Developmental Aspects of Sugar Transport by Isolated Dog Renal Cortical Tubules

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Summary

α-Methyl-D-glucoside (AMG) uptake was examined in isolated renal cortical tubules from newborn, 3-month-old, and adult dogs. All three age groups demonstrated active sugar transport. The initial rate of AMG uptake was similar in the 3-month-old and adult tubules which was twice that of the newborn. At steady-state, the adult and newborn tubules had achieved a similar intracellular AMG concentration which was 45% greater than that of the 3-month-old. Determination of the flux constants of these uptake patterns revealed that there was an age-dependent increase in both the net flux and the fractional influx rate constant. However, the 3-month-old had the highest fractional efflux rate constant and the newborn the lowest value with the adult in between. Kinetic analysis of AMG uptake showed a single saturable transport system for each age group. The newborn and adult had similar K_m values but the 3-month-old had a value that was 60% higher. The 3-month-old tubules had the highest V_{max} and the newborn tubules the lowest with the adult value in between. AMG uptake by tubules from each age group demonstrated a similar pattern of inhibition in a low sodium buffer and by glucose and phlorizin. This indicated that, aside from kinetic changes with maturation, the saturable transport system for AMG is similar in each age group.

Abbreviations

AMG, α-methyl-D-glucoside
KRB, Krebs-Ringer bicarbonate buffer
DR, distribution ratio
ICF, intracellular fluid

The developing kidney is characterized by a functional immaturity of both the glomerulus and tubule (35). One of the manifestations of this tubular immaturity is diminished maximal tubular reabsorption of glucose in the human (3, 6), dog (2), and sheep neonate (1). However, these studies are whole kidney clearance measurements which are influenced by numerous variables such as the state of extracellular volume, glomerular filtration rate, and the fractional excretion of sodium. Therefore, the intrinsic ability of the proximal tubule from these developing animals and the human neonate to reabsorb solute can only be approximated. Further, the mechanism of sugar transport at the level of the brush border membrane can only be inferred from such studies and not addressed directly.

The use of isolated renal cortical tubule fragments allows the study of sugar transport independent of the glomerular filtration rate, sodium excretion, or extracellular fluid volume. AMG is a nonmetabolizable model for the glucose-galactose transport sys-

tem (31) which Silverman (32) has shown is transported exclusively by the brush border membrane, making it an ideal substrate for evaluating luminal sugar transport in the developing animal.

A previous report of AMG transport by isolated renal cortical tubules from the newborn rat demonstrated two kinetically distinct transport systems with only the low affinity system present in the adult rat (26). However, initial uptake and steady-state values of AMG were lower in the immature rat in spite of the two systems. Since the loss of a transport system is an unusual developmental change, we examined AMG uptake by isolated renal cortical tubules from developing dogs to assess whether a similar pattern is present in species other than the rat.

MATERIALS AND METHODS

Mongrel dogs of either sex from three different age groups— 5–7 days old designated newborn, 3 months old, and greater than 1 year old—were used for the preparation of isolated renal tubules according to a modification (17, 26) of the method of Burg and Orloff (8). Kidneys were surgically removed from the dogs after sacrificing them with an arterial injection of 2 ml of T-61 euthanasia solution (Hoechst Corp.). The kidneys were immediately perfused at 4°C via the renal artery with a 0.375% collagenase solution (w/v) in KRB containing 10 mM sodium acetate, pH 7.4. The renal cortex was excised from the medulla, and cortical slices were made using a Stadie-Riggs microtome. The slices were then homogenized gently with four strokes of a pestle in a 15-ml loose Dounce homogenizer and the suspension (1 g/3 ml of KRB) was centrifuged for 1 min at 40 × g in an International Equipment Co. model UV centrifuge (Needham Hts., MA). The pellet was suspended in 3 ml of KRB containing 0.375% collagenase and 0.4% hyaluronidase (w/v) for each gram of original kidney cortex.

After a 45-min digestion at 37°C in a Dubnoff metabolic shaking incubator, three times the incubation volume of iced KRB was added to the suspension and this was centrifuged at 40 × g for 1 min. The supernatant was discarded and the tubules were resuspended in the same volume of iced KRB. This was repeated twice. After the final wash, the tubules were resuspended in KRB to a concentration of approximately 5–7 mg wet weight/ ml, and filtered through a 104-μm mesh. In studies examining the effect of low medium sodium on uptake, the tubules were resuspended in KRB that was modified by replacing the 118.5 mM NaCl with 118.5 mM choline chloride to give a final medium sodium concentration of 35 meq/liter. Fetal calf serum was added to a final concentration of 5% (v/v) in both KRB and the low sodium KRB.

Uptake studies were performed in Burg and Orloff flasks with

continuous bubbling of a 95% O_2-5% CO_2 mixture as described previously (17, 24, 26). Substrate uptake was initiated by the addition of [^{14}C]α-methyl-D-glucoside 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 33,000 × g and the supernatants were removed for counting. The pellet surface and the test tube wall were washed once with ice-cold KRB 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 a 0.1-ml aliquot of the water extract of the pellet and the original incubation supernatants 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 (22). The intracellular fluid volume was calculated as the difference between the total tissue fluid (wet weight minus dry weight after overnight desiccation) and the volume of "trapped fluid." The trapped fluid volume was determined using [^{14}C]polyethylene glycol as previously described (17).

In concentration-dependence studies, the uptake of [^{14}C]AMG by isolated tubules was measured after 5 min of incubation over the substrate concentration range of 0.05 to 10 mM AMG. These data were analyzed by Hofstee plots fitted by the least squares method to obtain the parameters of the equation:

$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

The data were analyzed for significance using Student's *t* test.

Collegenase grade II, was obtained from Worthington Biochemical Corp., Freehold, NJ with a specific activity of 161 U/mg. Hyaluronidase, type I-S, was obtained from Sigma Chemical Corp., St. Louis, MO. [U^{-14}C]α-methyl-D-glucoside (279 mCi/mmol), [^{14}C]polyethylene glycol (21.7 mCi/g), and OCS scintillation fluid were obtained from Amersham Corp., Arlington, IL. Additional chemicals were obtained from commercial sources and were of the highest purity available.

RESULTS

Time Course of AMG Uptake. The uptake of 2 mM AMG with time by isolated renal cortical tubules from newborn, 3-month-old, and adult dogs is shown in Figure 1. Isolated cortical tubules from all three age groups of dogs demonstrated active uptake of 2 mM AMG after 1 min of incubation with a DR of 1.56 ± 0.19 in the newborn, 2.93 ± 0.30 in the 3-month-old, and 3.11 ± 0.38 in the adult dogs. The uptake by adult dog tubules was maximal by 15 min of incubation with a DR of 9.86 ± 0.62 and dropped slightly after 30 min of incubation such that
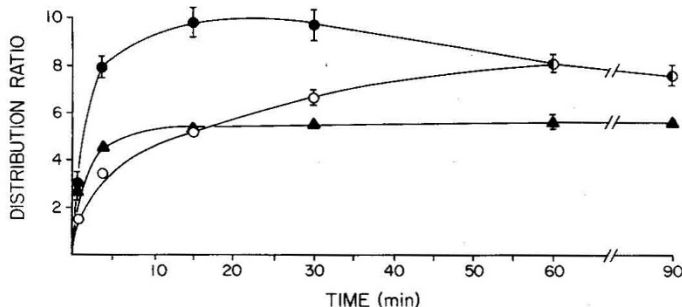

Fig. 1. Time-dependent uptake of 2 mM [^{14}C]AMG by isolated dog renal cortical tubules. Isolated tubules from newborn (O), 3-month-old (▲), and adult (●) dogs were prepared and incubated with labeled AMG as described in "Materials and Methods." Each *point* represents the mean ± SEM of at least six determinations. Standard errors not shown are within the size of the point.

a DR of 8.23 ± 0.38 was measured at 60 min of incubation. Uptake by 3-month-old dog tubules achieved a steady-state by 15 min of incubation with a DR of 5.61 ± 0.10. In tubules from newborn dogs, a steady-state was not attained until 60 min of incubation with a DR of 7.93 ± 0.23.

The initial rate of uptake, determined by measuring uptake after 1 min of incubation, was lower in tubules from newborn dogs than that observed for either adult or 3-month-old dogs ($P < 0.01$ for adult tubules and $P < 0.01$ for 3-month-old tubules). Uptake by newborn dog tubules exceeded that by 3-month-old tubules after 15 min of incubation since a steady-state had been reached by the 3-month-old tubules, but the newborn tubules continued to accumulate AMG. After 60 min of incubation, the uptake by newborn tubules reached that of adult tubules. This occurred in spite of the lower initial uptake rate by newborn tubules, suggesting that the efflux of AMG is slower in the newborn tubules to account for the similar DR at 60 min of incubation for adult and newborn tubules. A slower rate of AMG efflux from newborn tubules compared to that of 3-month-old tubules would also explain the higher DRs by newborn tubules at the later time points. The uptake by 3-month-old tubules appeared to be a composite of adult and newborn uptake in that the initial uptake was between adult and newborn values and the maximal DR was reached by 15 min.

Determination of Flux Constants. Differences in the time-dependent uptake curves can be evaluated in terms of flux constants (Table 1) based on a two-compartment model of medium and intracellular fluid (23). There was an age-dependent increase in both the net AMG flux and the fractional influx rate constant (λ_{IM}) with the adult values being slightly higher than the 3-month-old and nearly twice the newborn values. This was reflected in the age-dependent increase in the DR after 1 min of incubation. The fractional efflux rate constants showed a somewhat different pattern in that the highest value was observed with 3-month-old tubules, being 3-fold greater than newborn and 1.4-fold greater than the adult value. The newborn fractional efflux rate constant was the lowest value determined being only one-half that of the adult. Because of the low fractional efflux rate constant, the newborn tubules achieved a steady-state DR comparable to the adult in spite of a low fractional influx rate constant. The high fractional efflux rate constant led to the low steady-state DR observed in the 3-month-old.

Concentration Dependence of AMG Uptake. The concentration dependence of AMG uptake is shown in Figure 2. In all three age groups, only a single saturable transport system was observed. The apparent kinetic transport parameters of this system were $K_m = 5.14 ± 0.50$ mM, $V_{max} = 25.79 ± 1.63$ mmol/liter of ICF in the newborn; $K_m = 8.40 ± 0.81$ mM, $V_{max} = 52.01 ± 5.53$ mmol/liter of ICF in the 3-month-old dogs, and $K_m = 5.27 ± 0.75$ mM and $V_{max} = 37.64 ±3.38$ mmol/liter of ICF in the adult dogs. There was no difference in the apparent K_m between the newborn and the adult dogs. However, the apparent K_m was 63% higher in the tubules from 3-month-old dogs compared to newborn ($P < 0.01$), and 59% higher than that of the adult ($P < 0.05$).

There were corresponding changes in V_{max} with maturation. V_{max} was 102% higher ($P < 0.01$) in the tubules from 3-month-old puppies compared to newborns. By adulthood, however, this increase over the newborn was only 46% ($P < 0.02$). There was no statistical difference between the V_{max} obtained with 3-month-old tubules compared with adult tubules.

Effect of Low Na$^+$ Buffer and Inhibitors. The effect of a low Na$^+$ buffer and various inhibitors of AMG uptake is shown in Figure 3. Reducing the medium Na$^+$ concentration to 35 meq/liter significantly decreased the uptake of AMG by the tubules from all three age groups of dogs. Phlorizin was the most potent inhibitor tested, reducing AMG uptake to less than 20% of control. This marked inhibition by phlorizin, which preferentially affects the brush border glucose transporter (33), suggests that the predominant site of cellular uptake of AMG by isolated

Table 1. *Two-compartment model of medium and intracellular fluid**

$$\boxed{\text{Medium}} \overset{\lambda_{IM}}{\underset{\lambda_{MI}}{\rightleftharpoons}} \boxed{\text{intracellular space}}$$

Age group	Medium pool size (μmol)	Steady-state distribution ratio	Intracellular fluid pool size (μmol)	Fractional turnover rates/min		Flux (μmol/min)
				λ_{IM}	λ_{MI}	
Newborn	2.0	7.93 ± 0.23	0.0452	0.0044	0.197	0.0088
3-month-old	2.0	5.61 ± 0.10	0.0267	0.0069	0.522	0.0139
Adult	2.0	8.23 ± 0.38	0.0442	0.0084	0.378	0.0167

* All calculations are based on 5 mg of tubules suspended in 1 ml of incubation medium containing 2.0 mM AMG, and an intracellular space of 56.96 ± 1.51% ($n = 11$), 47.54 ± 0.99% ($n = 21$), and 53.83 ± 0.40% ($n = 6$) of wet tissue weight for the newborn, 3-month-old, and adult tubule preparation, respectively. The rate constants are related by the equation (M) (λ_{IM}) = (ICS) (λ_{MI}), where M is the medium pool size and ICS is the intracellular fluid substrate pool. Flux is expressed as μmol/min either into or out of the cell at a steady-state.
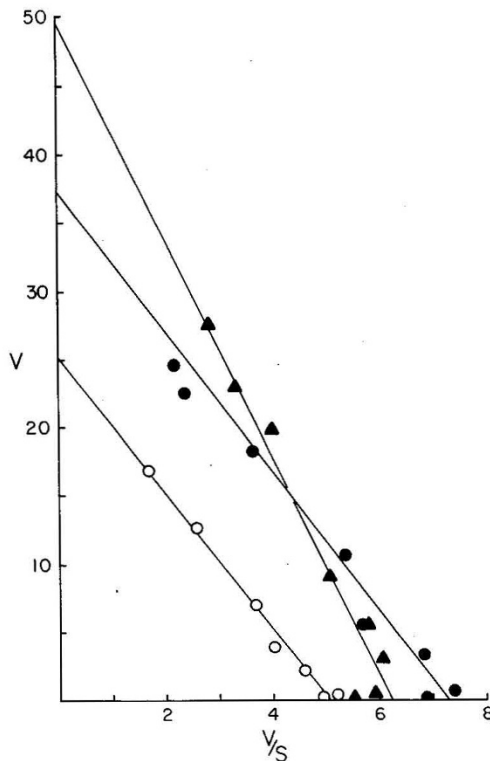

Fig. 2. Hofstee plot of the concentration dependence of AMG uptake by isolated renal tubules from newborn (O), 3-month-old (▲), and adult (●) dogs. Tubules were incubated for 5 min with 0.1 μCi/ml [^{14}C]AMG and sufficient unlabeled AMG to give the desired concentrations over the range of 0.05 to 10 mM. V represents the velocity of uptake in mmol/liter intracellular fluid per 5 min and S represents the substrate concentration in mM.

renal tubule fragments is across the luminal membrane. Glucose was also an effective inhibitor in each age group, but galactose, even at twice the glucose concentration, only significantly inhibited AMG uptake by newborn tubules. Galactose inhibited AMG transport slightly in the 3-month-old and adult tubules, but this was not statistically significant. These results suggest that, aside from the changes in the kinetic parameters with maturation, the saturable transport system for AMG is similar in each age group.

DISCUSSION

The developing kidney in a number of species, including man, has been characterized as being functionally immature. This immaturity extends to both glomerular and tubular function,

although there is a balance between their respective capacities (2, 3, 6). As the individual matures, there is a rise in glomerular function as measured by the glomerular filtration rate (34, 37). The maturation of tubular function is characterized by increased ability to reabsorb amino acids (7, 15, 17) sugars (1-3, 6), phosphate (18), bicarbonate (9), and other solutes. Also with maturation, there is an increased ability of the tubule to secrete solute as evidenced by an increasing p-aminohippurate clearance with age (14, 20, 28).

These functional changes with maturation are also accompanied by morphologic changes (10, 12, 13, 19). There is an increase in the number of nephrons with maturation, although nephronogenesis is complete by 36 weeks of conceptual age in the human (19) and 2 weeks of postnatal age in the dog (13). In addition to the number of nephrons, there are also increases in the length and volume of the proximal tubule, and in the surface area of the glomerulus. However, this rise in length and surface area of the proximal tubule with development does not appear to account completely for the increased transport capacity. Indeed, Schwartz et al. (29) have estimated that only one-third of the rise in the tubular transport of p-aminohippurate can be ascribed to an increase in tubular surface area, while two-thirds of the increase is due to an enhanced intrinsic transport function of the proximal tubule cell.

Enhanced intrinsic proximal tubule transport could arise by a number of mechanisms among which are the appearance of new transport systems with maturation, increasing uptake across the luminal membrane by augmenting either the number or the affinity of the membrane carriers, increasing the transfer of resorbate across the basolateral membrane into the blood, and enhancing the generation and transduction of cellular energy to transport. Although early studies suggested that new transport systems were acquired with maturation (4, 5), subsequent studies have shown that the newborn proximal tubule has a full complement of transport systems for sugars (26, 36), amino acids (15–17, 21), and organic acids (27) so that the functional immaturity of this nephron segment is not due to the lack of transport systems. Indeed in the newborn rat, there is a high affinity system for α-methyl-D-glucoside transport in addition to the single low affinity system found in the adult (26). However, in spite of these two transport systems in the newborn rat, the initial entry rate of AMG at both a high and low concentration is slower in the newborn compared to the adult. The K_m value for the system which is shared by both the mature and immature animal is the same in each age group, but the V_{max} for this system in the newborn is only one-half of that of the adult. Our data show that in the dog only one saturable system is present in all three age groups. There was, however, a progressive rise in the initial rate of AMG uptake with maturation as observed in the rat. Kinetic analyses of this increase showed that the K_m for this transport system was 63% higher in the 3-month-old compared to the newborn puppy, but that the adult value was the same as the
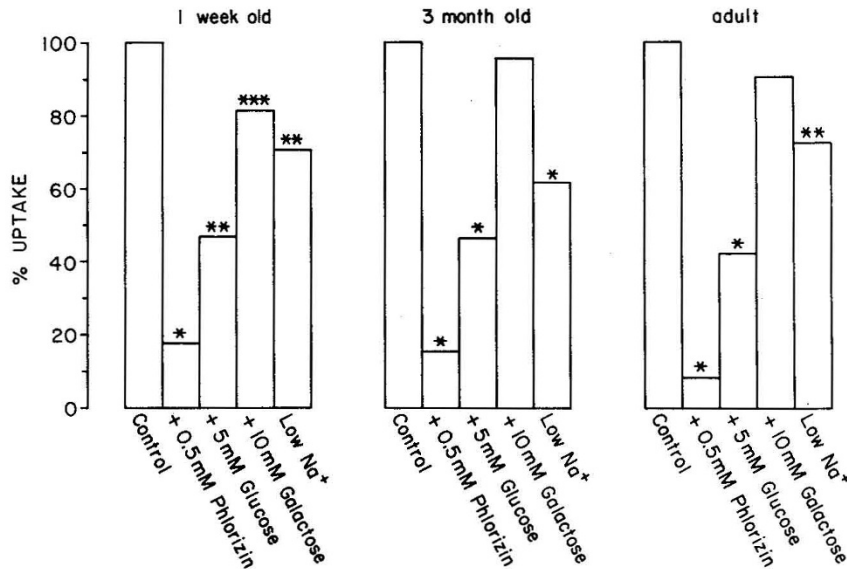
Fig. 3. The effect of low Na$^+$ buffer and inhibitors on AMG uptake. The uptake by isolated tubules incubated for 5 min with 2 mM AMG in a modified Krebs-Ringer buffer containing 35 meq/liter Na$^+$ was measured and plotted as a percentage of that obtained in Krebs-Ringer buffer containing a normal amount of Na$^+$ as described in "Materials and Methods." The effect of various inhibitors on the uptake of 2 mM AMG was examined after 5 min of incubation and is plotted as a percentage of the uptake in the absence of the inhibitors. *, $P < 0.001$; **, $P < 0.01$; ***, $P < 0.05$.

newborn value. The V_{max} for this sugar transport system was increased in both the adult and 3-month-old dog compared to the newborn with the highest value noted in the 3-month-old. These data suggest that the rise in initial rate of AMG uptake by dog isolated renal tubules is due to an increase in the number of carriers as evidenced by the rise in V_{max}. The 3-month-old had the highest value for V_{max}, but this was offset by a reduction in the affinity of the system for AMG leading to an initial rate that was intermediate between the adult and newborn.

Another phenomenon evident in uptake studies using immature renal tissue is decreased solute efflux rates compared to adult tissue. This can be surmised from Figure 1 where comparable steady-state DRs are achieved by both adult and newborn tubules in spite of a much lower influx rate with the newborn tubules. This has been noted previously for glycine uptake by renal tubules from newborn puppies (17). A slow efflux rate has also been shown for glycine (4, 21, 25, 30) proline (4, 16), taurine (11), and cystine (15) in immature rat renal tissue. The slow efflux rates characteristic of immature renal tubule cells have usually increased to the high values observed in the adult. Our observations in the dog provide the first instance when an intermediate stage occurs as in the 3-month-old when the efflux rate is actually higher than the adult. The explanation for this remains to be determined.

REFERENCES AND NOTES

1. Alexander DP, Nixon DA 1963 Reabsorption of glucose fructose and meso-inositol by the foetal and postnatal sheep kidney. J Physiol (Lond) 167:480
2. Arant BS Jr, Edelmann CM Jr, Nash MA 1974 The renal reabsorption of glucose in the developing canine kidney: a study of glomerulotubular balance. Pediatr Res 8:638
3. Arant BS Jr 1978 Developmental patterns of renal functional maturation compared in the human neonate. J Pediatr 92:705
4. Baerlocher KE, Scriver CR, Mohyuddin F 1970 Ontogeny of iminoglycine transport in mammalian kidney. Proc Natl Acad Sci USA 65:1009
5. Baerlocher KE, Scriver CE, Mohyuddin F 1971 The ontogeny of amino acid transport in rat kidney. II. Effects on distribution ratios and intracellular metabolism of proline and glycine. Biochim Biophys Acta 249:353
6. Brodehl J, Franken A, Gellissen K 1972 Maximal tubular reabsorption of glucose in infants and children. Acta Paediatr Scand 61:413
7. Brodehl J 1976 Postnatal development of tubular amino acid reabsorption. In Silvernagl S, Lang F, Greger R (eds) Amino Acid Transport and Uric Acid Transport. Georg Thieme Publishers, Stuttgart, pp 128–136
8. Burg MB, Orloff J 1962 Oxygen consumption and active transport in separated renal tubules. Am J Physiol 203:327

9. Edelmann CM Jr, Rodriguez-Soriano J, Boichis H, Gruskin AB, Acosta MI 1967 Renal bicarbonate reabsorption and hydrogen ion excretion in normal infants. J Clin Invest 46:1309
10. Fetterman GH, Shuplock NA, Phillip FJ, Gregg HS 1965 The growth and maturation of human glomeruli and proximal convolutions from term to adulthood. Pediatrics 35:601
11. Friedman AL, Jax DK, Chesney RW 1981 Developmental aspects of renal β-amino acid transport. III. Ontogeny of transport in isolated renal tubule segments. Pediatr Res 15:10
12. Hay DA, Evans AP 1982 Maturation of the proximal tubule in the puppy kidney: a comparison to the adult. Anat Rec 194:273
13. Horster M, Lewy JE 1971 Filtration fraction and extraction of PAH during the neonatal period in the rat. Am J Physiol 219:1061
14. Horster M, Kemler BJ, Valtin H 1971 Intracortical distribution of volume and number of glomeruli during postnatal maturation in the dog. J Clin Invest 50:796
15. Hwang SM, Foreman JW, Segal S 1982 Developmental pattern of cystine transport in isolated rat renal tubules. Biochim Biophys Acta 690:145
16. Hwang SM, Serabian MA, Roth KS, Segal S 1983 L-Proline transport by isolated renal tubules from newborn and adult rats. Pediatr Res 17:42
17. Medow MS, Foreman JW, Bovee KD, Segal S 1982 Developmental changes of glycine transport in the dog. Biochim Biophys Acta 693:85
18. McCrory WW, Forman CV, McNamara H, Barnett HL 1982 Renal excretion of phosphate in newborn infants. J Clin Invest 31:357
19. Potter EL, Thierstein ST 1943 Glomerular development of the kidney as an index of fetal maturity. J Pediatr 22:695
20. Rennick B, Hamilton B, Evans R 1961 Development of renal tubular transport of TEA and PAH in the puppy and piglet. Am J Physiol 201:743
21. Reynolds R, Roth KS, Hwang SM, Segal S 1978 On the development of glycine transport systems by rat renal cortex. Biochim Biophys Acta 571:274
22. Rosenberg LE, Blair M, Segal S 1961 Transport of amino acids by slices of rat kidney cortex. Biochim Biophys Acta 54:479
23. Rosenberg LE, Berman M, Segal S 1963 Studies of the kinetics of amino acid transport, incorporation into protein and oxidation in kidney-cortex slices. Biochim Biophys Acta 71:664
24. Roth KS, Hwang SM, Segal S 1976 The effect of maleic acid on the kinetics of alpha-methyl-D-glucoside uptake by isolated rat renal tubules. Biochim Biophys Acta 426:675
25. Roth KS, Hwang SM, London JW, Segal S 1977 Ontogeny of glycine in isolated rat renal tubules. Am J Physiol 233:F241
26. Roth KS, Hwang SM, Yudkoff M, Segal S 1978 The ontogeny of sugar transport in kidney. Pediatr Res 12:1127
27. Roth KS, Serabian MA, Medow MS 1982 A comparison of the uptake of 3-hydroxy-3-methylglutaric acid in newborn and adult rat kidney. Metabolism 31:406
28. Rubin ML, Bruick E, Rappaport M 1949 Maturation of renal function in childhood: clearance studies. J Clin Invest 28:1144
29. Schwartz GJ, Goldsmith DI, Fine LG 1978 Aminohippurate transport in the proximal straight tubule: development and substrate stimulation. Pediatr Res 12:793
30. Segal S, Rea C, Smith J 1971 Separate transport systems for sugars and amino acids in developing rat kidney cortex. Proc Natl Acad Sci USA 68:372
31. Segal S, Rosenhagen M, Rea C 1973 Developmental and other characteristics

of alpha-methyl-D-glucoside transport by rat kidney cortex slices. Biochim Biophys Acta 291:519

32. Silverman M 1976 Glucose transport in the kidney. Biochim Biophys Acta 457:303

33. Silverman M, Black J 1975 High affinity phlorizin receptor sites and their relation to the glucose transport mechanism in the proximal tubule of the dog kidney. Biochim Biophys Acta 394:10

34. Spitzer A, Edelmann CM Jr 1971 Maturational changes in pressure gradients for glomerular filtration. Am J Physiol 221:1431

35. Spitzer A 1982 The Kidney during development. Masson Publishing, New York

36. Turner RJ, Silverman M 1978 Sugar uptake into brush border vesicles from dog kidney. I. Specificity. Biochim Biophys Acta 507:305

37. Weil WB Jr 1955 Evaluation of renal function in infancy and childhood. Am J Med Sci 229:519

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Immunoregulation in an Isolated 12-year-old Boy with Congenital Severe Combined Immunodeficiency

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Summary

We report the evaluation of *in vitro* immunoregulation in a 12-year-old untreated boy with severe combined immunodeficiency (SCID). Severely hypogammaglobulinemic, the patient was incapable of a specific antibody response to either natural substances or administered antigens. Ficoll-Hypaque-isolated peripheral blood mononuclear cells (MNL) from the patient failed to respond to pokeweed mitogen (PWM) with the normal increment in immunoglobulin-secreting cells, as measured by a reverse hemolytic plaque assay. Since the patient was lymphopenic, his MNL were relatively enriched for monocytes (range = 51–81%). Removal of phagocytic cells or the addition of unrelated irradiated helper T lymphocytes resulted in enhanced, but still suboptimal response to PWM, suggesting some intrinsic defect in B lymphocyte function. Co-culture of patient MNL with normal MNL resulted in marked suppression (12% of predicted) of PWM-induced Ig-secreting cells. Suppressor activity was unaffected by prior irradiation of patient MNL, but was substantially reversed (99% of predicted) by removal of his phagocytic cells, whereas the combination of the two procedures further reversed suppression (184% of predicted). The patient's MNL consistently demonstrated subnormal percentages of T3+ and T4+ cells and subnormal to low normal percentages of T8+ cells. These data suggest both an intrinsic defect in B lymphocyte function, and a relative excess of monocytes which could further inhibit Ig secretion by B lymphocytes. Natural killer (NK) cell function was characterized by normal target cell binding by NK cells but severely depressed NK cell cytotoxicity.

Abbreviations

SCID, severe combined immunodeficiency
MNL, mononuclear cells
PWM, pokeweed mitogen
NK, natural killer
HBSS, Hanks' balanced salt solution
Ig-SC, immunoglobin-secreting cells
HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
EBV, Epstein-Barr virus
PHA, phytohemagglutinin
Con A, concanavalin A
SK-SD, streptokinase-streptodornase
WBC, white blood cells

SCID encompasses a heterogeneous group of congenital disorders which manifest varying degrees of dysfunction of both B and T lymphocytes (28). Although the majority of patients lack adequate numbers and function of both T and B lymphocytes, a minority of patients has been identified with normal circulating numbers of these cell populations (6, 10, 21, 22). The basis for this paradox is believed to be the abnormal differentiation of B and T cell precursors (22). Inadequate numbers of helper T cells have been implicated in a few patients (4, 24) and several authors have also suggested a role for excessive numbers of suppressor T cells, which has been documented in at least one case (21). Certainly other forms of immunodeficiency, including subgroups of patients with common variable hypogammaglobulinemia, selective IgA deficiency, and X-linked agammaglobulinemia, have been described with increased numbers or function of suppressor T lymphocytes (1, 25, 27). An intrinsic B cell abnormality in SCID has also been observed (7).

We have had the opportunity of serially evaluating immune functions in a 12-year-old white male with SCID, who has been maintained since birth in a gnotobiotic environment. This patient has been the subject of multiple investigations regarding