

# Postnatal Development of Renal Function: Micropuncture and Clearance Studies in the Dog

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**ABSTRACT** Postnatal renal development was studied in dogs between 2 and 77 days. Single, superficial nephrons were evaluated by micropuncture, concurrently with measurements of total renal function and morphometric analyses in the same animals.

Glomerular filtration rate for the entire kidney increased linearly from 0.13 ml/min per g kidney weight at 2 days to 0.91 at 77 days. Extraction of *p*-aminohippurate increased from about 20 to 80%, and renal plasma flow per g kidney weight, measured as  $C_{PAH}/E_{PAH}$ , increased threefold during the same period. Filtration fraction increased to the mature value during the first half of the postnatal period studied.

The clearance of urea per unit of renal mass increased with age, whereas the fraction of filtered urea reabsorbed declined during the early part of the postnatal period. The pattern of fractional urea reabsorption may be due mainly to increased medullary recycling of urea and to a rise in the reabsorption of water from the medullary collecting duct.

Urine osmolality was higher than plasma from birth onward and rose with age. Osmolal equality of collecting duct fluid and medullary interstitium reflected mature vasopressin (ADH)-induced water permeability. The rise in urinary concentration was predominantly due to increasing medullary sequestration of urea.

Glomerular filtration rate of the superficial nephron

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increased from 3.2 nl/min at 21 days, when subcapsular nephrons were uniformly patent, to 23.1 at 77 days. Despite this rise in filtered load, fractional reabsorption of sodium and water in superficial proximal tubules was constant and at the mature level from the onset of intratubular perfusion. Changes in arterial plasma protein concentration, in filtration fraction, and in the hydrostatic pressure gradient between proximal tubule and peritubular capillary may interact to maintain glomerulotubular balance.

The data, together with results of an accompanying morphological study, demonstrate a sequence of coordinated changes during postnatal renal maturation.

## INTRODUCTION

The postnatal development of renal function has previously been described for the kidney as a whole. Since morphologically renal maturation follows a centrifugal pattern, the oldest nephrons are at the corticomedullary junction, and the youngest at any stage of postnatal maturation are located in the subcapsular layer. Hence, clearance data in the developing kidney represent a composite from nephrons at different stages of maturity; they cannot quantify the development of the single nephron.

The dog strain used in the present experiments allowed a longitudinal study of the functional growth of single superficial nephrons, from the onset of intratubular perfusion to maturity. To the extent that the postnatal kidney (1), like the mature kidney (2), is composed of two major populations of nephrons, the micropuncture data reported here mirror the postnatal evolution of the superficial nephron type.

The present study permits comparison not only of the functional growth of single nephrons with that of the entire kidney, but also with age-related structural alterations (1). The results reveal a coordinated progression of nephron maturation during postnatal development.

## METHODS

Studies were performed on 26 neonatal dogs of a purebred beagle strain (Omis Beagle Kennels, Roscoe, Ill.), ranging in age from 2 to 77 days and weighing 310–2900 g. The pregnant mothers were shipped 2 wk before whelping. The newborn dogs were weaned at 30–40 days. They received a mixture of commercial dog food (Purina Dog Chow, 24% protein, Ralston Purina Co., St. Louis, Mo.; Ken-L Ration, 10% protein, Quaker Oats Co., Chicago, Ill.) during the weaning period, and had free access to food and water before the experiment.

**General procedures.** Anesthesia was initiated with an intraperitoneal injection of 20 mg/kg sodium pentobarbital (Nembutal Sodium, Abbott Laboratories, North Chicago, Ill.) and maintained at a constant level with intravenous infusion of 3 mg/kg per hr. The animal was placed on the heated surface of a micropuncture table, and body temperature was kept constant within 37.5°–38.0°C by means of a thermosensitive feedback device. Tracheotomy was performed in all experiments. Polyethylene catheters (Intramedic; Clay-Adams, Inc., Parsippany, N. J.) were inserted into the left jugular vein for administration of inulin and *p*-aminohippurate in a 0.9% NaCl solution, and into the left femoral artery for periodic sampling of blood and continuous measurement of arterial blood pressure. A small cannula (PE 50) was placed with its tip close to the orifices of the renal arteries for injection of Lissamine green.

The left kidney was exposed through a subcostal incision and dissected free of perirenal fat and peritoneal attachments, and a catheter was placed into the left ureter below the renal pelvis. The kidney was suspended in a plastic holder of adequate size which was positioned into a metal holder, thereby minimizing the transfer of respiratory and pulsatory movements. The renal vein was partially and gently dissected free of its surrounding tissue. The exposed surface was bathed in mineral oil maintained at a temperature of 37.5°C with a thermosensitive circuit (Slama, Göttingen, Germany), and the micropuncture area was illuminated by two fiber-optic systems (Dolan-Jenner Industries, Melrose, Mass., and Edmund Scientific Co., Barrington, N. J.). The transparency and consistency of the renal capsule allowed micropuncture access to superficial nephrons without removing the capsule. Blood pressure was continuously monitored by a Statham strain gauge (Statham Laboratories, Inc., Hato Rey, Puerto Rico) connected to a polygraph recorder (model 5D; Grass Instrument Co., Quincy, Mass.).

In all studies, a constant i.v. infusion of 0.9% NaCl was given at a rate equal to 0.5% of body weight per hr. This solution contained inulin (Inutest; Laevosan-Gesellschaft, Linz/Donau, Austria) and *p*-aminohippurate (Sodium Aminohippurate; Merck Sharp & Dohme, West Point, Pa.) in amounts calculated to sustain plasma concentrations of 150 mg/100 ml inulin and 2.0 mg/100 ml *p*-aminohippurate (PAH).

**Analytical.** After 45–60 min had been allowed for equilibration, 35- to 45-min clearance periods were begun. Urine was collected under mineral oil into glass containers, and its volume was determined by weight (model H 10; Mettler Instrument Corp., Princeton, N. J.). Clearance periods were obtained continuously, and each period was bracketed by two samples of arterial blood (0.4 ml). Renal venous blood was collected in two samples twice during the experiment by puncture of the renal vein as previously described for the rat (3). PAH concentration in arterial and venous samples and in urine was determined by the method of Bratten and

Marshall as modified by Smith, Finkelstein, Aliminosa, Crawford, and Graber (4). Inulin concentration in plasma and urine was determined by the method of Führ, Kaczmarczyk, and Krüttgen (5). Concentrations of urea in plasma, urine, and renal tissue were measured colorimetrically (6), and those of sodium and potassium with an internal standard flame photometer (model 143; Instrumentation Laboratory, Inc., Lexington, Mass.). Osmolality in serum and urine was measured in a standard osmometer (Osmette; Precision Systems, Inc., Waltham, Mass.) or with a cryostat for nanoliter volumes (Clifton Technical Physics, New York). Arterial protein concentration was measured electrophoretically (Sepraphore III, Gelman Instrument Co., Ann Arbor, Mich.). Clearances of inulin ( $C_{in}$ ), PAH ( $C_{PAH}$ ), and urea ( $C_{urea}$ ) as well as extraction ratio for PAH ( $E_{PAH}$ ), renal plasma flow (RPF), and filtration fraction (FF) were calculated from standard equations.

The last clearance period was terminated by clamping the left renal hilum. The kidney was taken out and prepared immediately for analysis of renal tissue by two methods, i.e. chemical and cryoscopic, as described previously (7). Concentrations of urea, sodium, and potassium were determined in four sections representing cortex, outer medulla, outer zone of inner medulla, and inner medulla. Osmolality in the same sections was measured in nanoliter volumes of tissue fluid (7). Renal tissue water content was determined in eight animals between 2 and 60 days of age by drying duplicate samples from cortex, outer medulla, and inner medulla at 43°C to constant weight.

**Micropuncture protocol, techniques, and analyses.** Superficial nephron function was evaluated in 17 dogs between 21 and 77 days applying a uniform protocol. Quantitative collection of proximal tubular fluid, tubular transit time, proximal intratubular and peritubular capillary pressure, and tubular diameter were obtained under free flow conditions while monitoring glomerular filtration rate (GFR), RPF ( $C_{PAH}/E_{PAH}$ ), and sodium excretion. These data allowed calculation of superficial nephron filtration rate (SGFR), water reabsorption ( $TF/P_{in}$ ), and transtubular hydrostatic pressure gradient.

Proximal tubular transit time was determined after intra-aortic injection of 0.1 ml of buffered Lissamine green (10%) measuring the interval between diffuse staining of the renal surface and the star configuration of dye-filled segments. The time between star formation and appearance of dye in a distal segment was taken as transit time through the loop of Henle. The last surface segments of several proximal tubules were located by observing the passage of dye. Identification of these segments as the last accessible ones was confirmed later by injecting a minute oil droplet and observing its immediate disappearance below the surface. Sharpened micropipettes (Friedrich & Dimmock, Inc., Millville, N. J.), with 8–10  $\mu$  tip diameter and otherwise constant characteristics (wall thickness, tip to shoulder length), were filled with Sudan black-stained polymer oil shortly before use. An oil block was inserted distal to the puncture site sufficient in length to insure complete separation of collected proximal fluid from that distal to the oil block. The timed quantitative collection was carried out according to criteria described previously (8). Only those samples were analyzed in which a spontaneous collection was maintained throughout the sampling period, i.e., constant position of the oil block without downstream drift or contact with the pipette tip.

Volume of the tubular fluid sample was measured in a constant bore tubing calibrated with a radioactive solution

of known concentration. The length of the fluid column enclosed in polymer oil was read at a 32-fold magnification from an eyepiece micrometer. Concentration of inulin (Inu-test) in tubular fluid was determined by the method of Hilger, Klümper, and Ullrich (9), modified for sample volumes of 7  $\mu$ l (8).

Proximal intratubular and peritubular capillary pressure was measured manometrically (Diefanbach, Frankfurt, Germany) with the Landis method (10). Proximal segments of three to five nephrons were selected at random, and at least three determinations were obtained in each. Interspersedly, five to seven peritubular capillaries were evaluated in the same manner.<sup>1</sup>

Intratubular stop-flow pressure (SFP) was measured in seven kidneys. Using the technique described by Gertz, Mangos, Braun, and Pagel (11), two to four early proximal segments were identified, and a long oil block was placed into the nephron distal to the punctured segment. Intratubular pressure proximal to the oil block was recorded at 2-min intervals and the mean value of at least six readings at steady state taken as SFP.

Measurements of tubular diameter were made during the experiment in proximal segments by a Leitz adjustable eyepiece micrometer at a 100-fold magnification and compared later with photomicrographs. The micropuncture area was photographed with a Nikon camera mounted on a Leitz binocular stereomicroscope ( $\times 32$  magnification). Illumination was provided by an electronic flash and by two fiber-optic systems. Diameters of segments during free flow were taken from a black and white photograph at  $\times 240$  magnification utilizing a caliper and a photograph of a micrometer standard at the same magnification.

In eight experiments covering the age range from 21 to 77 days, nephrons were injected with a silicone compound (Microfil; Cantor Biomedical Products, Swarthmore, Pa.). The section of the kidney containing these nephrons was kept in isotonic NaCl for 12 hr and then immersed in 12.5% NaOH for 2 hr. Subsequent dissection was carried out under a Leitz binocular stereoscopic microscope. The length of dissected nephron segments was measured directly with a Leitz adjustable eyepiece micrometer.

Calculation of single nephron filtration rate was made from the measured tubular fluid inulin concentration and the intratubular flow rate at the point of quantitative collection. The fraction of filtrate reabsorbed up to the puncture site in the late segment of the proximal convoluted tubule was calculated as  $1 - (P/TF_{in})$ .

The results were evaluated statistically using the computer programs of Dartmouth College.<sup>2</sup>

## RESULTS

In this homogeneous dog strain, both body weight and kidney weight increased almost linearly with age (Table I). Hence, kidney weight and body weight were closely correlated over the age range studied (Fig. 1). The

<sup>1</sup> The size of the peritubular capillary did not vary with age. However, similar to the rat kidney (12), the maturing dog kidney in addition has short, wider peritubular segments from which several capillaries branch off. Measurements, taken systematically in both structures, varied equally with age.

<sup>2</sup> Kiewit Computation Center, Dartmouth College, Hanover, N. H. 1970. Statistical Programs: STAT01, for single groups; STAT10, for one variable linear regression.

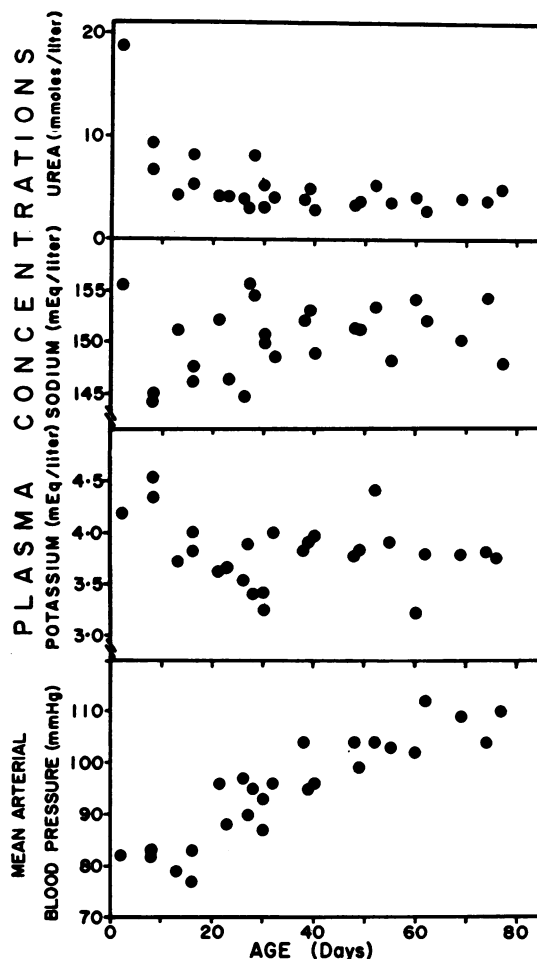
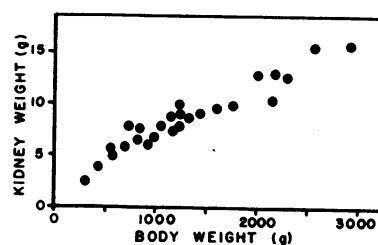


FIGURE 1 Basic data which were monitored in all dogs of this study. Each symbol in this and all subsequent figures depicts the mean of the data obtained in one animal, and all animals studied are represented in each figure. The close correlation between body weight and renal weight indicates the uniformity of the purebred beagle strain used in these experiments.

data of this study have been expressed per unit of renal tissue mass in order to gauge the development of function in relation to the concomitant anatomical growth. Since the increase in renal weight was shown in an accompanying morphological study (1) to be a consequence mainly of tubular growth, the data also permit

TABLE I  
Total Renal Function in 26 Dogs during Postnatal Development from 2 to 77 Days after Birth

Age	Body weight	Kidney weight	C <sub>IN</sub> *	C <sub>PAH</sub>	E <sub>PAH</sub>	RPF	Filtration fraction	Filtered sodium reabsorbed
days	kg	g	ml/min · gKW	ml/min · gKW	%	ml/min · gKW		%
2(7)†	0.31	2.43	0.13 ± 0.007§	0.15 ± 0.008	16.8	0.91 ± 0.050	0.14	99.37 ± 0.020
8(9)	0.44	3.74	0.20 ± 0.019	0.29 ± 0.030	29.1	1.00 ± 0.103	0.20	99.73 ± 0.056
8(8)	0.71	5.85	0.20 ± 0.036					99.79 ± 0.075
13(8)	0.52	5.67	0.27 ± 0.007	0.44 ± 0.017	38.5	1.14 ± 0.045	0.24	99.95 ± 0.006
16(9)	0.59	5.10	0.25 ± 0.011	0.59 ± 0.035	43.4	1.36 ± 0.081	0.18	99.75 ± 0.058
16(5)	1.06	7.98	0.12 ± 0.017	0.18 ± 0.026	35.0	0.52 ± 0.074	0.23	99.15 ± 0.048
21(11)	0.73	8.18	0.27 ± 0.040	0.45 ± 0.063	36.6	1.23 ± 0.173	0.22	99.39 ± 0.073
23(5)	1.18	7.52	0.28 ± 0.035	0.38 ± 0.050	28.0	1.34 ± 0.177	0.21	99.36 ± 0.068
26(8)	0.86	7.95	0.37 ± 0.004	0.63 ± 0.008	46.5	1.35 ± 0.016	0.27	99.80 ± 0.022
27(7)	0.84	6.45	0.37 ± 0.010	0.74 ± 0.015	50.0	1.47 ± 0.029	0.25	99.45 ± 0.065
28(9)	1.25	10.15	0.35 ± 0.039					99.25 ± 0.161
30(7)	1.16	8.85	0.47 ± 0.028	0.72 ± 0.015	41.0	1.75 ± 0.037	0.27	99.72 ± 0.061
30(7)	0.93	5.77	0.47 ± 0.023					99.57 ± 0.101
32(8)	1.23	7.62	0.35 ± 0.012	0.55 ± 0.016	46.3	1.18 ± 0.035	0.29	99.94 ± 0.006
38(7)	1.00	7.11	0.49 ± 0.006					99.57 ± 0.074
39(9)	1.26	9.08	0.43 ± 0.013	0.77 ± 0.018	53.0	1.46 ± 0.035	0.30	99.45 ± 0.057
40(9)	1.32	8.73	0.63 ± 0.027	1.15 ± 0.057	59.0	1.95 ± 0.096	0.32	99.32 ± 0.100
48(11)	2.02	12.97	0.58 ± 0.014	0.86 ± 0.035	55.1	1.56 ± 0.064	0.37	99.51 ± 0.035
49(8)	1.45	9.01	0.49 ± 0.020	0.91 ± 0.009	52.1	1.75 ± 0.018	0.28	99.55 ± 0.064
52(7)	1.62	9.74	0.71 ± 0.010	1.66 ± 0.027	74.0	2.24 ± 0.036	0.32	99.40 ± 0.051
55(11)	1.76	10.08	0.55 ± 0.024	1.46 ± 0.061	81.0	1.80 ± 0.075	0.31	99.05 ± 0.130
60(6)	2.20	13.27	0.79 ± 0.056	1.79 ± 0.055	72.0	2.49 ± 0.076	0.32	99.75 ± 0.050
62(3)	2.16	10.48	0.78 ± 0.014	1.87 ± 0.044	69.0	2.71 ± 0.064	0.29	99.43 ± 0.041
69(5)	2.30	12.74	0.83 ± 0.041	2.40 ± 0.049	81.5	2.95 ± 0.060	0.28	99.88 ± 0.024
74(4)	2.94	15.80	1.20 ± 0.113	2.61 ± 0.258	83.3	3.13 ± 0.297	0.38	99.97 ± 0.010
77(8)	2.58	15.83	0.91 ± 0.015	2.38 ± 0.089	78.6	3.03 ± 0.113	0.30	99.79 ± 0.038

\* Abbreviations: C<sub>IN</sub> = clearance of inulin; C<sub>PAH</sub> = clearance of *p*-aminohippurate; E<sub>PAH</sub> = extraction of *p*-aminohippurate; RPF = renal plasma flow (C<sub>PAH</sub>/E<sub>PAH</sub>); gKW = g kidney weight.

† Numbers in parentheses denote number of clearance periods.

§ Mean ± SEM.

|| PAH was not infused in these four experiments.

TABLE II  
Summary of Statistical Analyses for the Data Presented in Table I

	Body weight	Kidney weight	C <sub>IN</sub> *	C <sub>PAH</sub>	E <sub>PAH</sub>	RPF	Filtration fraction (2-40 days)	Filtered sodium reabsorbed
								%
No. of samples	26	26	196	165	44	165	99	196
No. of dogs	26	26	26	22	22	22	13	26
Slope†	0.031	0.142	0.011	0.033	0.828	0.031	0.04	0.001
y intercept	0.19	3.61	0.06	0.19	21.60	0.58	0.15	99.56
F-ratio for slope§	241.29	104.55	281.36	163.43	154.24	116.96	53.10	0.43

\* Abbreviations: C<sub>IN</sub> = clearance of inulin; C<sub>PAH</sub> = clearance of *p*-aminohippurate; E<sub>PAH</sub> = extraction of *p*-aminohippurate; RPF = renal plasma flow (C<sub>PAH</sub>/E<sub>PAH</sub>).

† Slope refers to the relationship between each characteristic (y) and age (x).

§ Significance of slope to abscissa. All slopes except that for filtered sodium reabsorbed are significantly different from zero (P < 0.01).

an approximation of development of various functions in relation to the increase in tubular mass.

Despite changes in dietary intake, body composition, and renal function, plasma concentrations of sodium, potassium, and urea remained relatively constant except during the 1st wk (Fig. 1).

Systemic arterial blood pressure, which was monitored continuously in each experiment, rose—perhaps at different rates—throughout the postnatal period (Fig. 1). Between 21 and 77 days, when the most dramatic changes in functional development of the superficial nephron occurred (see below), mean arterial blood pressure rose from 90 to 105 mm Hg. For reasons given

in the Discussion, this change is not considered to be a major determinant of the rise in glomerular filtration rate.

**Clearance data.** A synopsis of total renal function in 26 dogs between 2 and 77 days of postnatal age is given in Table I. Statistical analyses of these clearance data are summarized in Table II. Glomerular filtration rate per gram kidney weight increased sevenfold from 0.13 ml/min at 2 days to 0.91 at 77 days (Fig. 2a). The clearance of PAH also increased with age (Table I). As in the rat (3), the extraction of PAH in the dog rose strikingly and directly with age (Fig. 3), reaching the mature value at approximately 10 wk after birth.

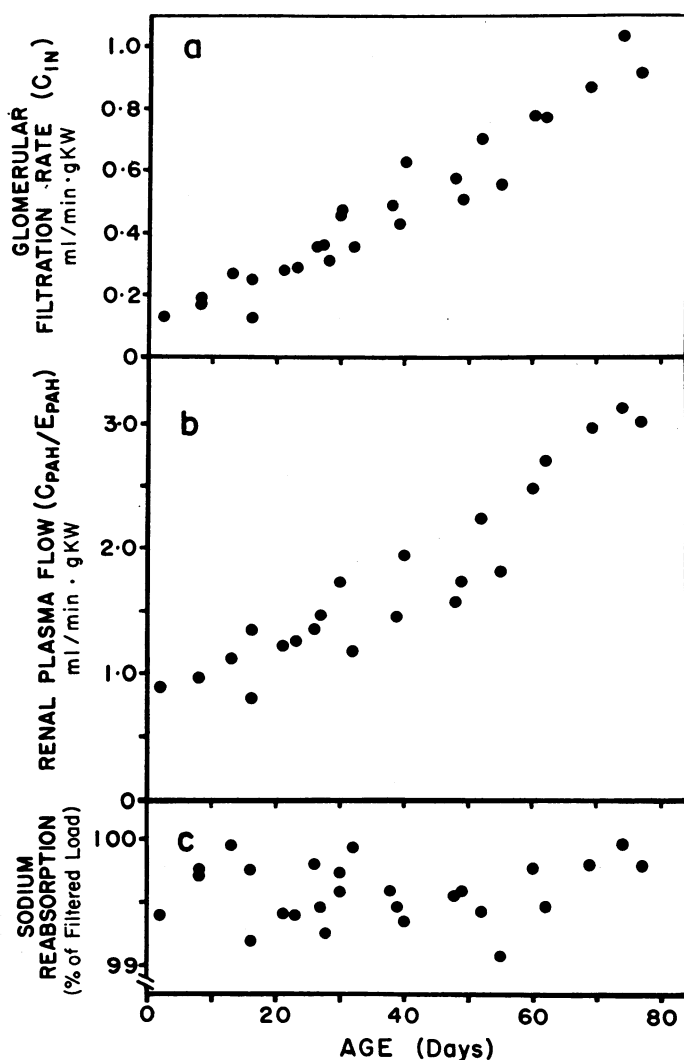


FIGURE 2 Relation of glomerular filtration rate (a) and renal plasma flow (b) to postnatal age. Despite the steadily increased filtered load of sodium, fractional sodium reabsorption for the whole kidney (c) remained constant. In this and subsequent figures, gKW = gram of kidney weight.

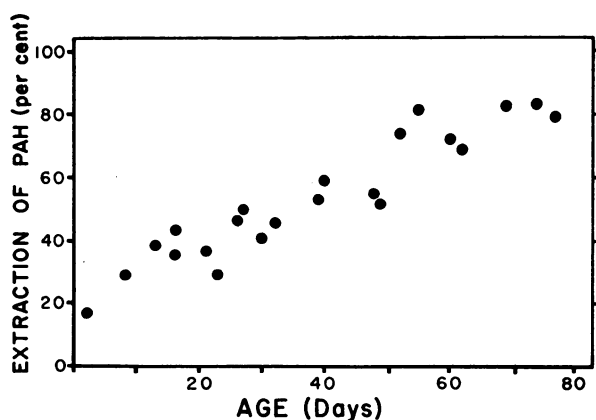


FIGURE 3 Changes in renal extraction of *p*-aminohippurate ( $E_{PAH}$ ) during postnatal development in the dog.

Accordingly, the ability of the kidney to extract PAH must be taken into consideration for determination of renal plasma flow during postnatal development. Renal plasma flow per gram kidney weight, measured as  $C_{PAH}/E_{PAH}$ , increased from 0.91 ml/min at 2 days of age to 3.03 at 77 days (Fig. 2*b*). The range of mature values for GFR and RPF (13, 14), expressed per gram kidney weight, was reached between 60 and 77 days after birth.

Inspection of Fig. 2*a* and *b* shows that during the earlier half of the postnatal period, GFR increased about fourfold, whereas RPF rose less than twofold. Consequently, there was a significant (Table II) increment in the filtration fraction up to 40 days (Fig. 4). Thereafter, it remained relatively constant at about 0.3 in this series.

Since serum sodium concentration was relatively constant with age ( $150 \pm 0.72$  mEq/liter, mean  $\pm$  SEM), the rate of increase in the filtered load of sodium was determined by the rise in GFR. This increase in filtered

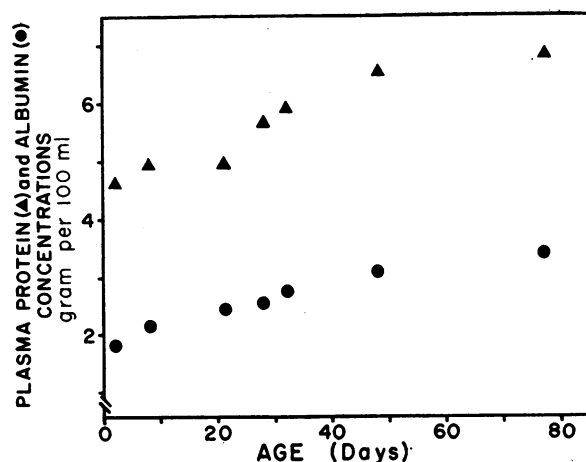


FIGURE 5 Changes with age in arterial plasma protein and albumin concentrations in samples from the aorta.

load was accompanied by a proportional rise in tubular sodium reabsorption, for fractional reabsorption of sodium remained constant within the narrow limits of 99.05–99.97 over the entire postnatal period (Fig. 2*c*).

The change in total plasma protein concentration (Fig. 5) is similar to the pattern described previously (15). The major increase occurred during the earlier postnatal period from 4.3 g/100 ml at 2 days to 5.9 at 32 days. Plasma albumin accounted for about one-third of this rise. It can be calculated (16) that this change results in a rise of plasma oncotic pressure of 8–10 mm Hg during postnatal development.

**Urea and urinary concentration.** The filtered load of urea increased almost proportionately to the increase in GFR since the plasma concentration of urea was relatively constant (Fig. 1). The urea clearance rose with age from 0.05 ml/min per g kidney weight at 2

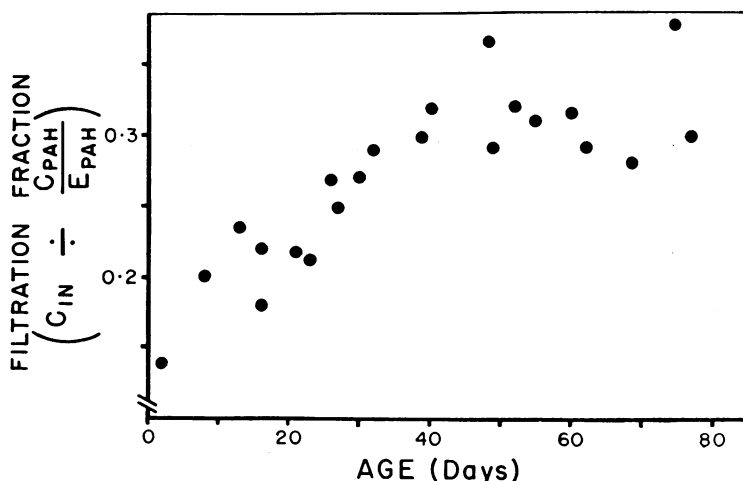


FIGURE 4 Relation of filtration fraction to postnatal age.

days to 0.54 at 77 days (Fig. 6 *a*). This rise was due not only to the increased filtered load of urea (i.e. the increased GFR) but also to a change in its reabsorption.

The fraction of filtered urea which was reabsorbed decreased with age from a mean value of 0.73 during the 1st wk to about 0.45 during the latter half of the study (Fig. 6 *b*). In contrast, the fractional reabsorption of water did not change systematically with age up to about 50 days, after which it is increased, leading to a  $U/P_{in}$  of about 445 at 74 days (Fig. 6 *c*).

As is shown in Fig. 7, urine flow (milliliter/minute per gram kidney weight) did not vary primarily as a function of age. In the older age group (40–77 days), the relationship between urine flow and  $U/P_{in}$  was similar to that which is seen in adult animals. At the younger ages there appeared to be no hyperbolic correlation between urine flow and  $U/P_{in}$ , and at any given urine flow, the younger the animal, the smaller the fraction of filtered water which was reabsorbed.

Urine was hypertonic to plasma at all ages. The ratio

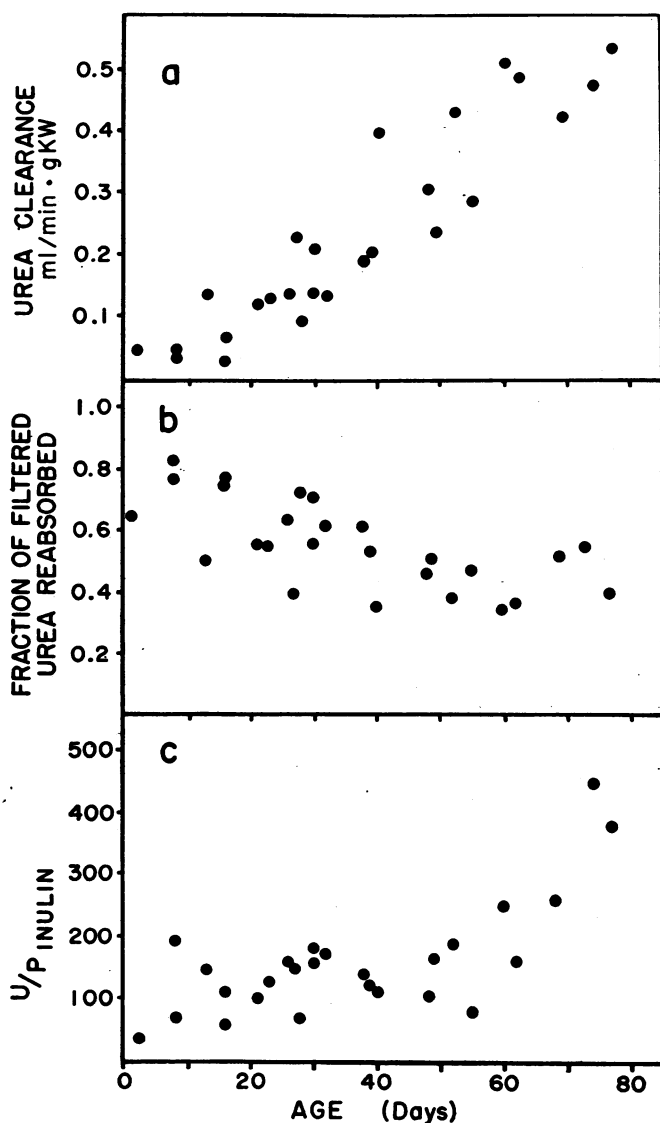


FIGURE 6 Relation between postnatal age and urea clearance (*a*), fractional reabsorption of urea (*b*), and urine to plasma inulin concentration ratio (*c*). Fractional reabsorption of urea decreased strikingly during the early postnatal period when fractional water reabsorption changed little. Possible explanations for this seeming paradox have been discussed in the text.

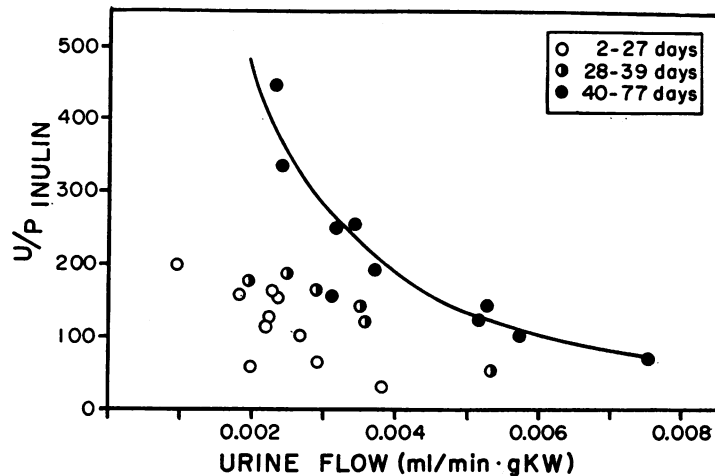


FIGURE 7 Correlation between urine flow and urine to plasma inulin concentration ratio at three stages of postnatal maturity. The line was not calculated.

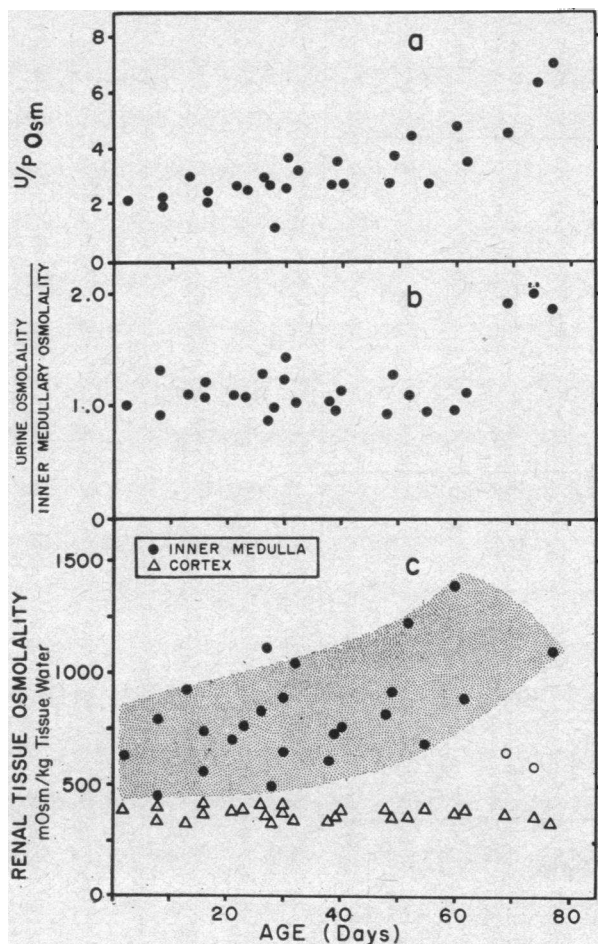


FIGURE 8 Aspects of the urinary concentrating system during postnatal maturation. Figures from top to bottom are *a*, *b*, and *c*, respectively. The open circles represent falsely low values (see text) and account for the high ratios in *8b*.

of urine to plasma osmolality rose from 2.0 at an early postnatal age to 7.0 at the end of the period under study (Fig. 8*a*). Urine osmolality was equal to or exceeded inner medullary osmolality at all ages (Fig. 8*b*). This fact probably reflects osmotic equilibration between fluid in the medullary collecting duct and medullary interstitium.

The osmolality of the inner medulla as determined by two methods (7), rose with age (Fig. 8*c*). The two open circles at 69 and 74 days represent falsely low values because of dissipation of the corticomedullary gradient for urea after the last urine sample had been obtained. Dissipation of the urea gradient in these two instances is reflected by the low content of urea in the inner medulla in the face of a maintained sodium content (Fig. 9). The justification for excluding the points is further strengthened by the high urine to plasma osmolal and inulin ratios in these two experiments (Figs. 8*a* and 6*c*). Unlike the osmolality of the inner medulla, that of the cortex (Fig. 8*c*) remained constant ( $366 \pm 4.9$  mOsm/kg tissue water, mean  $\pm$  SEM). There also was no systematic, age-related change in the osmolality of the outer medulla ( $433 \pm 7.6$ ) or outer zone of the inner medulla ( $496 \pm 11.9$ ).

Fig. 9 shows that the rise in the osmolality of the inner medulla was due predominantly to an accumulation of urea, which rose about threefold. Two other changes in the early postnatal period may have contributed to the rise in inner medullary solute concentration (i.e., mmoles/liter per kg tissue H<sub>2</sub>O as distinct from content, mmoles/liter per 100 g dry solid). Water in the inner medulla decreased from 87.5 to 82.5% of wet tissue weight (Fig. 10), and there may have been a slight increase in the sequestration of sodium (Fig. 9).

*Single nephron evaluation.* Data derived through



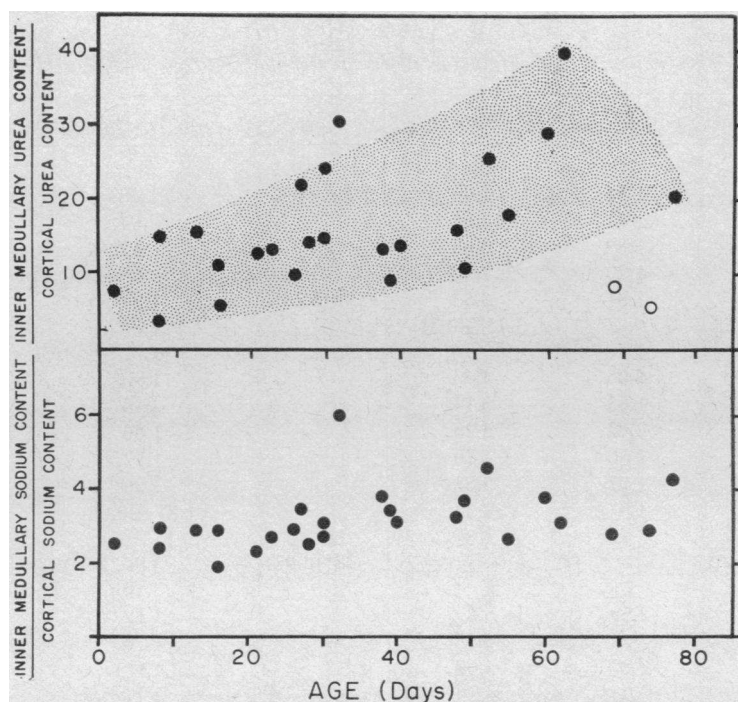


FIGURE 9 Corticomedullary gradients for urea and sodium in the maturing dog kidney. Tissue content refers to mmoles/100 g of urea-free dry solid. The open circles represent falsely low values (see text).

micropuncture evaluation of 103 nephrons in 17 dogs between 21 and 77 days of age are given in Table III. A summary of statistical analyses is presented in Table IV. In this dog strain, perfusion of proximal tubules located in the surface layer of the cortex with glomerular filtrate was present uniformly during the 3rd postnatal wk. Brisk flow in peritubular capillaries was visible about 2 wk earlier (see below). The filtration rate of superficial nephrons, determined in the last accessible surface segment of proximal tubules (see Methods), increased sevenfold within 2 months of postnatal development from 3.2 nl/min at 21 days to 23.1 at 77 days. A constant level of SGFR per gram renal weight appeared to be reached at 7–8 wk after birth (Fig. 11). The fraction of filtered volume reabsorbed ( $1 - P/TF_{1a}$ ) to the site of fluid collection in the proximal tubule remained constant with age and was at a mature level from the onset of intratubular perfusion (Fig. 12).

Hydrostatic pressures in proximal tubules and peritubular capillaries are presented in Table IV. Proximal intratubular pressure was constant between 21 and 77 days after birth (Fig. 13). This level of intratubular pressure appeared to be set even at the low flow rates observed at the very beginning of visible intratubular perfusion (Table III). However, peritubular capillary pressure increased significantly (Table IV and Fig. 13)

from 3.7 mm Hg at 13 days<sup>3</sup> to 9.2 mm Hg at 40 days after which it appeared to level off. During the latter period from 48 to 77 days, peritubular capillary pressure remained slightly below intratubular pressure (Table IV and Fig. 13).

Proximal tubular diameter was determined by two techniques (see Methods) as the distance between the

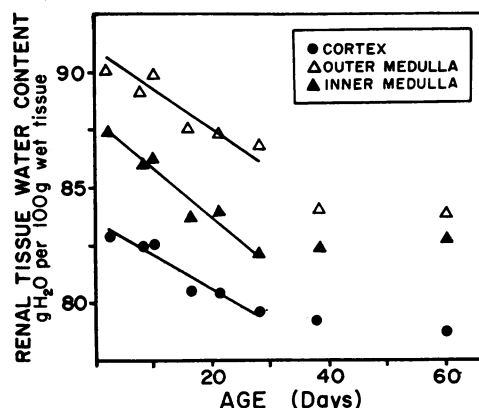


FIGURE 10 Changes with age in renal cortical and medullary tissue water content.

<sup>3</sup> Since blood flow in peritubular capillaries was vigorous before the onset of intratubular perfusion, it was relevant to determine capillary pressure earlier than age 21 days.

TABLE III  
Single Nephron Function in 17 Dogs during Postnatal Development from 21 to 77 Days after Birth

Age	TF/P <sub>1n</sub> *†	V*	SGFR*	Age	TF/P <sub>1n</sub> *†	V*	SGFR*
<i>days</i>		<i>nl/min</i>	<i>nl/min</i>	<i>days</i>		<i>nl/min</i>	<i>nl/min</i>
21 (8.18)§	1.85	1.84	3.40	39 (9.08)	1.88	4.51	8.48
	1.76	1.71	3.01		2.04	3.89	7.94
	1.93	1.89	3.65		1.99 ± 0.04		8.01 ± 0.44
	2.08	1.20	2.50	40 (8.73)	1.97	2.91	5.73
	1.95	1.81	3.53		1.85	3.68	6.81
	1.91 ± 0.05		3.22 ± 0.21		1.71	4.25	7.27
27 (6.45)	2.15	2.06	4.43		1.90	4.84	9.20
	1.90	1.65	3.14		2.16	3.05	6.59
	1.86	2.82	5.25		1.66	4.30	7.14
	1.88	2.50	4.70		1.72	2.95	5.07
	1.97	1.97	3.88		1.96	4.47	8.76
	2.14	1.97	4.22		1.87 ± 0.06		7.07 ± 0.49
	1.96	2.47	4.84	48 (12.97)	1.96	6.47	12.68
	1.98 ± 0.04		4.35 ± 0.26		2.10	6.57	13.80
28 (10.15)	2.27	2.59	5.89		1.82	5.04	9.17
	2.02	2.22	4.48		1.77	9.32	16.50
	1.75	2.89	5.07		1.95	5.60	10.92
	2.21	2.03	4.49		2.17	4.26	9.24
	1.96	2.70	5.29		1.93	8.70	16.79
	2.04 ± 0.09		5.05 ± 0.26		1.90	7.74	14.71
30 (8.85)	2.07	2.53	5.24		1.95 ± 0.05		12.98 ± 1.06
	1.96	2.16	4.23	49 (9.01)	1.95	6.46	12.60
	1.88	3.22	6.05		1.91	8.21	15.68
	1.94	3.94	7.64		2.12	6.79	14.39
	2.08	2.18	4.53		1.87	7.21	13.48
	1.99 ± 0.04		5.54 ± 0.61		2.08	7.83	16.29
30 (5.77)	2.01	2.14	4.30		2.25	5.24	11.79
	1.84	2.85	5.24		2.16	7.77	16.78
	1.93 ± 0.08		4.77 ± 0.47		2.05 ± 0.05		14.44 ± 0.72
32 (7.62)	1.91	2.68	5.12	52 (9.74)	1.90	7.74	14.71
	2.03	2.09	4.24		1.73	11.56	20.00
	1.81	3.30	5.97		1.88	8.08	15.19
	1.97	2.29	4.51		2.04	8.57	17.48
	1.95	3.29	6.42		1.86	10.53	19.59
	1.98	3.53	6.99		2.00	7.55	15.10
	1.95 ± 0.03		5.54 ± 0.45		1.75	8.28	14.49
38 (7.11)	2.09	4.11	8.59		1.88 ± 0.04		16.67 ± 0.90
	2.21	2.47	5.46	55 (10.08)	2.15	9.58	20.60
	1.78	3.61	6.43		1.98	9.69	19.19
	2.03	2.62	5.32		1.91	8.97	17.13
	2.03 ± 0.09		6.45 ± 0.76		2.08	8.70	18.10
39 (9.08)	2.09	3.54	7.40		1.92	9.79	18.80
	1.87	3.39	6.34		2.20	7.59	16.70
	2.06	4.44	9.15		2.04 ± 0.05		18.43 ± 0.58
	2.11	3.41	7.20	60 (13.27)	1.72	11.90	20.47
	1.92	5.31	10.20		1.69	7.80	13.18
	1.85	3.96	7.33		1.80	10.80	19.44
					1.78	8.30	14.77

TABLE III—(Continued)

Age	TF/P <sub>1a</sub> *†	V*	SGFR*	Age	TF/P <sub>1a</sub> *†	V*	SGFR*
<i>days</i>		<i>nl/min</i>	<i>nl/min</i>	<i>days</i>		<i>nl/min</i>	<i>nl/min</i>
60(13.27)	1.94	10.60	20.56	74(15.80)	2.11	8.81	18.59
	1.85	11.20	20.72		1.71	14.67	25.09
	1.80 ± 0.04		18.22 ± 1.36		1.99	10.70	21.29
69(12.74)	2.04	8.50	17.34		1.83	9.23	16.89
	1.79	13.16	23.56		1.93	10.67	20.59
	2.23	9.60	21.41		1.91 ± 0.07		20.50 ± 1.39
	2.01	9.14	18.37	77(15.83)	1.96	10.70	20.97
	2.16	10.30	22.25		2.05	9.69	19.86
	1.86	13.30	24.74		1.90	10.94	20.79
	1.97	10.04	19.78		1.82	17.29	31.47
	2.14	11.79	25.23		1.98	11.19	22.16
	1.87	12.33	23.06		1.94 ± 0.04		23.05 ± 2.14
	2.01 ± 0.05		21.82 ± 0.94				

\* Determined in last accessible proximal tubular segment on renal surface (see Methods).

† Abbreviations: TF/P<sub>1a</sub> = ratio of inulin concentration in tubular fluid to that in plasma; V = intratubular flow rate; SGFR = glomerular filtration rate of single superficial nephron.

§ Numbers in parentheses denote weight of one kidney (g).

|| Mean ± SEM.

light refractory lines bordering the darker tubular lumen. Measured in vivo ( $n=82$ ), it increased about three-fold from 8.6  $\mu$  at 21 days to 27.6  $\mu$  at 77 days; the range of mature values (13, 14) was approached linearly and was reached by about 49 days. Measurements taken from photomicrographs of the renal surface in 11 of these 17 experiments yielded a similar pattern with values of 8.2  $\mu$  at 21 days and 25.8  $\mu$  at 77 days. Tubular

volume was not determined because its calculation may involve inexact assumptions; the refractory line may not represent the luminal boundary (14), and the contribution of the brush border to the surface area cannot be estimated.

Transit time through the visible portion of the proximal convoluted tubule was constant between 21 and 77 days. The value in 53 nephrons was  $11.6 \pm 0.4$  sec (mean

TABLE IV  
Summary of Statistical Analyses for the Data Presented in Table III and for Hydrostatic Pressures  
in Proximal Tubules and in Peritubular Capillaries

	TF/P <sub>1a</sub> *	SGFR	Intratubular hydrostatic pressure (21–40 days)	Peritubular capillary pressure (13–40 days)†	Intratubular hydrostatic pressure (48–77 days) <sup>1</sup>	Peritubular capillary pressure (48–77 days)‡
			<i>mm Hg</i>	<i>mm Hg</i>	<i>mm Hg</i>	<i>mm Hg</i>
Mean ± SEM	1.96 ± 0.014		11.95 ± 0.244		12.24 ± 0.28	11.28 ± 0.40
No. of nephrons or capillaries	103	103	46	58	46	44
No. of dogs	17	17	8	10	9	9
Slope§	0.0007	0.396	0.025	0.205	0.060	0.068
y intercept	1.99	−6.379	11.15	0.861	8.60	7.13
F ratio for slope	0.465	276.77	0.410	20.186	10.63	4.04

\* Abbreviations: TF/P<sub>1a</sub> = ratio of inulin concentration in tubular fluid to that in plasma; SGFR = glomerular filtration rate of single superficial nephron.

† For statistical analysis, data on hydrostatic pressures were divided into two groups because peritubular capillary pressure changed with age (Fig. 13).

§ Slope refers to the relationship between each characteristic (y) and age (x).

|| Significance of slope to abscissa. Slopes for SGFR and for peritubular capillary pressure (13–40 days) are significantly different from zero ( $P < 0.001$ ).

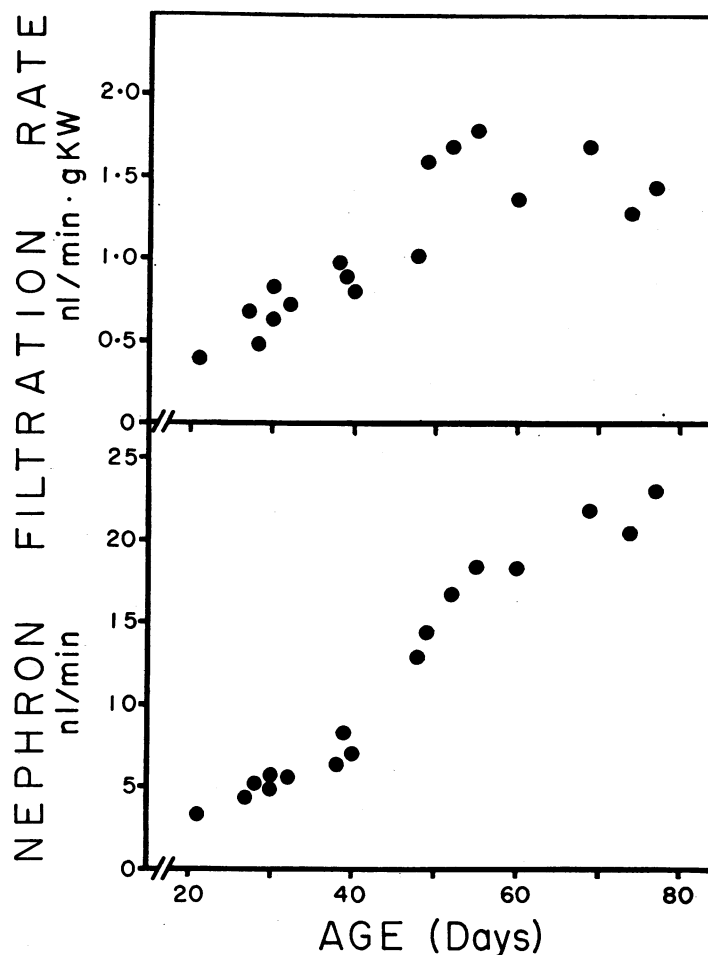


FIGURE 11 Correlation of glomerular filtration rate in superficial nephrons with postnatal age. Micropuncture data are presented individually with statistical analyses in Tables III and IV. Note that the age range covered in this and the next figure begins at 21 days because superficial nephrons in this dog strain are not uniformly patent during the first 3 wk after birth (see text).

$\pm$ SEM). Linear flow velocity of dye through the loop of Henle did not vary during the same period. Loop transit time in 38 nephrons had a value of  $32.7 \pm 1.8$  sec (mean  $\pm$ SEM). The constancy of tubular transit time and intratubular hydrostatic pressure points to the possible role of the loop of Henle in setting the resistance to flow in the proximal tubule as suggested for the mature kidney (12).

The length of the proximal tubule, measured as the distance between glomerulus and transition into the descending loop of Henle,<sup>4</sup> increased more than twofold during the postnatal period from 21 to 77 days. Micro-

dissection of 19 nephrons from eight animals showed a value of 3.1 mm at 21 days with an almost linear rise to 7.0 mm at 77 days. As in the mature dog (17), 40–50% of the total proximal length in each of the kidneys dissected was located on the surface and therefore accessible to micropuncture.

Intratubular stop-flow pressure in 21 proximal segments of seven dogs, aged 21–77 days, was constant and had a value of  $37.5 \pm 4.9$  mm Hg (mean  $\pm$ SEM).

## DISCUSSION

The animal model used in these experiments appeared to be particularly suited for the study of postnatal renal maturation. (a) The newborn dog remained stable in an acute experiment. This fact is reflected in the average duration of 7 hr for these experiments, as well

<sup>4</sup>In contrast with unmacerated, uninjected specimens, the nephron preparation used in this study did not permit exact location of the end of the pars recta.

as in the minimal variation of function in any given experiment (Tables I and III). (b) The spectrum of maturational changes occurred over a span of time which permitted a description of the sequence of development from newborn to the mature level (i.e., constancy of function per unit of renal weight). This constancy was reached for the kidney as a whole as well as for the single superficial nephron within the period of this study. (c) The morphological homogeneity of the beagle strain permitted correlation between functional changes and postnatal age since strain differences such as body and renal size were eliminated.

**Glomerular filtration rate.** Early postnatal development of the kidney is characterized by a disproportionate increase in functional as opposed to anatomical growth. This fact has been recognized specifically for GFR ever since the early observations in children (18). The present study documents the phenomenon not only for a number of other functions but also for glomerular filtration rate in the single superficial nephron (SGFR; Fig. 11). The mechanisms responsible for the early postnatal rise in glomerular filtration rate have not been ascertained. Those that have been considered in the past include changes in systemic arterial blood pressure, in afferent and efferent arteriolar resistance (19), in glomerular capillary permeability (20), and in glomerular size (21).

Neogenesis of glomeruli in this dog strain may continue during the first 2 wk after birth (1), and thus enhance GFR during this period. However, the number of glomeruli was constant throughout the major part of postnatal functional maturation. Judging by measurements of glomerular dimensions (1) in the same dogs

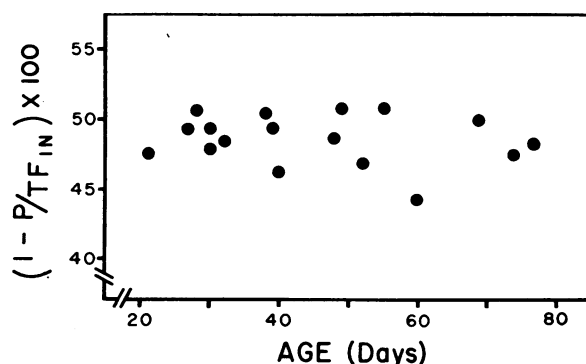


FIGURE 12 Fractional water reabsorption in the maturing superficial proximal tubule of the dog.

for which functional studies are reported here, the increase in glomerular size is probably of minor importance. During the same time span that the glomerular filtration rate of single superficial nephrons increased sevenfold, the volume of these glomeruli increased by only 33% (1). These figures may be compared with data from adult rats in which a similar difference in volume between superficial and juxtamedullary glomeruli (22) is accompanied by at most a threefold difference in SGFR under varying physiological conditions (2). Further, although glomerular surface area available for filtration might have increased at a greater rate than glomerular volume, this change still could not account for the observed magnitude of increase in GFR (1).

The constancy of intratubular stop-flow pressure in the face of constant intratubular pressure during free

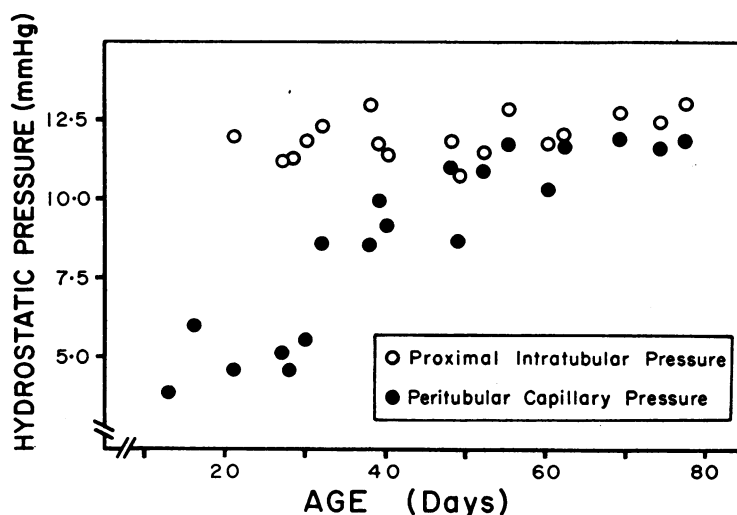


FIGURE 13 Relation of proximal intratubular to peritubular capillary hydrostatic pressure at various postnatal ages. Peritubular pressure could be measured before 21 days (see footnote 3).

flow (Fig. 13) and of rising plasma oncotic pressure during the early postnatal period of this study, must have resulted from an increase in glomerular capillary pressure. This increment may have been the consequence of a rise in either systemic blood pressure (Fig. 1) or in peritubular capillary pressure (Fig. 13) during the same time span. The relative contributions of these two effects cannot be estimated from the present study since no attempt was made to localize or quantify intrarenal vascular resistances, i.e., in afferent and efferent arterioles or in the renal venous circulation (see below).

By exclusion, therefore, the present data point to changes in permeability of the glomerular filtering membrane to water as possibly the major determinant of the postnatal increase in glomerular filtration rate. This view is in fact supported by a recent report that the renal clearance of dextran of mol wt 15,000 is close to zero in newborn infants, whereas in the adult this clearance is about 90% of the inulin clearance (20).

*Urea, renal tissue solutes, and urinary concentration.* The data suggest that the age-related changes in fractional reabsorption of water occur mainly in the medullary collecting duct. This conclusion is based partly on the  $U/P_{18}$  ratios, which range with age from 33 to 445, and partly on evidence which suggests that fractional reabsorption proximal to the medullary collecting duct increased in proportion to the filtered load of water. The proximal tubule reabsorbed a constant fraction of the filtered load of sodium and water (Fig. 12), and the mature fractional sodium reabsorption for the entire kidney (Fig. 2) suggests that this remained constant also in tubular segments beyond the proximal convolution. Further, the osmotic equilibration between urine and inner medullary interstitium reflected high endogenous ADH-induced water permeability of the medullary collecting duct and presumably of the distal convolution and cortical collecting duct as well. Finally, the increase with age in the length of loops of Henle (see below) and the rise in medullary osmolality (Fig. 8c) will enhance the absolute water reabsorption from the loops and thereby contribute to constant fractional reabsorption.

The major portion of filtered urea is probably reabsorbed passively and thus in part determined by the rate of water reabsorption. Since changes in fractional water reabsorption during the postnatal period occurred mainly in the medullary collecting duct, changes in the fractional reabsorption of urea may be attributed largely to this segment. However, the data (Fig. 6b and c) seem to present a conflict in that fractional reabsorption of urea decreased during the early postnatal period when fractional water reabsorption changed little. Several explanations might be invoked to resolve the seeming paradox. (a) The reflection coefficient for urea may

have increased with age. This is possible even though the present data suggest high water permeability due to ADH, which in the mature kidney also increases the urea permeability of this segment (23). (b) Urine flow per unit of kidney weight did not increase systematically with age. However, since the rate of increase in cortical mass exceeded that of medullary tissue (1), volume flow per unit of medullary weight probably rose with age. This may have led to increased linear flow velocity in the medullary collecting duct, and hence to decreased *fractional* reabsorption. (This does not, of course, imply a decrease in absolute reabsorption which probably increased.) (c) During early postnatal renal maturation, net addition of urea to loops of Henle may increase as the loops gradually descend from the cortex toward the inner medulla (24). This is a reasonable postulate not only because even at birth the medullary urea concentration is higher than that of the cortex (Fig. 9), but also because there appears to be a positive relation between the length of loops of Henle and the amount of urea recycled (25). As the loops increase in length, some of the urea which was previously removed from the medullary region via the blood will now enter the loops and be delivered to more distal segments, i.e., recycling can increase the fraction of filtered urea entering the medullary collecting duct without decreasing the reabsorptive rate in the proximal segment. The consequent steady increase with age of the load of urea added to the collecting duct might well contribute to a steady decrease in *fractional* reabsorption.

During the latter part of the postnatal period, the hyperbolic relationship between  $U/P_{18}$  and urine flow (Fig. 7) suggests that these variables were correlated in the mature fashion. It might therefore be anticipated that by this time the relationship between urea and water reabsorption in the medullary collecting duct resembled that seen in the mature kidney. This is in fact so, for this relationship from about 40 days onward is similar to that which Shannon described in adult dogs (26).

The accumulation of solutes in the inner medulla is a consequence of the maturational changes which have been described. The descent of loops of Henle toward the inner medulla augments the countercurrent multiplication of sodium as well as the medullary recycling of urea during the early postnatal period. This process may continue into the latter part of the period when a rise in fractional water reabsorption might also contribute to the amount of urea reabsorbed into the inner medulla.

Changes with age in urine concentration in the newborn have been attributed to low water permeability of the distal nephron (27). In contrast, the data of the present study demonstrate that the urine and medullary

interstitium were in osmotic equilibrium at any postnatal age. This also appears to be true in rats (28). Furthermore, this study extends the view, based on dehydration of young rats (28) as well as on acute and chronic urea loading of newborn infants (29), that the accumulation of medullary urea is a major change leading to the ability of the neonatal kidney to concentrate urine.

**Filtration fraction.** The age-related increase in the renal extraction of PAH observed in the present study on dogs (Fig. 3) confirms observations in infants (30) and in neonatal rats (3). Factors which may contribute to the increase, such as increase in proximal tubular mass and transport capacity for PAH of the proximal tubular cell (31), were considered in an earlier report (3).

The rise in RPF per gram kidney weight agrees with earlier reports on the piglet (19) and dog (32). The simultaneous determination of GFR and RPF in this study shows that in the newborn dog, as in the rat (3), filtration fraction increased during the early phase of postnatal development (Fig. 4). In contrast with the rat, in which nephron neogenesis may be the major determinant of the increase in filtration fraction, other mechanisms are responsible for this increase in the dog, and they were considered above as those which affect GFR.

**Reabsorption of sodium and water in single nephrons.** The glomerular filtration rate of single superficial nephrons increased sevenfold between 21 and 77 days (Fig. 11). The filtered load of sodium increased in proportion to that of filtered fluid in face of a constant serum sodium concentration. In view of the notion that the kidney of the newborn may have difficulty maintaining glomerulo-tubular balance, it was of considerable interest that the proximal tubule of the single nephron reabsorbed a constant fraction (Fig. 12) of a greatly increasing filtered load. This held true from the very beginning of intratubular perfusion of superficial nephrons to the end of the observed postnatal period during which time proximal tubular length increased twofold and proximal luminal diameter threefold.

Glomerulo-tubular balance for sodium and water may be achieved through intra-epithelial and peritubular influences. The present study provides some information about the latter. It has been proposed that an increase in filtration fraction (33) and in plasma oncotic pressure (34) augments proximal tubular reabsorption by enhancing removal of the tubular reabsorbate from the peritubular interstitial space. In the dog strain used in these experiments, circulation in peritubular capillaries was visible for about 2 wk before ultrafiltrate could be seen within the tubules on the renal surface. Under the assumption that this peritubular blood was postglomeru-

lar (35) and by extrapolation from the filtration fraction for the whole kidney (Fig. 4), that for single superficial nephrons must have increased from close to zero to about 0.3 during the period of micropuncture evaluation. This rise might have abetted the increase in oncotic pressure within peritubular capillaries which would be predicted from the increase in systemic plasma protein concentration (Fig. 5). Since both filtration fraction and plasma protein concentration rose steadily during the early postnatal period, the influence of peritubular oncotic pressure on tubular reabsorption must have been relatively minor at the beginning of intratubular perfusion and increased with age.

There is at present no information on the profile of a hydrostatic pressure gradient between tubular lumen and peritubular capillary which was observed in this study. However, if small hydrostatic pressure differences across any of the compartment boundaries interposed between tubular and peritubular capillary lumen have any influence at all upon the net flux of solute and fluid between these two structures, then the direction of this gradient would favor the net rate of sodium and water movement toward the capillary lumen. Thus, during the early postnatal period of this study, the effect of low filtration fraction and plasma protein concentration might be opposed in direction by a hydrostatic pressure gradient.

The rise in peritubular capillary hydrostatic pressure with age may be related—apart from a possible change in efferent arteriolar resistance—to an increase in intrarenal venous resistance. The venous pressure gradient in the mature kidney is 10–15 mm Hg between surface capillaries and renal vein. The low peritubular capillary pressure suggests that this gradient was minimal early during the neonatal period (Fig. 13). In the mature kidney, this pressure drop may be localized to the junction of arcuate and interlobar veins (36), an area in which venous valves have been demonstrated (37). If these valves influence renal venous resistance, then their growth during the postnatal period may have contributed to the rise in peritubular capillary pressure.

In the preceding discussion, constancy of proximal fractional reabsorption for sodium has been inferred from the proven constancy for water. This inference is strengthened by the finding in the present experiments that fluid from the late proximal tubule was isosmotic to plasma at all phases of maturation.<sup>6</sup> The constancy of fractional reabsorption, however, does not necessarily imply maturity of sodium transport (38). It is conceivable that early in postnatal development, maturational changes in the active component of net sodium transfer are complemented by the observed changes in Starling forces originating from the peritubular environment to result in glomerulo-tubular balance.

<sup>6</sup>Horster, M., and H. Valtin. Unpublished observation.

Maturity has been defined in this study as a constant relation between renal function and tissue mass or between different functions (e.g., GFR and RPF). This state was reached during the later part of the postnatal period covered by these experiments, whereas the early postnatal period (2–40 days) was characterized by disproportionate development. Functional balance, such as constancy of proximal fractional reabsorption, was evident during the entire postnatal period. Thus, the present study provides experimental evidence for an earlier suggestion (39) that postnatal renal maturation may be divided into two phases. The first, distinguished by disproportionate but coordinated development, eventuates in the constant, mature pattern; during the second, both function and structure continue to grow *pari passu* to the absolute values of adulthood.

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