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development
DNA
extracellular fluid
intracellular fluid
kidney
potassium
protein
sodium

Maturation of the Developing Rabbit Kidney: Variations in Cellular Size and Contents

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Summary

Because the rabbit kidney is being used as an experimental model with increasing frequency, this study was designed to measure the relationships between cell number, size, and contents in the developing rabbit. Kidney slice extracellular and intracellular fluid spaces, high in the fetus and neonate, declined as the rabbits matured, being paralleled by changes in body fluid spaces. Although the cellular contents of both sodium and potassium were increased in the young kidney, intracellular sodium concentration was slightly lower in the fetal (43.6 mEq/liter) and 2-wk kidneys (44.5 mEq/liter) than in the mature kidney (51.7 mEq/liter). Intracellular potassium concentrations were similar in all age groups (163 to 167 mEq/liter). Tissue protein content was similar during development. In contrast, DNA content and the number of nuclei in kidney tissue were high in the fetus (DNA, 59.9 mg/g solids; nuclei, 3.9 × 10^9/g solids), decreasing in postnatal life (DNA in adult, 18.2 mg/g solids; nuclei in adult, 1.0 × 10^9/g solids). In association with this, the diameter of proximal tubular cells increased with maturation. These data should be valuable to those interested in kidney development.

The improved survival of premature human neonates has increased the need for better understanding of the functional capabilities of the neonatal kidney. Many investigations of tubular function in kidneys from adult animals and maturation of these functions in younger animals have been performed in the rabbit (10, 11, 13, 16–18).

The present studies establish values for the increases in cellular number, size, and contents during growth of the rabbit kidney. They also examine the relationships of changes in renal fluid spaces to those in body fluid spaces that occur during development of the fetal rabbit to an adult animal at age 6 months.

MATERIALS AND METHODS

FLUID SPACES IN ANIMALS

Measurements of total body water and of extracellular fluid spaces were undertaken using the methods established by Prentice *et al.* (19) and Berne and Levy (2). Rabbits 1, 2, 4, 8, and 26 wk of age were anesthetized with intravenous pentobarbital. Flank incisions were made, and the renal vessels were ligated to prevent excretion of the radioisotopes. After obtaining a venous blood sample to use as blank, tared amounts of [^{3}H]water (New England Nuclear; specific activity, 100 mCi/g) and [^{14}C]carboxy inulin (New England Nuclear; specific activity, 2.45 mCi/mg) were injected intravenously. The animal was kept warm under light anesthesia during a 2-hr equilibration period at which time a repeat venous blood sample was drawn. Aliquots of the initial injection solution and the blood were counted in a Tri-Carb liquid scintillation Counter (Packard model 3375) using standard techniques to separate ^{14}C from ^{3}H counts. The distribution spaces of ^{3}H and ^{14}C were taken as estimates of total body water and extracellular space, respectively, and expressed as percentage of body weight.

FLUID SPACES AND ELECTROLYTE CONTENT OF KIDNEYS

Kidney cortical tissue was prepared and incubated using methodology described previously (7), studying animals ranging in age from 29 days gestation to 26 wk. In brief, 0.3 to 0.4 mm cortical slices were cut with a Stadie Riggs microtome and then were incubated in a Ringers-phosphate solution oxygenated with 100% O_2 in a shaking water bath at 27°C. After an hour's incubation, the slices were blotted, weighed, and homogenized in 10% trichloroacetic acid (TCA) in a manual tissue grinder. The homogenates were filtered, and the filtrate, as well as incubating medium, was analyzed.

In addition to the determinations of inulin previously described (7), measurements of sodium and potassium were performed on a flame photometer. Measurements were made on both tissue filtrates and the incubation medium. The lithium concentration of the diluent was adjusted (12) to allow measurement of the very low levels of these cations in tissue filtrate. Because of these low levels of sodium, it was necessary to take great care to avoid sodium contamination or erroneous sodium measurements in these studies. Acid-washed flasks containing no cortical tissue were run with each experiment, and the values for any sodium measurements from these blank flasks were subtracted from the values observed in the tissue containing samples before further calculations were undertaken. In addition, the sodium concentration in the TCA was measured before it was used for tissue homogenization to ensure that significant quantities of sodium had not been leached either from the containers used to store the TCA or from the glass with which it had come into contact.

The amounts of sodium and potassium in the extracellular fluid space of the tissue were calculated from the tissue inulin space and from the measurements of sodium and potassium in the incubation medium. These values were subtracted from the measured total tissue values to yield an estimate of intracellular sodium and potassium contents. Intracellular sodium and potassium concentrations could be calculated from these values and from the estimates of intracellular water content.

PROTEIN AND DNA CONTENT OF KIDNEY

Cortical tissue was incubated as described above. The tissue was homogenized and poured into a polycarbonate tube where it then stood on ice for 30 min. The extraction procedure was modified from previously described procedures (3–6) as follows: after centrifuging the sample in TCA, the pellet was washed in ethanol and spun. One N perchloric acid was added to the pellet and incubated at 80°C for 20 min. This mixture was centrifuged, additional 0.5 N perchloric acid was added to the pellet, and the tube was respun. One hundred μl of supernate was added to 2000 μl of diphenylamine reagent, incubated at 37°C for 18 hr, and read on a Coleman Spectrophotometer (model 55) at 500 nm.

Five-tenths N NaOH was added to the remaining pellet, incu-

bated overnight at 37°C, and analyzed for protein by the method of Lowry *et al.* (14).

DETERMINATION OF NUMBER OF NUCLEI IN KIDNEY

The method of isolating nuclei to be counted under a light microscope was an adaptation of several procedures (1, 8, 9). Kidneys were obtained as described above. Approximately 1 g of cortical slice was weighed and homogenized in 10 ml 2% citric acid using a motor-driven Teflon homogenizer (A. H. Thomas). The hypotonicity of the solution and the homogenizer were effective in lysing cells. The acidity of the solution was instrumental in preserving the integrity of the nuclei (9).

To the homogenate 30 ml 2% citric acid were added, and the sample was spun in a refrigerated centrifuge at 1500 rpm for 10 min. The supernate was discarded, and 40 ml 2% citric acid were added to the sediment. This suspension was spun at 1000 rpm for 10 min. After discarding the supernate, the sediment was resuspended in another 40 ml 2% citric acid and spun at 700 rpm for 10 min. The sediment was resuspended in 40 ml 2% citric acid, and after thorough mixing, the nuclei were counted in a Levy double counting chamber (Fisher Scientific Co.). The mean of five counts was taken as one observation. Examination of the suspension with high magnification verified the absence of whole cells or significant debris.

MEASUREMENT OF PROXIMAL TUBULAR CELL DIAMETER

Cell diameter was measured as follows: individual nonincubated kidneys from each age group were fixed in formalin and sectioned. Sections were stained by routine procedures with hematoxylin and eosin. Photomicrographs of superficial cortical and juxtamedullary regions were taken using a Polaroid camera at a magnification of × 400. Proximal tubular cells were identified by their histologic characteristics with diameters of these cells being measured from the photomicrographs using a millimeter rule. Only cells thought to be sectioned through their equator were included in these measurements.

STATISTICAL METHODS

Values from each age group were compared with those for the 26-wk animals using the Student *t* test for nonpaired data. In addition, correlation coefficients were determined by linear regression analysis to determine linearity of change from fetal to mature ages.

RESULTS

BODY FLUID SPACES (TABLE 1)

The rabbits grew rapidly after birth, their weights increasing in a near-linear pattern up to 8 wk of age. At 1 wk of age when the rabbits The rabbits grew rapidly after birth, their weights increasing in a near-linear pattern up to 8 wk of age. At 1 wk of age when the rabbits were first large enough to obtain reliable measurements, total body water ([^{3}H]water space) and extracellular fluid ([^{14}C]inulin space) were quantitated. Values for both spaces when expressed per unit of body weight were higher than those observed in the adult rabbits. The extrapolated value for intracellular fluid space was also increased in the 1-wk-old animals. Regression analyses for total water and extracellular fluid spaces (ECF) demonstrated significant linear change with age.

KIDNEY SLICE FLUID SPACES (TABLE 2)

The sizes of the fluid spaces in the kidney slices obtained from rabbits of different ages are compared in Table 2. As in the intact animals, water comprised more than 80% of tissue weight in the fetal and very young animals. This was due to higher values for both intracellular fluid spaces and ECF in the younger animals. When expressed as ml/kg tissue solids, both ECF and intracellular

Table 1. *Alterations in body weight and fluid spaces in the developing rabbit*

Age (wk)	No. of animals	Rabbit wt (kg)	Total water[1] (% body wt)	ECF[2] (% body wt)	Intracellular fluid spaces Total water-ECF (% body wt)
1	3	0.12 ± 0.01[3,4]	82.9 ± 1.2[4]	21.8 ± 0.8[5]	61.1 ± 0.2[4]
2	5	0.34 ± 0.01[4]	72.4 ± 2.1	19.3 ± 2.2	53.1 ± 4.2
4	3	0.98 ± 0.06[4]	77.0 ± 1.4	20.2 ± 1.3	56.8 ± 2.5[5]
8	3	1.76 ± 0.14[4]	65.7 ± 2.0	17.0 ± 0.4	48.7 ± 2.4
26 (adult)	7	3.79 ± 0.13	65.7 ± 3.2	16.1 ± 1.2	49.3 ± 2.3
r value[6]			0.55[4]	0.4[5]	0.21

[1] Measured as [^{3}H]water space.
[2] Measured as [^{14}C]inulin space.
[3] Mean ± S.E.
[4] Significantly different than $P < 0.01$.
[5] Significantly different with $P < 0.05$.
[6] *r* values are those calculated by linear regression; asterisks indicate *P* values calculated from *t* values.

Table 2. *Comparison of sizes of fluid compartments in the developing rabbit kidney*

Age group	No. of animals	Fluid space (% wet tissue wt)			Fluid space (ml/kg tissue solids)		
		Total water	ECF	Intracellular fluid space	Total water	ECF	Intracellular fluid space
Fetal	51	84.5 ± 0.2[1,2]	36.2 ± 1.0[3]	48.3 ± 1.0[2]	5381 ± 13[2]	2304 ± 37[2]	3077 ± 33[2]
1 wk	36	82.6 ± 0.4[2]	32.5 ± 0.6	49.8 ± 0.5[2]	4712 ± 23[2]	1890 ± 30[2]	2822 ± 36[2]
2 wk	25	79.2 ± 0.4[2]	32.1 ± 1.3[3]	47.7 ± 0.9[2]	3958 ± 20[2]	1586 ± 44	2372 ± 10[2]
4 wk	17	78.8 ± 0.3	31.7 ± 0.8[3]	47.0 ± 0.8[2]	3734 ± 14	1517 ± 43	2217 ± 57[2]
6 wk	23	77.0 ± 0.3	29.6 ± 0.8[2]	47.2 ± 0.8[2]	3343 ± 13	1302 ± 20[2]	2041 ± 37[2]
8 wk	12	75.6 ± 0.3[3]	32.0 ± 0.8	43.9 ± 0.8	3141 ± 12	1325 ± 29[2]	1816 ± 33
10 wk	6	77.4 ± 1.0	31.7 ± 0.1	45.7 ± 1.2	3383 ± 44	1384 ± 28	1999 ± 56
Normal adult	28	76.9 ± 0.3	33.8 ± 0.7	42.9 ± 0.5	3347 ± 13	1499 ± 31	1848 ± 33
r value		0.55[2]	0.05	0.47[2]		0.46[2]	−0.69[2]

[1] Mean ± S.E.
[2] Significantly different than $P < 0.01$.
[3] Significantly different with $P < 0.05$.

Table 3. *Tissue and intracellular sodium and potassium levels in kidney cortex slices obtained from animals of different ages*

Age group	No. of animals	Tissue sodium (mEq/kg tissue solids)	Intracellular sodium (mEq/kg tissue solids)	Intracellular sodium (mEq/liter intracellular fluid spaces)	Tissue potassium (mEq/kg tissue solids)	Intracellular potassium (mEq/kg tissue solids)	Intracellular potassium (mEq/liter intracellular fluid spaces)
Fetal	41	453 ± 9.0[1,2]	134.7 ± 8.7[2]	43.6 ± 3.9[3]	522 ± 6.9[2]	393 ± 8.0[2]	163 ± 2.0
1 wk	39	393 ± 8.2[2]	129.2 ± 7.4[2]	46.1 ± 2.8	486 ± 10.7[2]	468 ± 10.0[2]	166 ± 7.0
2 wk	24	332 ± 7.8[3]	105.1 ± 7.7	44.5 ± 3.3[3]	411 ± 6.5[2]	395 ± 6.0[2]	167 ± 2.1
4 wk	17	329 ± 12.5	102.3 ± 8.4	45.7 ± 3.6	372 ± 5.9[2]	357 ± 5.6[2]	163 ± 3.7
6 wk	23	280 ± 8.0	104.0 ± 4.7	51.3 ± 2.3	337 ± 5.0[2]	330 ± 4.7[2]	162 ± 1.6
8 wk	12	279 ± 7.8	85.4 ± 4.8	48.3 ± 2.4	305 ± 7.2	298 ± 5.8	165 ± 3.9
10 wk	10	316 ± 6.4	101.3 ± 4.5	51.4 ± 1.3	323 ± 9.6	310 ± 9.4	162 ± 2.1
26 wk (adult)	28	310 ± 9.5	95.6 ± 5.2	51.7 ± 2.2	313 ± 0.9	300 ± 3.3	163 ± 2.1
r values			0.69[2]	0.27[2]		0.25[3]	0.008

[1] Mean ± S.E.
[2] Significantly different than $P < 0.01$.
[3] Significantly different with $P < 0.05$.

Table 4. *Comparison of cellular constituents in developing kidney*

Age group	Tissue solids (% wet wt)	Tissue protein (mg/g)[1]	Tissue DNA (mg/g)[2]	No. of nuclei ($\times 10^9$/g)[2]
Fetal	15.5 ± 0.2[2]	724 ± 42	59.9 ± 3.5[3]	3.9 ± 0.5[3]
	($n = 51$)	($n = 11$)	($n = 11$)	($n = 7$)
2 wk	20.8 ± 0.4	586 ± 20	50.3 ± 1.2[3]	2.7 ± 0.2[3]
	($n = 25$)	($n = 22$)	($n = 22$)	($n = 6$)
4 wk	21.2 ± 0.3	630 ± 13	23.0 ± 0.9[3]	1.9 ± 0.2[4]
	($n = 17$)	($n = 12$)	($n = 12$)	($n = 6$)
6 wk	23.0 ± 0.3	561 ± 28	17.8 ± 1.3	1.6 ± 0.1[4]
	($n = 23$)	($n = 12$)	($n = 12$)	($n = 6$)
26 wk (adult)	23.1 ± 0.3	634 ± 33	18.2 ± 0.7	1.0 ± 0.2
	($n = 28$)	($n = 21$)	($n = 21$)	($n = 3$)
r value	0.56	0.17	0.64[3]	0.39

[1] Expressed per gram of tissue solids.
[2] Mean ± S.E.
[3] Significantly different than $P < 0.01$.
[4] Significantly different with $P < 0.05$.

fluid spaces values gradually declined to adult levels as the animals matured.

SODIUM AND POTASSIUM CONTENTS OF KIDNEY TISSUE (TABLE 3)

Total tissue sodium, expressed per unit of tissue solids, was significantly higher in the fetal and 1- and 2-wk-old animals than in the more mature rabbits. Because the fluid spaces were greater in these younger animals, however (Table 2), total tissue sodium remained similar in all age groups when the values were expressed in terms of wet tissue weight. Intracellular sodium content expressed per kg of tissue solids was also increased in the fetal and 1-wk-old rabbits, and when intracellular sodium concentrations were calculated (Table 3), only the fetal and 2-wk-old kidneys showed statistically significant differences. Linear regression did, however, show a significant, albeit small, increase with age.

Maturation of total tissue and intracellular potassium content and concentrations revealed a similar pattern to that seen with sodium. Total tissue and intracellular potassium contents, expressed per unit of tissue solids, were increased in the younger animals. However, intracellular potassium concentration, expressed as mEq/liter of intracellular fluid, was remarkably similar in animals of all ages.

To determine whether the depth of slice had influence on water content or intracellular sodium and potassium concentrations, four slices were cut from the kidneys of 2-wk-old animals. Slices from the respective levels were incubated together, and values were compared. No statistically significant differences were seen in values among the various levels.

PROTEIN DNA AND NUCLEAR CONTENTS OF KIDNEYS (TABLE 4)

As expected from the data on fluid spaces (Table 2), tissue solids represented a smaller percentage of total tissue weight in the kidneys from the neonatal animals than in kidneys from more mature animals. The value progressively increased with increasing age, the adult value of 23.1% being reached in 6-wk-old animals. Tissue protein represented a constant percentage of tissue solids. Although this value was higher in the neonatal kidney than in those at any other age, the differences did not reach statistical significance.

In contrast, tissue DNA represented a significantly higher percentage of tissue solids in the fetal kidneys. The relative amount of DNA declined progressively with increasing maturation to reach the adult value in the 6-wk-old animals. In keeping with the data on DNA, the number of nuclei per g of tissue solids was also higher in the kidneys from the fetal animals than in those from older animals.

Table 5. *Measurement of the diameter of proximal tubular cells during development of the rabbit kidney*

Age group	Cellular diameter (μ) (Region) Superficial cortical	Cellular diameter (μ) (Region) Juxtamedullary
Fetal	10.1 ± 0.15[1,2]	12.5 ± 0.18[2]
2 wk	11.3 ± 0.26[2]	15.0 ± 0.20[2]
4 wk	16.0 ± 0.30	14.5 ± 0.25[2]
6 wk	15.8 ± 0.30[3]	18.0 ± 0.28
8 wk	15.3 ± 0.15[3]	16.0 ± 0.18[2]
Adult	20.3 ± 0.20	18.8 ± 0.50
r values	0.70[2]	0.63[2]

[1] Mean ± S.E.
[2] Significantly different than $P < 0.01$.
[3] Significantly different with $P < 0.05$.

Table 6. *Maintenance of cellular integrity in the absence of exogenous substrate*

	% of control value			
Age group	ECF	Intracellular fluid spaces	Intracellular sodium	Intracellular potassium
Fetal	90.4 ± 13.6[1]	96.3 ± 4.1	117.3 ± 10.2	102.7 ± 2.5[2]
1 wk	102.6 ± 4.0	95.0 ± 3.9	92.6 ± 4.3	100.6 ± 4.6
2 wk	106.0 ± 2.6	95.8 ± 1.7	99.1 ± 4.0	98.3 ± 2.2
4 wk	99.9 ± 0.8	97.6 ± 1.8	106.4 ± 4.9	97.7 ± 2.0
6 wk	95.5 ± 3.5	101.8 ± 2.1	101.4 ± 4.9	91.4 ± 1.8
8 wk	100.3 ± 1.6	99.2 ± 1.2	102.3 ± 2.3	91.3 ± 2.6
10 wk	107.3 ± 1.8	94.0 ± 1.3[2]	101.8 ± 6.9	101.5 ± 1.2[2]
26 wk	103.1 ± 2.0	98.9 ± 1.6	102.0 ± 4.7	95.6 ± 2.2

[1] Mean ± S.E.

[2] $P < 0.05$ compared to the adult values using the Student t test for nonpaired data.

Table 7. *Comparison of values from kidney tissue obtained from pregnant and nonpregnant adult rabbits*

Measurement	Adult	Pregnant adult	P[1]
Tissue ECF (ml/kg tissue solids)	1499 ± 31[2] (n = 28)	1743 ± 39 (n = 16)	<0.01
Tissue intracellular fluid spaces (ml/kg tissue solids)	1848 ± 33 (n = 28)	1794 ± 78 (n = 16)	NS[3]
Tissue sodium (mEq/kg tissue solids)	310 ± 9.5 (n = 28)	339 ± 18.4 (n = 16)	<0.05
Intracellular sodium (mEq/liter)	51.7 ± 2.2 (n = 28)	52.6 ± 2.2 (n = 16)	NS
Tissue K (mEq/kg tissue solids)	313 ± 0.9 (n = 28)	295 ± 9.3 (n = 16)	NS
Intracellular K (mEq/liter)	163 ± 2.1 (n = 28)	155 ± 3.6 (n = 16)	NS
Tissue protein (g/kg tissue solids)	634 ± 33 (n = 21)	743 ± 28 (n = 12)	<0.05
Tissue DNA (g/kg tissue solids)	18.2 ± 0.7 (n = 21)	20.6 ± 0.8 (n = 12)	<0.05
Nuclear count ($\times 10^9$/g tissue solids)	1.0 ± 0.2 (n = 3)	0.87 ± 0.2 (n = 5)	NS

[1] Using Student t test for paired data.

[2] Mean ± S.E.

[3] NS, not significant.

PROXIMAL TUBULAR CELL SIZES (TABLE 5)

There was a progressive increase in the size of proximal tubule cells in both the superficial cortical and juxtamedullary regions of the cortex. The mean diameter of the superficial cortical cell increased from 10.1 μ in the fetus to 20.3 μ in the adult rabbit while the mean diameter of the juxtamedullary cell rose from 12.5 to 18.8 μ during development.

MAINTENANCE OF CELLULAR INTEGRITY IN THE ABSENCE OF EXOGENOUS SUBSTRATE (TABLE 6)

Slices from rabbit kidneys of each age group were incubated in paired flasks of incubation medium, one containing 10 mM sodium acetate and the other no exogenous substrate, the sodium acetate being replaced by equimolar amounts of sodium chloride. Despite the absence of exogenous substrate, the kidneys slices from animals of all ages were able to maintain the intracellular sodium and potassium concentrations found in tissue incubated in the presence of exogenous substrate.

INFLUENCE OF PREGNANCY (TABLE 7)

In th studies of fetal kidney tissue, the kidneys from the pregnant animals were also studied. Table 7 compares these results to those from nonpregnant adult animals. Kidney cortex from the pregnant animals had significantly higher contents of tissue water, sodium, protein, and DNA. However, intracellular sodium concentrations were maintained at levels comparable to those of the nonpregnant animal adults.

COMMENT

Nash and Edelmann (15) have suggested that use of the term "immature" may be inappropriate for describing renal function during development. Although immature describes a difference between function in the developing organism and the adult, such differences may well be appropriate for the state of the development of that organism. The composition of the fetal tissue differs from that of more mature animals. These changes do not reflect those induced by pregnancy in the mother. The fetus, living in a fluid environment, has a high content of water. Postnatally, the rabbit, as well as other mammals living in a terrestial environment, lose water from both extracellular and intracellular spaces so that tissue solids represent an increased proportion of wet tissue weight with normal growth and development. The present study demonstrates the importance of expressing data from young animals in the appropriate manner. Failure to do so could result in observations that are liable to be misinterpreted as representing "immaturity" in the younger animal. Thus, comparing tissue contents at different ages using total tissue water as the denominator may underestimate the values at young ages because water comprises a higher percentage of tissue weight at these ages. Conversely, factoring by tissue solids may overestimate values of the young. Only by factoring cellular contents by cellular water at each age can one demonstrate the constancy of the cellular composition and appreciate the maturity of the internal milieu during growth.

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