Quantitative urinalysis in healthy Beagle puppies from 9 to 27 weeks of age

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Objectives—To evaluate indices of renal function in healthy, growing Beagle puppies from 9 to 27 weeks of age and to determine whether indices change with age during this period.

Animals—6 healthy Beagle puppies.

Procedure—Urine collections were performed at 2-week intervals in puppies 9 to 27 weeks old. Daily excretion of urinary creatinine, protein, sodium, potassium, chloride, phosphorus, and calcium were determined, as were quantitative urinalyses including endogenous creatinine clearance, urine protein-to-creatinine ratios (UPr/C), and fractional clearances of sodium (FNa), potassium (FK), chloride (FCl), calcium (FCa), and phosphorus (FP).

Results—Significant differences among age groups were detected for endogenous creatinine clearance, and daily urinary protein, potassium, calcium, and phosphorus excretion. Significant differences also existed among age groups for UPr/C, FNa, FK, FCl and FP. Age-related effects fit a linear regression model for FNa, UPr/C, daily phosphorus excretion, and daily protein excretion. Quadratic regression models were judged most appropriate for endogenous creatinine clearance, FK, daily chloride excretion, and daily potassium excretion. Endogenous creatinine clearance measurements higher than adult reference ranges were observed from 9 to 21 weeks of age. The FNa, FK, FCl, FCa, and FP were slightly higher than those reported for adult dogs.

Conclusions and Clinical Relevance—Selected results of quantitative urinalyses in healthy 9- to 27-week-old Beagle puppies differ with age and differ from those measured in adult dogs. Diagnostic measurements performed in puppies of this age range should be compared with age-matched results when possible. (*Am J Vet Res* 2000;61:577–581)

Disorders of renal function and urinary electrolyte secretion, including juvenile renal disease, congenital tubular defects, and urolithiasis, are occasionally encountered in young animals.[1] Although postnatal development of the urinary system has been studied in neonatal puppies and kittens, clinically useful indices of renal function have not been investigated in juvenile dogs. Randomly collected urine samples can be used to measure protein or electrolyte excretion, whereas timed urine collections can be used to estimate glomerular filtration rate.[2] In kittens, endogenous creatinine clearance and measurements of urinary excretion varied between 4 to 30 weeks of age.[3] Creatinine clearances different from adult values were observed in kittens between 9 and 19 weeks of age, whereas urinary excretion of electrolytes and inorganic phosphorus differed from adults in kittens < 7 weeks old.[3] The purpose of the study reported here was to evaluate indices of renal function in puppies during the age range when they are likely to be brought to clinicians for pediatric disorders of the urinary system. Changes in indices over time and differences from reported adult values are assessed.

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Materials and Methods

Puppies—Six healthy 9-week-old Beagle puppies (3 male, 3 female) from 2 litters of approximately uniform size (initial mean body weight, 2.8 ± 0.4 kg) were obtained from the Canine Conditioning Unit at the Ontario Veterinary College and transported to the animal housing area of the Atlantic Veterinary College (facilities approved by the Canadian Council on Animal Care). Puppies were studied at 2-week intervals at the ages of 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27 weeks.

Puppies were considered healthy on the basis of physical examination findings and results of CBC, serum biochemical analyses, and fecal examinations. Puppies were group housed with 2 to 4 other sex-matched puppies between collection periods, and twice daily were fed dry puppy growth diet[a] calculated for optimal growth requirements. Immunizations against canine distemper virus, adenovirus-2, parainfluenza, parvovirus, and coronavirus were administered at 6, 9, 12, and 16 weeks of age, with rabies vaccines administered at 16 weeks of age. In a separate study, urodynamic studies were completed on the same puppies at 10, 12, 16, 20, and 24 weeks of age. At least 5 days elapsed between urodynamic tests and urine collection periods. Urinalyses were completed prior to each urodynamic procedure to detect any urinary tract infections. The University of Prince Edward Island Animal Care Committee approved all protocols.

Sample collection—For urine collection periods, puppies were moved and acclimatized to individual modified metabolic cages. Puppies were fed individually twice daily during urine collections, and water was available at all times. Urine was collected from removable trays every 8 hours for a period of 48 hours. Urine was stored in closed glass containers and refrigerated after collection. A steel mesh covering attached to the bottom of the slatted cage floor prevented fecal contamination. Collection times began and ended approximately 1 hour after morning feeding periods, at a time when puppy excretory activity facilitated emptying of the urinary bladder. The urinary bladder was palpated at the end of the collection time and expressed manually or emptied by catheterization if distended.

At the midpoint of the 48-hour collection period, body weight was recorded and blood samples were collected for determination of serum creatinine, sodium, chloride, potassium, calcium, and phosphorus concentrations. At the end of each collection period, the total urine volume was measured and an aliquot of the pooled urine sample was submitted for determination of creatinine, total protein, sodium, chloride, potassium, calcium, and phosphorus concentrations. Serum and urine assays were completed within 4 to 24 hours after the end of the collection period.

Analytic methods—Reagent kits and an automated discrete analyzer[b] were used to determine concentrations of urine and serum creatinine, inorganic phosphorus, calcium, and serum total protein. Urine protein concentration was measured by use of a Coomassie brilliant blue G dye-binding method.[c] Concentrations of sodium, potassium, and chloride in urine and serum samples were determined by use of ion-selective electrodes.

Calculations—Endogenous **creatinine clearance (CrCl)** was calculated as[2]:

$$CrCl = [U_{cr} \times Uvol/S_{cr}]/\text{min/kg of body weight}$$

Fractional clearances of sodium (FNa), **potassium (FK)**, **chloride (FCl)**, **calcium (FCa)**, and **phosphorus (FP)** were calculated as[2]:

$$F_x = [U_x / S_x] \times [S_{cr}/U_{cr}] \times 100$$

Twenty-four hour **urine protein-to-creatinine ratio (UPr/C)** was calculated as:

$$UPr/C = U_{Pr} \text{ (expressed in mg/dl)}/U_{Cr} \text{ (expressed in mg/dl)}$$

Measurements of the amounts of **urine protein (UPr)**, **urine sodium (UNa)**, **urine potassium (UK)**, **urine chloride (UCl)**, **urine calcium (UCa)**, and **urine phosphorus (UP)** in 24 hours also were expressed by body weight (UPr/kg in mg/kg/d, and UNa/kg, UK/kg, UCl/kg, UCa/kg, UP/kg in mmol/kg/d). Twenty-four hour measurements were calculated by dividing 48-hour measurements by 2.

Statistical analysis—Analysis of variance was used to determine significant differences between measurements over time.[1,d] Regression over time was completed for variable means and individual puppy data. Quadratic and linear models were fitted to each variable.[4] If the resulting quadratic term was not significant, the linear model was adopted. The regression method used was suggested by Crowder and Hand,[5] which takes into account the fact that repeated measures were made on each puppy studied. Significance was determined at $P \le 0.05$ for all tests.

Results

Results of ANOVA for quantitative urinalysis measurements indicated significant differences between age groups for endogenous CrCl ($F = 15.7$; **degrees of freedom (df)** for groups = 9, df for error = 50; $P < 0.001$;

Table 1—Mean (± SD) results of creatinine and selected fractional clearances in 6 healthy Beagle puppies

Age (wk)	Creatinine clearance (ml/min/kg)	FC sodium (%)	FC potassium (%)	FC chloride (%)	FC calcium (%)	FC phosphorus (%)
9	4.05[a] (0.61)	0.18[a] (0.11)	18.12[a] (3.23)	0.59[a] (0.08)	0.42 (0.06)	22.92[a] (5.85)
11	4.75[a] (0.66)	0.30[b] (0.11)	17.17[a] (3.22)	0.54[a] (0.07)	0.73 (0.57)	26.93[a] (5.03)
13	4.43[a] (0.51)	0.33[b] (0.12)	20.47[a] (3.54)	0.63[b] (0.08)	0.53 (0.09)	23.19[a] (7.53)
15	4.50[a] (0.53)	0.33[b] (0.07)	20.31[a] (2.55)	0.57[a] (0.05)	0.44 (0.18)	23.63[a] (4.42)
17	3.72[b] (0.38)	0.39[b] (0.12)	21.10[a] (4.36)	0.69[b] (0.06)	0.44 (0.26)	27.44[a] (3.27)
19	3.92[b] (0.47)	0.43[b] (0.13)	21.17[a] (2.89)	0.72[b] (0.10)	0.57 (0.27)	24.29[a] (3.14)
21	3.58[b] (0.44)	0.36[b] (0.09)	21.55[a] (1.59)	0.63[b] (0.04)	0.58 (0.17)	37.69[b] (12.08)
23	3.10[b] (0.40)	0.40[b] (0.04)	17.62[a] (1.14)	0.63[b] (0.09)	0.44 (0.17)	28.48[a] (4.47)
25	2.81[b] (0.19)	0.42[b] (0.05)	17.00[a] (0.98)	0.64[b] (0.01)	0.48 (0.17)	25.55[a] (1.75)
27	2.49[b] (0.10)	0.42[b] (0.05)	15.90[b] (1.47)	0.61[b] (0.04)	0.50 (0.16)	27.69[a] (2.41)
ANOVA *P* value	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P < 0.001$	NS	$P = 0.004$
Best fit model	Quadratic	Linear	Quadratic	None	None	None

Mean values with any superscript letter in common are not significantly different at $P \le 0.05$. FC = Fractional clearance. NS = Not significant.

Table 2—Mean (± SD) selected quantitative urinary solute and protein excretion values and urine protein-to-creatinine ratios in 6 healthy Beagle puppies

Age (wk)	Sodium excretion (mmol/kg/d)	Potassium excretion (mmol/kg/d)	Chloride excretion (mmol/kg/d)	Calcium excretion (mmol/kg/d)	Phosphorus excretion (mmol/kg/d)	Protein excretion (mg/kg/d)	Protein-to-creatinine ratio
9	1.67 (3.12)	5.26[a] (0.45)	3.77[a] (0.41)	0.07[a] (0.01)	4.29[a] (1.14)	5.73[a] (2.08)	0.21[a] (0.08)
11	3.12 (1.45)	5.35[a] (0.57)	4.04[a] (0.63)	0.13[a] (0.08)	5.35[b] (0.35)	5.32[b] (2.20)	0.16[a] (0.08)
13	3.09 (1.29)	5.9[a] (0.43)	4.34[a] (0.34)	0.09[a] (0.01)	4.43[a] (1.45)	4.85[b] (0.35)	0.15[a] (0.02)
15	3.20 (0.95)	5.68[a] (0.50)	3.99[a] (0.53)	0.08[a] (0.03)	4.07[a] (0.57)	5.03[b] (1.45)	0.16[a] (0.04)
17	2.77 (0.94)	4.78b (1.14)	3.78[a] (1.03)	0.06[b] (0.04)	3.75[a] (0.94)	2.88[b] (0.36)	0.09[b] (0.01)
19	3.64 (0.89)	5.95[a] (0.29)	4.61[a] (0.36)	0.09[a] (0.04)	3.97[a] (0.21)	3.94[b] (0.83)	0.11[b] (0.03)
21	2.87 (0.98)	5.12[a] (0.45)	3.82[a] (0.73)	0.08[a] (0.03)	3.86[a] (0.42)	4.77[b] (2.41)	0.14[a] (0.06)
23	2.71 (0.50)	3.54[b] (0.24)	3.16[b] (0.45)	0.05[b] (0.02)	2.98[a] (0.19)	2.94[b] (0.22)	0.10[b] (0.01)
25	2.49 (0.43)	3.08[b] (0.18)	2.88[b] (0.15)	0.05[b] (0.02)	2.62[a] (0.10)	3.50[b] (2.94)	0.11[b] (0.09)
27	2.25 (0.47)	2.63[b] (0.26)	2.47[b] (0.35)	0.05[b] (0.02)	2.35[a] (0.22)	2.80[b] (0.27)	0.10[b] (0.01)
ANOVA *P* value	NS	$P < 0.001$	$P < 0.001$	$P = 0.01$	$P < 0.001$	$P < 0.0001$	$P < 0.02$
Best fit model	None	Quadratic	Quadratic	None	Linear	Linear	Linear

See Table 1 for key.

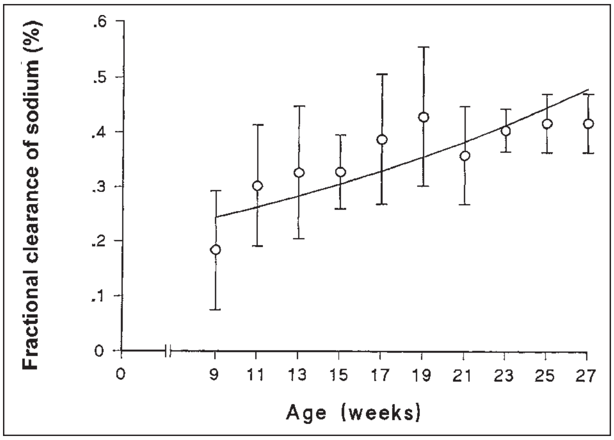

Figure 1—Relationship between urinary fractional clearance of sodium and age in healthy, growing Beagle puppies. Open circles represent mean values; bar values represent ± 1 standard deviation.

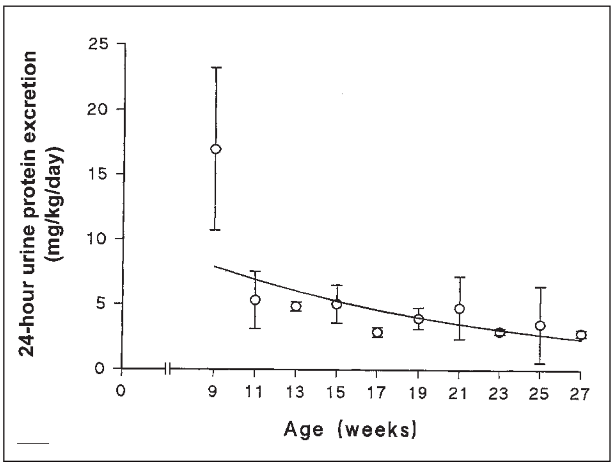

Figure 2—Relationship between 24-hour excretion of protein and age in healthy, growing Beagle puppies. *See* Figure 1 for key.

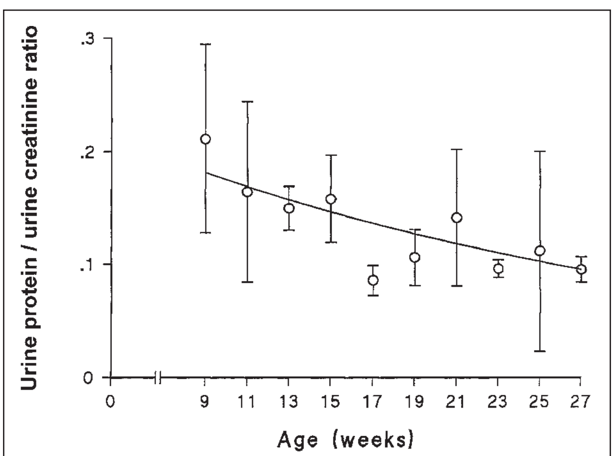

Figure 3—Relationship between urine protein-to-creatinine ratios and age in healthy, growing Beagle puppies, on the basis of 24-hour collections. *See* Figure 1 for key.

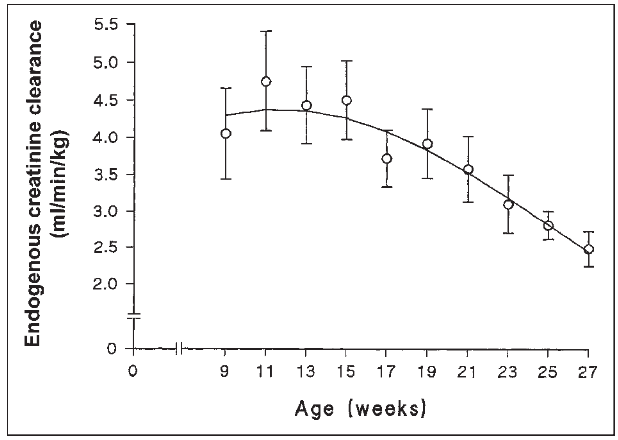

Figure 4—Relationship between endogenous creatinine clearance and age in healthy, growing Beagle puppies. *See* Figure 1 for key.

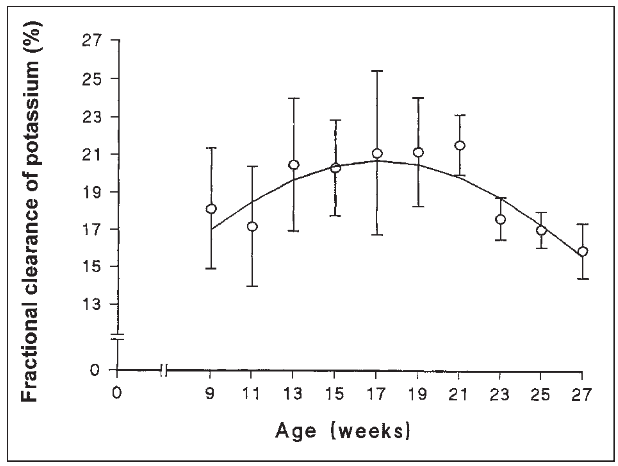

Figure 5—Relationship between urinary fractional clearance of potassium and age in healthy, growing Beagle puppies. *See* Figure 1 for key.

Table 1). Endogenous CrCl measurements were significantly higher in younger puppies (9 to 15 weeks old), compared with older puppies (17 to 27 weeks old).

Significant differences between age groups also were observed for FNa ($F = 3.77$; df = 9, 50; $P < 0.001$), FK ($F = 3.51$; df = 9, 50; $P = 0.002$), FCl ($F = 3.97$; df = 9, 50; $P < 0.001$), and FP ($F = 3.25$; df = 9, 50; $P = 0.004$; Table 1). The FNa at 9 weeks of age was significantly lower than that for all other sampling times, which were not significantly different from each other. The FK was significantly lower at 27 weeks of age, compared with those for all other sampling times, which were not significantly different from each other. The FCl at 9, 11, and 15 weeks of age were not significantly different from each other, but were significantly lower than FCl for the remaining sampling times; values for the latter times were not significantly different from each other.

There was no significant change over time in FCa. The FP at week 21 was significantly higher than all other sampling times, which were not significantly different from each other.

Significant differences also existed between age groups with respect to 24-hour UPr/C ($F = 3.43$; df = 9,50; $P = 0.002$), 24-hour UPr/kg ($F = 32.5$; df = 9,50; $P < 0.001$), UK/kg ($F = 8.68$; df = 9,50; $P < 0.001$), UCa/kg ($F = 2.71$; df = 9,50; $P = 0.012$), and UP/kg ($F = 9.45$; df = 9,50; $P < 0.001$; **Table 2**). For protein excretion, the value from week 9 was significantly higher than values from weeks 11 to 27, which were not significantly different from each other. The UPr/C had a decrease in values over time, with values from weeks 9 to 15 and 21 being significantly higher than those from weeks 17 to 19 and 23 to 27, respectively.

There was no significant change over time in 24-hour sodium excretion. Values of chloride excretion decreased over time, with values for weeks 9 to 21 being significantly higher than those for weeks 23 to 27. For 24-hour potassium and calcium excretion, values decreased over time, with values for weeks 9 to 15 and weeks 19 and 21 being significantly higher than the values for weeks 17 and weeks 23 to 27, respectively. For phosphorus excretion, only the value for week 11 was significantly higher than all the others. There were no variables for which the individual (puppy-specific) slopes of the regression lines were significantly different from each other.

Linear regression of the logarithmically transformed variable provided for the best-fit model for FNa (P = 0.034; **Fig 1**), UP/kg (P = 0.008), UPr/kg (P = 0.008; **Fig 2**), and UPr/C (P = 0.04; **Fig 3**). A linear model also appeared appropriate for UCa/kg, although the change in the slope was not significant (P = 0.067). The following variables had the best fit to quadratic models: CrCl (**Fig 4**), FK (**Fig 5**), UK/kg, and UCl/kg; all P values were ≤ 0.05. Either model could determine no significant relationship with respect to age for FCa, FCl, and FP (Tables 1 and 2).

Discussion

Puppies are periodically brought to clinicians for evaluation of renal failure, proteinuria, urinary calculi, or electrolyte or mineral imbalances.[1] Interpretation of diagnostic tests including serum biochemical analyses and urinalyses must be considered in comparison with age-matched reference values. Adult reference values can be expected for most serum biochemical variables by 9 to 12 months of age.[6,7] To our knowledge, results of endogenous CrCl measurements, urine protein quantification, and other urinary indices have not been reported for puppies.[7]

In our study, timed urine collections were performed to obtain initial data regarding quantitative urinalysis results in healthy Beagle puppies. Our collections were performed in puppies from 9 to 27 weeks of age, a time when puppies are likely to be brought to clinicians for recognizable clinical disorders. Measurements from kittens[3] as young as 4 weeks have been reported, and certainly 6 to 8 week measurements would have provided valuable canine data. However, in the study reported here, the puppies were transported from another facility and earlier weaning and transportation were considered potentially dangerous. Some error may have been introduced by the collection method, as we could not ensure that the urinary bladder was completely empty at the start and completion of the collection period. Incomplete collection may result in falsely decreased clearance values. However, the extended time of collection (48 hours) helped minimize this potential error. The sedation and invasiveness that would have been required for repeated urinary catheterizations in the youngest puppies was considered more likely to affect results.

Renal development continues postnatally in the dog, with the most rapid maturation happening within the first few weeks of life.[8-10] **Glomerular filtration rate (GFR)** increases during this time, reaching adult values by 2 months of age in micropuncture studies.[8] Postnatal development of GFR depends on development of renal vasculature and blood flow, a gradual increase in systemic blood pressure, and intrinsic morphologic development of glomeruli and filtration surface area.[8-10] In anesthetized neonates and 3, 8, and 12 week old puppies, inulin and CrCl measurements were described as equivalent to measurements in adult controls, but results were not given.[11] In another study, GFR were low in German Shepherd Dog neonates (mean inulin clearance 0.40 ml/min/kg at 1 day to 1.84 ml/min/kg at 13 days) and rose to 3.326 ± 0.05 ml/min/kg and 3.799 ± 0.239 ml/min/kg in 4 and 8 week old puppies, respectively.[12] Reported mean endogenous CrCl measurements for adult Beagles range from 2.1 ml/min/kg[13] in fasted 13 to 22 month-old Beagles to 3.7 ml/min/kg in 6 to 12 month-old female Beagles.[14]

Mean endogenous CrCl values in the Beagle puppies reported here were greater than adult reference ranges from 9 to 21 weeks of age before returning to more closely approximate adult values (Table 1 and Fig 4). A similar pattern was observed in kittens from 9 to 19 weeks of age, following values that were lower than adult values at 4 and 5 weeks of age.[3] An increase in creatinine filtration during growth was speculated.[3] However, muscle mass and serum creatinine concentrations are lower in puppies than adults, making increased creatinine filtration during this phase of growth less likely. The gradual decline in CrCl measurements from 11 to 27 weeks of age may be a result of increases in serum creatinine during this time. Serum creatinine concentrations rise slowly with age[6] as creatinine production by muscle increases. Assessment of renal function tests in puppies and kittens during these age ranges must be considered in light of these results.

Protein excretion, as measured by UPr/C and 24-hour protein excretion, steadily decreased over the study period (Table 2, Fig 2 and 3). The highest protein excretion was observed at 9 weeks of age, which may partially have been attributed to fecal contamination as the puppies had soft stools during this first collection period. From kittens, similar values were reported (UPr/C, 0.14 to 0.29).[3] In kittens, the lowest UPr/C were observed from 16 to 22 weeks of age, whereas the lowest 24 hour protein excretion was observed at 4 weeks of age when kittens were still suckling.[3] An increase in glomerular capillary pore density and surface area results in increased ultrafiltration as puppies mature.[15] However, permeability to larger macromolecules such as protein would be expected to be constant. Maturing tubular function and reabsorption of protein may explain small changes in protein excretion with age. At no time was mean urine UPr/C found to be ≥ 0.21 in the puppies in our study (reference range, < 0.2 in healthy 9- to 11-month-old Beagles)[16] so mild age-related changes in protein excretion are unlikely to be clinically important in growing puppies.

Fractional clearance of electrolytes was variable in our study. The FNa (Fig 1) increased linearly over time; whereas, fractional clearance (Fig 5) and 24-hour excretion of potassium and chloride increased slightly from 9 to 19 weeks of age, then decrease. Age-related changes were not seen for 24-hour excretion of sodium. Sodium, potassium, and chloride excretion and FK and FCl were slightly higher at some sampling times than reported adult measurements in Beagles[13] and varied breeds.[2] This difference was greatest for FK at 13 to 21 weeks of age, where mean FK exceeded 20%, and greatest for daily excretion of sodium in puppies 11 and 23 weeks of age, where mean 24-hour sodium excretion exceeded 2.65 mmol/kg. Excretion of electrolytes is affected by dietary intake, serum concentrations, fluid balance, and urinary concentrating ability. Although puppies were on a constant diet, food intake was not quantified, so minor changes in total food or fluid intake may have influenced electrolyte clearance.

Although there was some variability with age, clear changes for calcium and phosphorus excretion during growth were not established. Overall, phosphorus and calcium excretion declined over time, but individual measurements were variable. The FP and FCa did not differ among most age groups. The FCa was slightly higher at all ages studied than that described for adult dogs (0.15 ± 0.13% in varied breeds[2] and 0.16 ± 0.11% in fed Beagles[13]) as were mean FP (FP, 21.0 ± 9.0%[2] in adult dogs of varied breeds). Increased turnover or altered renal tubular handling of minerals during growth may explain these differences. The FCa was calculated using total calcium measurements, which reflects protein-bound and free calcium. Clearance values calculated on the basis of ionized calcium measurements, which is the primary form of calcium filtered,[17] theoretically would more accurately reflect calcium homeostasis. However, accurate measurement of ionized calcium in urine is impractical[17] because of sample handling requirements for the ionized calcium ion-selective electrode.

In summary, results of quantitative urinalyses changed subtly in the growing Beagle puppies of our report. When compared with reported adult measurements, the most important deviations from adult measurements were in CrCl values in puppies at 9 to 21 weeks of age. Clinicians also may expect FK, FCl, FCa, and FP to be slightly higher in 9 to 27 week old puppies than those reported for adult dogs, although measurements may vary in puppies consuming other diets and of other breeds. With the exception of FK and FCa, however, fractional clearances still fell within established adult reference ranges. The number of puppies studied here is small; quantitative urinalyses results from larger numbers of puppies of various breeds are needed to confirm our findings.

[a]Hill's Canine Growth, Hill's Pet Nutrition, Topeka, Kan.
[b]Hitachi 911, Boehringer Mannheim Corp, Indianapolis, Ind.
[c]Micro protein determination, Sigma Diagnostics, St Louis, Mo.
[d]SAS/STAT Version 6.4, SAS Institute Inc, Cary, NC.

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