Biochem. J. 178: 253 (1979).

13. Goncharevskaya, O. A. and Dlouha, H.: The development of various generations of nephrons during postnatal ontogenesis in the rat. Anat. Rec. 182: 367 (1975).

14. Greth, W. E., Thier, S. O. and Segal, S.: The transport and metabolism of L-proline-^{14}C in the rat in vivo. Metabolism 27: 975 (1978).

15. Hammerman, M. R. and Sacktor, B.: Transport of amino acids in renal brush border membrane vesicles. Uptake of L-proline. J. Biol. Chem. 252: 591 (1977).

16. Hay, D. and A. and Evan, A. P.: Maturation of the proximal tubule in the puppy kidney: a comparison to the adult. Anat. Rec. 195: 273 (1979).

17. Hillman, R. and E. and Rosenberg, L. E.: Amino acid transport by isolated mammalian renal tubules. II. Transport systems for L-proline. J. Biol. Chem. 244: 4494 (1969).

18. Holtzapple, P., Genel, M., Rea, C. and Segal, S.: Metabolism and uptake of L-proline by human kidney cortex. Pediatr. Res. 7: 818 (1973).

19. Horster, M. and Larsson, L.: Mechanisms of fluid absorption during proximal tubule development. Kidney Int. 10: 348 (1976).

20. Horster, M. and Valtin, H.: Postnatal development of renal function: micropuncture and clearance studies in the dog. J. Clin. Invest. 50: 779 (1971).

21. Jokelainen, P.: An electron microscope study of the early development of the rat metanephric nephron. Acta Anat. 52: 7 (1963).

22. Lambert, P. P., Vanderveiken, J. P., Dekoster, R., Kahn, J. and DeMyttenaere, M.: Study of phosphate excretion by the stop-flow technique. Effects of parathyroid hormone. Nephron 1: 103 (1964).

23. Larsson, L.: The ultrastructure of the developing proximal tubule in the rat kidney. J. Ultrastructure Res. 51: 119 (1975).

24. Lasley, L. and Scriver, C. R.: Ontogeny of amino acid reabsorption in human kidney. Evidence from the homozygous infant with familial renal iminoglycinuria for multiple proline and glycine systems. Pediatr. Res. 13: 65 (1979).

25. Malvin, R. L., Wilde, W. S. and Sullivan, L. P.: Localization of nephron transport by stop-flow analysis. Am. J. Physiol. 194: 135 (1958).

26. McNamara, P. D., Ozegovic, B., Pepe, L. M. and Segal, S.: Proline and glycine uptake by renal brush-border membrane vesicles. Proc. Natl. Acad. Sci. USA 73: 4521 (1976).

27. McNamara, P. D., Pepe, L. M. and Segal, S.: Sodium gradient dependence of proline and glycine uptake in rat renal brush-border membrane vesicles. Biochim. Biophys. Acta 556: 151 (1979).

28. Mohyuddin, F. and Scriver, C. R.: Amino acid transport in mammalian kidney: multiple systems for imino acids and glycine in rat kidney. Am. J. Physiol. 219: 1 (1970).

29. Nash, M. A. and Edelmann, Jr, C. M.: The developing kidney. Immature function or inappropriate standard. Nephron 11: 71 (1973).

30. Nutzenadel, W. and Scriver, C. R.: Uptake and metabolism of β-alanine and L-carnosine by rat tissues in vitro: role in nutrition. Am. J. Physiol. 230: 643 (1976).

31. Osathanondh, V. and Potter, E. L.: Development of human kidney as shown by microdissection. Arch. Pathol. 82: 391 (1966).

32. Roth, K. S., Hwang, S. M., Yudkoff, M. and Segal, S.: On the transport of sugars and amino acids by newborn kidney: use of isolated proximal tubule. Life Sci. 18: 1125 (1976).

33. Schafer, J. A. and Barfuss, D. W.: Membrane mechanisms for transepithelial amino acid absorption and secretion. Am. J. Physiol. 238: F335 (1980).

34. Scriver, C. R.: Renal tubular transport of proline, hydroxyproline, and glycine. III. Genetic basis for more than one mode of transport in human kidney. J. Clin. Invest. 47: 823 (1968).

35. Scriver, C. R.: Familial Iminoglycinuria. In: J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson: The Metabolic Basis of Inherited Disease. 5th ed. (McGraw Hill Book Co., New York, 1981) in press.

36. Scriver, C. R. and Bergeron, M.: Amino acid transport in kidney. The use of mutation to dissect membrane and transepithelial transport. In: W. L. Nyhan: Heritable Disorders of Amino Acid Metabolism p. 515 (Wiley and Son, New York, 1974).

37. Scriver, C. R., Bergeron, M. and Arthus, F.-F.: Ontogeny of amino acid reabsorption in mammalian kidney. The proline model. Adv. Physiol. Sci. 11: 97 (1981).

38. Scriver, C. R., Chesney, R. W. and McInnes, R. R.: Genetic aspects of renal tubular transport. Diversity and topology of carriers. Kidney Int. 9: 149 (1976).

39. Scriver, C. R., Davies, E. and Lamm, P.: 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 (1968).

40. Scriver, C. R., McInnes, R. R. and Mohyuddin, F.: Role of epithelial architecture and intracellular metabolism in proline uptake and transtubular reclamation in PRO/Re mouse kidney. Proc. Natl. Acad. Sci. USA 72: 1431 (1975).

41. Scriver, C. R. and Mohyuddin, F.: Amino acid transport in kidney: heterogeneity of AIB uptake. J. Biol. Chem 243: 3207 (1968).

42. Silbernagl, S.: Renal transport of amino acids. Klin. Wochenschr. 57: 1009 (1979).

43. Silbernagl, S. and Deetjen, P.: Glycine reabsorption in rat proximal tubules. Microperfusion studies. Pflügers Arch. 323: 342 (1971).

44. Slack, E. M., Liang, C.-C. T. and Sacktor, B.: Transport of L-proline and D-glucose in luminal (brush-border) and contraluminal (basal-lateral) membrane vesicles from the renal cortex. Biochem. Biophys. Res. Comm. 77: 891 (1977).

45. Sokal, R. R. and Rohlf, F. J.: Biometry. Freeman, San Francisco 1969.

46. Spitzer, A. and Brandis, M.: Functional and morphological maturation of the superficial nephrons. J. Clin. Invest. 53: 279 (1974).

47. Suzuki, Y.: An electron microscopy of the renal differentiation. I. Proximal tubule cells. J. Electromicroscopy 6: 52 (1958).

48. Tavani, Jr, N., Calcagno, P., Zimmet, S., Flamenbaum, W., Eisner, G. and Jose, P. Ontogeny of single nephron filtration distribution in canine puppies. Pediatr. Res. 14: 799 (1980).

49. Ullrich, K. J. Sugar, amino acid, and Na^+ cotransport in the proximal tubule. Ann. Rev. Physiol. 41: 181 (1979).

50. Ullrich, K. J., Reimrich, G. and Klöss, S.: Sodium dependence of the amino acid transport in the proximal convolution of the rat kidney. Pflügers Arch. 351: 49 (1974).

51. Völkl, H. and Silbernagl, S.: Molecular specificity of tubular reabsorption of L-proline. A microperfusion study in rat kidney. Pflügers Arch. 387: 253 (1980).

52. Völkl, H., Silbernagl, S. and Deetjen, P.: Kinetics of L-proline reabsorption in rat kidney studied by continuous microperfusion. Pflügers Arch. 382: 115 (1979).

53. Wedeen, R. P. and Weiner, B.: The distribution of p-aminohippuric acid in rat kidney slices. I. Tubular localization. Kidney Int. 3: 205 (1973).

54. Presented in part at the International Congress of Physiology, Budapest, Hongary, July 1980 (37).

55. Work performed during a sabbatical leave from the MRC Genetics Group, Human Genetics Center McGill University (CRS).

56. This is publication number 82012 of the McGill University-Montreal Children's Hospital Research Institute.

57. We thank Christiane Mazini Schwab for her essential technical assistance. We are also grateful to: Mme Marie Villeneuve for the quantitative amino acid analysis; Dr. Patrick Vinay for evaluation of our tubule preparation; Dr. Russell Chesney for advice about preparation of isolated tubules from newborn rat kidney; Professor Stefan Silbernagl for discussion of proline transport systems in mammalian nephron. This work was supported in part by CRS (Medical Genetics Group Award) and grant MT-2862 (MB, U. de M.) from the Medical Research Council of Canada.

58. Address requests for reprints to:
Dr. Charles R. Scriver, De Belle Laboratory for Biochemical Genetics, Montreal Children's Hospital, 2300 Tupper Street, Montreal, Quebec, Canada H3H 1P3.

59. Received for publication June 2, 1981.

60. Accepted for publication April 1, 1982.

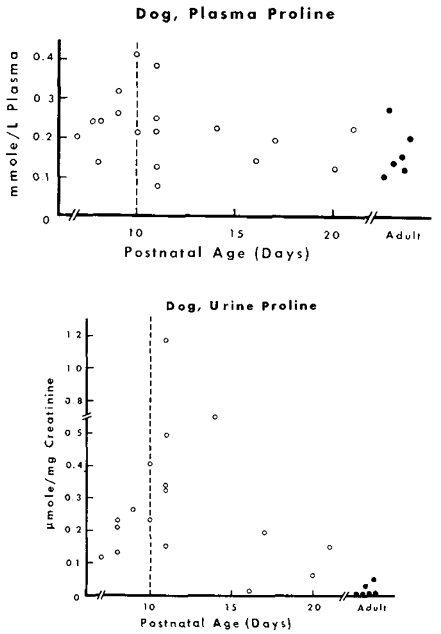

Figure 1
Plasma proline concentrations (upper panel) and urine proline content (lower panel) in puppies (o) and adult mongrel dogs (•). The increase in urinary proline during the first 10 days after birth is attributed to increasing single nephron filtration in the dog during this period (47).