

Age-related variations in hematologic and plasma biochemical test results in Beagles and Labrador Retrievers

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Objective—To investigate age-related variations in results of hematologic and plasma biochemical tests performed on dogs of 2 common breeds.

Design—Prospective cohort study.

Animals—34 Beagles and 44 Labrador Retrievers.

Procedure—Blood samples were collected throughout the dogs' lives; 589 samples were collected from the Beagles and 964 samples were collected from the Labrador Retrievers (age at the time of sample collection ranged from 22 days to 15 years). White blood cell and RBC counts; hemoglobin concentration; Hct; mean cell volume; mean cell hemoglobin concentration; alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities; and calcium, phosphorus, cholesterol, urea, protein, and albumin concentrations were measured.

Results—For all tests, there were significant effects of age on test results. There was a significant interaction between age and breed for all tests except hemoglobin, albumin, and phosphorus concentrations.

Conclusions and Clinical Relevance—Results suggested that there were age-related changes in hematologic and plasma biochemical test results in these 2 breeds of dogs. Changes were most evident during the first year of life, reflecting growth and maturation of the puppies. In some instances, values for puppies diverged markedly from those for adults, necessitating the use of age-specific reference ranges for the interpretation of clinical data. (*J Am Vet Med Assoc* 2003;223:1436–1442)

Reference ranges are essential to the interpretation of hematologic and plasma biochemical test results, with values typically considered normal if they fall within established reference ranges.¹ The accurate assessment of laboratory test results, therefore, is dependent on the use of reference ranges that are appropriate for the patient in question. Because there may be some variation associated with the analytical methodology used, it is important to use reference ranges supplied by the laboratory performing the test, rather than ranges published in reference texts. In addition, ranges that are developed through testing of a population of healthy animals demographically similar to the patient being tested should be used. In particular, reference ranges that have been obtained from adult animals of a particular breed or sex may not be suitable for individuals of a different breed, sex, or age.

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Breed- and age-related variations in hematologic and plasma biochemical test results in dogs have not been well documented. Much of what is known about age and sex effects has been derived from studies performed on Beagles to obtain reference ranges needed for interpretation of measurements from toxicologic studies.²⁻¹³ Beagles used in these studies were bred from research stock and were maintained in laboratory environments. They were often housed in closed colonies and usually kept sexually intact. Thus, it can be questioned whether these data can be applied to dogs other than the Beagles and, more importantly from a clinical perspective, how relevant the data are for companion dogs that may be very different in terms of their genetic background, environment, neuter status, and diet. Differences between hematologic values for Basenji dogs and dogs selected at random have been demonstrated,¹⁴ raising the possibility that interbreed differences could be important in the interpretation of certain diagnostic test results.

The purposes of the study reported here were to examine age-related variations over the entire lifespan, from the preweaning period through old age, in results of hematologic and plasma biochemical tests performed on companion dogs and determine whether there were differences in results for dogs from 2 common breeds. Our aim was to determine whether age-specific reference ranges are required for interpretation of clinical data.

Materials and Methods

Animals and samples—Data used in the study were retrieved from the clinical laboratory database at the Waltham Centre for Pet Nutrition, Leicestershire, UK. Thirty-four Beagles (26 male and 8 female) and 44 Labrador Retrievers (10 male and 34 female) housed at the Waltham Centre for Pet Nutrition were selected for the study, as these were the most numerous breeds of dogs housed at the facility. All animals were fed various commercially available, complete diets^a throughout the study period. Puppies ≤ 6 months old were fed a diet formulated for growth; dogs > 6 months old were fed an adult maintenance-type diet. All dogs were housed in purpose-built, environmentally enriched facilities¹⁵ and treated in accordance with the centre's research ethics and UK Home Office regulations.

Blood samples were collected from the dogs by means of cephalic venipuncture; food was withheld overnight prior to blood sample collection. A total of 589 samples were collected from the Beagles; Beagles were between 22 days and 13 years, 4 days old at the time blood samples were collected. Similarly, a total of 964 samples were collected from the Labrador Retrievers; Labrador Retrievers were between 29 days and 14 years, 242 days old at the time blood samples were collected. All dogs underwent periodic veterinary examinations, and only samples from dogs con-

sidered healthy at the time of sampling were entered into the database.

All puppies received standard vaccinations against parvovirus infection, distemper, and leptospirosis and received annual booster injections throughout their lives. None of the dogs were used for breeding during the study. Twenty-three of the male Beagles and 7 of the female Beagles were neutered; mean ages at the time of neutering were 7.0 years (range, 2.3 to 12.8 years) and 8.8 years (range, 8.3 to 8.8 years), respectively. Nine of the male Labrador Retrievers and 33 of the female Labrador Retrievers were neutered; mean ages at the time of neutering were 3.6 years (range, 1.3 to 8.4 years) and 5.1 years (range, 1.2 to 10.8 years), respectively.

Hematologic and plasma biochemical testing—White blood cell count, RBC count, and mean cell volume (MCV) were determined with an automated cell counter^b that also derived values for hemoglobin (Hb) concentration, Hct, and mean cell hemoglobin concentration (MCHC). For these analyses, blood was collected into a tube containing EDTA. Tubes were stored on a roller-mixer at room temperature after sample collection, and samples were analyzed within 1 hour. Samples containing clots were discarded.

The following plasma biochemical values were measured with an automated analyzer^c: protein, calcium, phosphorus, albumin, urea, and total cholesterol (free cholesterol plus cholesterol esters) concentrations and activities of aspartate aminotransferase (AST), alanine aminotrans-

ferase (ALT), and alkaline phosphatase (ALP). Globulin concentration was calculated as the difference between protein and albumin concentrations. For these analyses, blood was collected into a tube containing lithium heparin. Tubes were stored on ice for up to 1 hour prior to harvesting of plasma. Harvested plasma was analyzed immediately or stored at -20°C for up to 2 days. Grossly hemolyzed or lipemic samples were discarded.

Prior to each sample run, analyzers were calibrated according to manufacturers' directions and control samples were analyzed. The coefficient of variation was $\pm 5\%$. Control samples consisted of various commercially available standards with values within the manufacturers' recommended limits.

Statistical analyses—Data from all dogs were divided into 13 age groups: 3.1 to 8 weeks (nursing period), 8.1 to 16 weeks (postweaning period), 16.1 weeks to 1 year (juvenile period), 1 to 2 years, 2 to 3 years, 3 to 4 years, 4 to 5 years, 5 to 6 years, 6 to 7 years, 7 to 8 years, 8 to 9 years, 9 to 10 years, and > 10 years. Mean ages of the Beagles and Labrador Retrievers at the time of sample collection for each age group were, respectively, 6.7 and 6.4 weeks, 11.8 and 12.0 weeks, 23.4 and 31.1 weeks, 1.5 and 1.6 years, 2.4 and 2.5 years, 3.5 and 3.5 years, 4.6 and 4.5 years, 5.4 and 5.6 years, 6.6 and 6.5 years, 7.5 and 7.5 years, 8.5 and 8.7 years, 9.4 and 9.4 years, and 10.8 and 11.3 years.

Statistical analyses were performed with commercially available software^d; values of $P < 0.05$ were considered significant. Data were assessed for normality and homogeneity of vari-

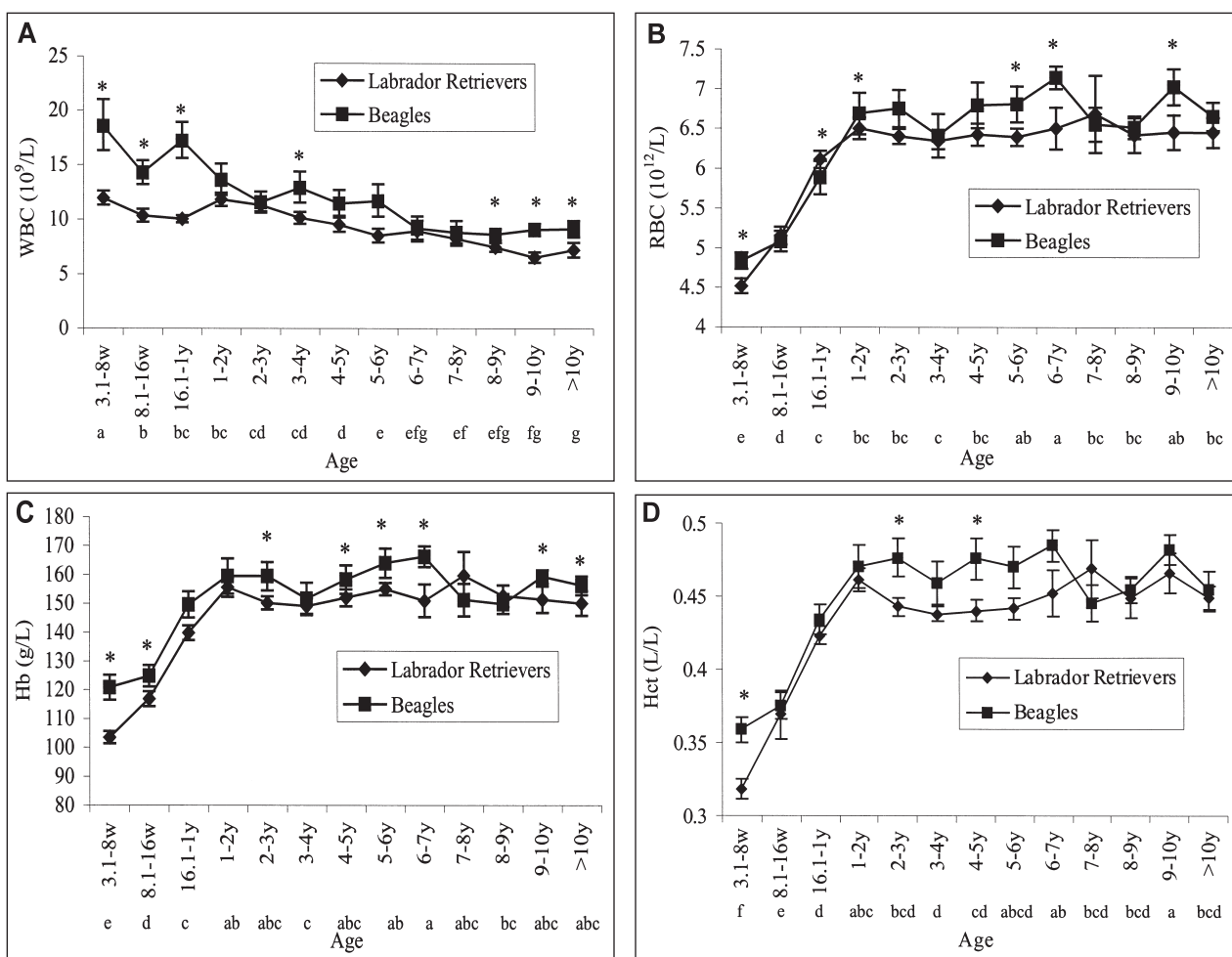


Figure 1—White blood cell counts (A), RBC counts (B), hemoglobin (Hb) concentrations (C), and Hct (D) in Beagles and Labrador Retrievers grouped on the basis of age. Error bars represent SE. *Values for the 2 breeds were significantly ($P < 0.05$) different. ^{a-g}Values for age groups with different letters were significantly ($P < 0.05$) different.

ance, and any data set that did not meet these criteria (WBC count and ALP, ALT, and AST activities) was log-transformed prior to analysis. For each laboratory test, the effects of age at the time of sample collection on test results were examined with a general linear model analysis that included age, breed, and the interaction between age and breed as fixed factors and dog (nested within breed) as a random factor. Differences between age groups were examined by use of the Student-Newman-Keuls test. Significant age-breed interactions were investigated for each

age group by use of *t* tests, identifying 4 critical periods (3.1 to 8 weeks, 8.1 to 16 weeks, 16.1 weeks to 1 year, and > 1 year).

For each age group, the mean value of the untransformed data for each dog was calculated and used to construct reference ranges. Reference ranges were constructed nonparametrically, with the lower reference limit being the 2.5th percentile and the upper reference limit being the 97.5th percentile. Following calculation of the mean value for each dog in each age group, line graphs of age group versus mean value

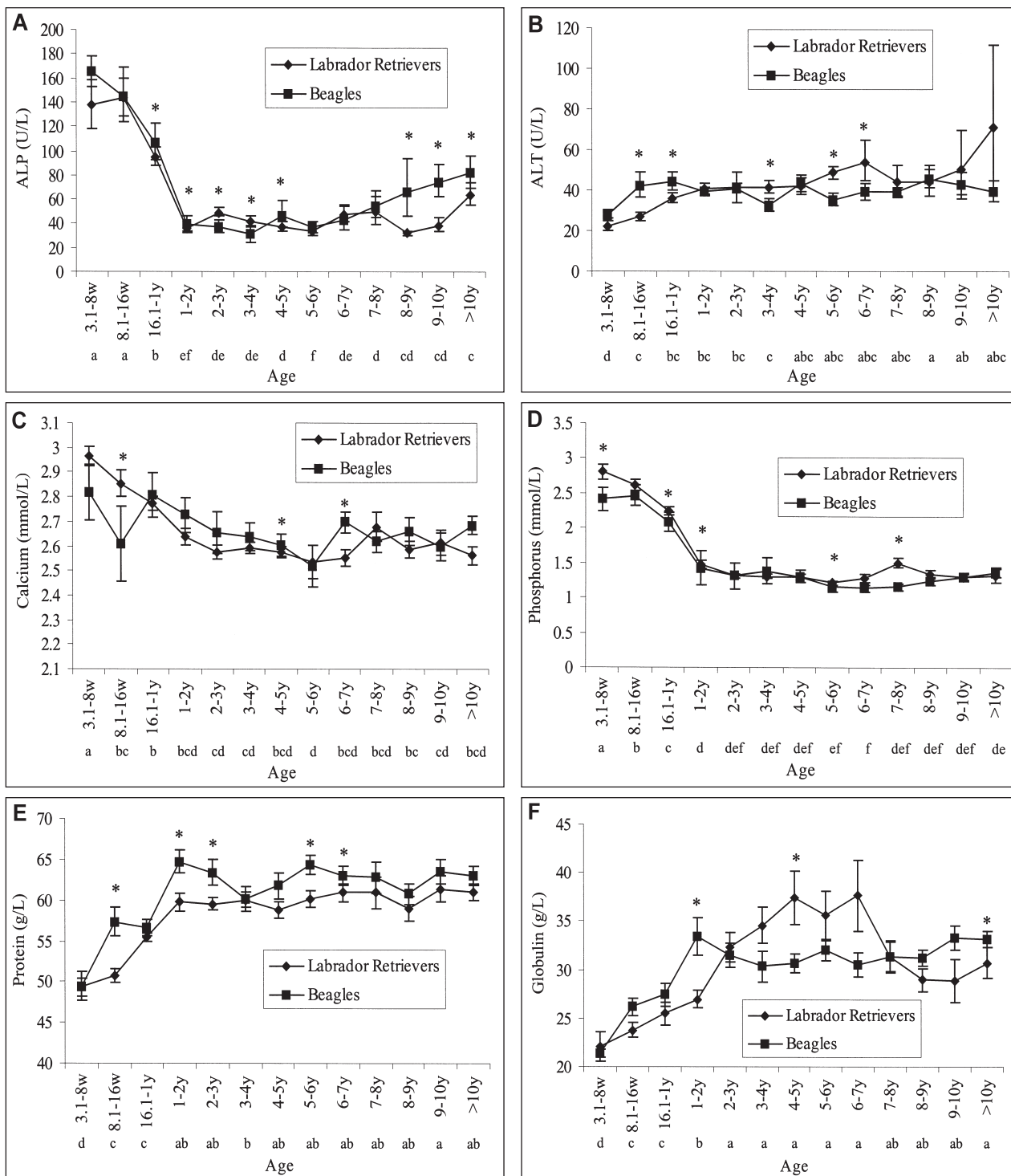


Figure 2—Plasma alkaline phosphatase (ALP) activity (A); alanine aminotransferase (ALT) activity (B); and calcium (C), phosphorus (D), protein (E), and globulin (F) concentrations in Beagles and Labrador Retrievers grouped on the basis of age. See Figure 1 for key.

were constructed. For those variables that had been log transformed, data were back-transformed prior to plotting.

Results

There was a significant interaction between age and breed for all tests except Hb, albumin, and phosphorus concentrations.

Effects of age on hematologic test results—White blood cell count decreased with age (Fig 1). White blood cell counts were significantly higher for the first age group (3.1 to 8 weeks old) than for any other age group. Although significantly lower than values for puppies between 3.1 and 8 weeks old, WBC counts for puppies between 8.1 and 16 weeks old were significantly higher than counts for dogs > 2 years old. White blood cell counts in dogs between 8.1 weeks and 2 years old were significantly higher than counts in dogs > 4 years old, and counts in dogs < 5 years old were significantly higher than counts in older dogs. Significant breed differences were detected for dogs < 1 year old and dogs > 8 years old, with Beagles having significantly higher WBC counts than Labrador Retrievers.

In dogs of both breeds, RBC counts, Hb concentration, and Hct increased during the first year of life, reaching a plateau after 1 year of age (Fig 1). Red blood cell count, Hb concentration, and Hct were significantly lower in the 3.1- to 8-week age group than in all other age groups. Red blood cell count, Hb concentration, and Hct were significantly lower in the 8.1- to 16-week age group than in all older age groups and significantly higher than in the 3.1- to 8-week age group.

There was a significant effect of age on MCV with a gradual decrease as age increased. This was particularly marked for Beagles, which had a significantly lower MCV than did Labrador Retrievers from the age of 5 years onward. Although there was a significant effect of age on MCHC, no specific trends were identified.

Effects of age on plasma biochemical test results—Alkaline phosphatase activities were highest during the first 16 weeks of life, then decreased and became stable from 1 year of age onward (Fig 2). Alkaline phosphatase activities for the 3.1- to 8-weeks and 8.1- to 16-weeks age groups were not significantly different, and values for these groups were significantly higher than values for all other age groups. Alkaline phosphatase activities for dogs in the 16.1-weeks to 1-year age group were significantly lower than values for younger dogs and significantly higher than values for older dogs.

Alanine aminotransferase activities were significantly lower in the 3.1- to 8-weeks age group than in all other age groups (Fig 2). Although there was a significant effect of age, no specific trends for AST activity were identified.

Plasma calcium concentration fluctuated with age, although it was significantly higher for puppies in the 3.1- to 8-weeks age group, compared with all other age groups (Fig 2). Plasma phosphorus concentration decreased progressively during the first year of life and then remained stable. Plasma phosphorus concentration was significantly higher in puppies in the 3.1- to 8-weeks age group, compared with all older dogs. Although significantly lower than the value for younger

puppies, plasma phosphorus concentration was significantly higher in dogs in the 8.1- to 16-weeks age group than in all older dogs. Although significantly lower than the value for younger puppies, plasma phosphorus concentration was significantly higher in dogs in the 16.1-weeks to 1-year age group than in all older dogs.

Plasma concentrations of cholesterol and urea fluctuated with age in both breeds.

Plasma protein concentration increased significantly in both breeds during the first year of life and was stable from 1 year of age onward (Fig 2). Plasma protein concentration was significantly lower in puppies in the 3.1- to 8-weeks age group, compared with all other age groups. Although significantly higher than values for younger puppies, plasma protein concentration was significantly lower in dogs in the 8.1- to 16-weeks and 16-weeks to 1-year age groups, compared with all older age groups. Although there was a significant effect of age on albumin concentration, no specific trends were identified. Globulin concentration increased steadily during the first 2 years of life and was significantly lower in puppies in the 3.1- to 8-weeks age group, compared with all older dogs. Although significantly higher than values for younger puppies, plasma globulin concentration was significantly lower in dogs in the 8.1- to 16-weeks and 16-weeks to 1-year age groups, compared with all older age groups.

Age-specific reference ranges—Distinctive, age-specific patterns were identified for WBC count; RBC count; Hct; ALP activity; and Hb, calcium, phosphorus, protein, and globulin concentrations. Each of these patterns was typically characterized by differences within or during the first year of life, relative to the remainder of the lifespan. Age-specific reference ranges were generated for these tests (Table 1 and 2). For the remaining tests, reference ranges were generated with data from all age groups (Table 3).

Table 1—Age-specific reference ranges for results of hematologic tests in Beagles and Labrador Retrievers

| Variable | Breed | Age range | | | |
|----------------------------|--------------------|---------------------|---------------------|----------------------|---------------------|
| | | 3.1 to 8 weeks | 8.1 to 16 weeks | 16.1 weeks to 1 year | > 1 year |
| WBC ($\times 10^9/L$) | | | | | |
| | Beagle | 6.9–36.8 (19.0) | 9.1–25.4 (14.5) | 8.6–32.0 (18.5) | 6.6–17.8 (9.9) |
| | Labrador Retriever | 6.2–19.8 (11.9) | 6.1–20.0 (10.2) | 7.2–14.4 (10.0) | 5.6–13.4 (9.5) |
| RBC ($\times 10^{12}/L$) | | | | | |
| | Beagle | 4.1–5.9 (4.9) | 3.8–6.6 (5.1) | 3.7–7.7 (5.8) | 5.8–8.3 (7.1) |
| | Labrador Retriever | 2.9–5.3 (4.6) | 3.7–6.1 (5.2) | 4.4–6.9 (6.3) | 5.6–7.5 (6.6) |
| Hemoglobin (g/L) | | | | | |
| | Beagle | 82–142 (123) | 86–149 (126) | 119–186 (145) | 142–191 (165) |
| | Labrador Retriever | 66–117 (107) | 79–142 (117) | 101–161 (145) | 137–173 (154) |
| Hct (L/L) | | | | | |
| | Beagle | 0.29–0.41 (0.36) | 0.28–0.46 (0.38) | 0.37–0.54 (0.42) | 0.42–0.56 (0.48) |
| | Labrador Retriever | 0.20–0.38 (0.32) | 0.20–0.69 (0.37) | 0.32–0.49 (0.43) | 0.38–0.51 (0.45) |

Numbers in parentheses represent median.

Table 2—Age-specific reference ranges for results of plasma biochemical tests in Beagles and Labrador Retrievers

| Variable | Breed | Age range | | | |
|----------------------------|--------------------|---------------------|---------------------|----------------------|---------------------|
| | | 3.1 to 8 weeks | 8.1 to 16 weeks | 16.1 weeks to 1 year | > 1 year |
| Alkaline phosphatase (U/L) | | | | | |
| | Beagle | 91–273 (162) | 33–416 (172) | 19–285 (114) | 16–122 (48) |
| | Labrador Retriever | 6–241 (170) | 27–370 (165) | 30–205 (105) | 22–87 (42) |
| Calcium (mmol/L) | | | | | |
| | Beagle | 1.90–3.35 (2.87) | 0.78–3.35 (2.75) | 1.95–3.43 (2.88) | 2.45–2.95 (2.64) |
| | Labrador Retriever | 2.58–3.30 (2.99) | 2.10–3.38 (2.85) | 2.53–3.18 (2.79) | 2.37–2.80 (2.62) |
| Phosphorus (mmol/L) | | | | | |
| | Beagle | 1.55–3.09 (2.70) | 0.94–3.20 (2.68) | 0.81–2.91 (2.22) | 0.68–3.44 (1.20) |
| | Labrador Retriever | 0.97–3.46 (2.84) | 1.42–3.33 (2.60) | 1.42–2.78 (2.28) | 1.08–1.79 (1.31) |
| Protein (g/L) | | | | | |
| | Beagle | 43–58 (49) | 45–73 (55) | 49–67 (56) | 57–69 (63) |
| | Labrador Retriever | 40–84 (48) | 44–60 (50) | 48–63 (56) | 54–68 (59) |
| Globulin (g/L) | | | | | |
| | Beagle | 19–25 (21) | 22–35 (25) | 22–45 (26) | 25–37 (31) |
| | Labrador Retriever | 14–50 (19) | 16–33 (23) | 19–57 (25) | 22–50 (31) |

See Table 1 for key.

Table 3—Reference ranges for results of hematologic and plasma biochemical tests that do not vary significantly ($P < 0.05$) with age in Beagles and Labrador Retrievers

| Variable | Beagles | Labrador Retrievers |
|----------------------------------|---------------------|---------------------|
| MCV (fL) | 63.2–74.3 (68.1) | 63.7–73.9 (68.9) |
| MCHC (g/L) | 314–350 (339) | 316–352 (340) |
| Alanine aminotransferase (U/L) | 20–106 (39) | 28–90 (40) |
| Aspartate aminotransferase (U/L) | 21–83 (27) | 15–37 (26) |
| Cholesterol (mmol/L) | 3.48–6.61 (5.38) | 3.91–7.14 (5.44) |
| Urea (mmol/L) | 3.5–13.3 (4.8) | 3.3–8.2 (5.2) |
| Albumin (g/L) | 26–37 (30) | 27–40 (30) |

See Table 1 for key.

Discussion

In this study, we found significant age-related variations in hematologic and plasma biochemical test results for dogs of 2 common breeds. In contrast to previous studies of this type, dogs in the present study were bred from domestic pets, housed in conditions resembling those in which companion dogs are kept, and fed commercially available pet foods. Various complete diets were fed to the dogs throughout the study period. Diet is well known to affect some hematologic and plasma biochemical test results (eg, dietary protein content can affect plasma urea concentration, and

dietary cholesterol content can affect plasma cholesterol concentration) and was undoubtedly a factor in the values measured in the present study. This likely was most evident in differences observed between puppies and adult dogs, because puppies < 6 months old were fed a diet formulated with growth-specific contents of protein, calcium, and phosphorus. On the other hand, although these differences in diets between puppies and older dogs may have contributed to age-related variations observed in the present study, most puppies in the general pet population would also be fed similar diets. Thus, results of the present study are likely clinically relevant and warrant further evaluation in studies with larger sample sizes and a wider variety of diets.

Analysis of age relationships identified clear patterns for some, but not all, tests in the present study. Most significant changes occurred during the first year of life, as was the case for WBC count; RBC count; Hct; ALP activity; and Hb, calcium, phosphorus, protein, and globulin concentrations.

White blood cell counts in puppies of both breeds were higher than counts in adults, and this was particularly apparent in the Beagles. This is consistent with the previous observation⁵ that total WBC counts increase soon after birth in Beagles, so that values in puppies up to 8 weeks old are higher than those of adults. The observed decrease in WBC count with age was consistent with results of 2 previous longitudinal studies in Beagles,^{3,9} but not with results of a cross-sectional study⁸ in which WBC count did not vary significantly with age. The cause of the high WBC count in young Beagles in the present study was not obvious in the absence of differential WBC counts, but it did not appear to be attributable to bacterial infection, as the dogs were found to be healthy during multiple clinical examinations. In addition, globulin concentrations were low, compared with adult values. Our results, therefore, indicate that care should be taken in interpreting WBC counts in Beagles < 1 year old, as values up to twice the upper reference limit for adults may be normal, especially in suckling puppies.

There were well-defined increases in RBC count, Hb concentration, and Hct in both breeds during the first year of life in the present study. This was consistent with previous observations that RBC count, Hb concentration, and Hct decrease rapidly during the first month of life.^{3,5,14} While decreases in RBC count, Hb concentration, and Hct during this period may be clinically important,¹⁶ it is also important to realize that low values are normal in young puppies and that they attain adult values between 6 and 12 months of age.^{3,5,14}

Newborn puppies have large, relatively immature RBCs, so MCV values are higher than those of adults, but MCV decreases rapidly after birth, and adult values are attained by 4 weeks of age.⁵ This may explain why MCV values were not significantly lower in the youngest age group in the present study, compared with older age groups, as most puppies in that group were > 4 weeks old when blood samples were first collected. In contrast, MCHC is reported to remain stable from birth through maturity in Beagles and Basenji dogs,^{5,14} and this was also the case for dogs in the present study. Therefore, caution should be exercised

when interpreting hematologic test results for puppies < 1 year old, and particularly those < 16 weeks old, because they may be misinterpreted as being anemic if adult reference ranges are used.

It is well recognized that ALP activities are high in young animals, with values up to 6 times the upper reference limit for adults reported for dogs and cats < 6 months old.¹⁷ High ALP activities in young animals are a result of ALP of bone origin, reflecting the activity of osteoblasts. Plasma activities of this isoenzyme decrease as maturity approaches and epiphyses close.¹⁸ Consistent with this, the present study found that ALP activities were significantly higher in dogs < 1 year old than in older dogs and that there were significant differences in ALP activities within the first year of life. These data agree with previous observations^{7,10,13} in Beagles that ALP activities are high throughout the first 3 months of life and then decrease to adult values by around 12 months.

The pattern of ALP activity was mirrored by that of plasma phosphorus concentration, which was significantly higher in puppies < 1 year old than in older dogs. Other studies^{7,10,13} have shown that serum phosphorus concentration is high in Beagle puppies through 6 months of age and then decreases to adult values by 12 months. Plasma phosphate concentrations up to 3.2 mmol/L have been reported in puppies, with a mean of 2.3 mmol/L at 6 months of age.¹⁹ Reference ranges generated with the present data indicated that plasma phosphorus concentrations as high as 3.5 mmol/L may be considered normal in growing puppies. Higher plasma phosphate concentrations in puppies reflect the processes of bone growth and remodeling, as well as increased renal reabsorption attributed to the effects of growth hormone.²⁰ These higher concentrations should be considered in the evaluation of clinical data to avoid falsely diagnosing hyperphosphatemia in dogs < 1 year old.

Plasma calcium concentrations were high in Labrador Retriever puppies, compared with older dogs, before and after weaning. It is not apparent why this was not seen in the Beagles, in which plasma calcium concentrations fluctuated, and data from previous studies are contradictory. Two studies^{7,10} found no change in serum calcium concentrations in Beagles during the first year of life, whereas calcium concentration in a third study¹³ decreased from a mean of 3.26 mmol/L at 2 weeks to 2.74 mmol/L at 1 year of age. We measured total calcium concentration, which is a composite of circulating ionized calcium, calcium bound to proteins (mainly albumin), and calcium complexed with anions such as phosphate. However, there were no significant differences in plasma protein or albumin concentrations between Beagles and Labrador Retrievers that might explain the differences in plasma calcium concentrations observed in the present study.

The finding that plasma protein concentration increased in both breeds during the first year of life was consistent with previous observations in Beagles^{7,10,13} and Basenjis.¹⁴ The increases in protein concentrations in the present study were probably a result of increases in globulin concentrations. This may have reflected changes in concentrations of immunoglobulins as well

as other proteins in this fraction (eg, transferrin, complement, lipoproteins, fibrinogen, and haptoglobin). Serum protein electrophoresis has demonstrated increases in γ -, α -2, and β -2 globulin concentrations during the first year of life in Beagle puppies.¹³ The present finding that albumin concentration was relatively unchanged in Beagles during the first year of life is, however, in conflict with results of a previous study¹⁰ in Beagles in which mean serum albumin concentration increased from 23 g/L at 2 to 3 months of age to 32 g/L at 11 to 14 months of age.

It is worth noting that in adult dogs in the present study, there were no significant changes in any of the hematologic or plasma biochemical test results with increasing age, with the exception of ALT activity in Labrador Retrievers. Inspection of data for the older Labrador Retrievers revealed a large SD that was attributed to 3 dogs having ALT activities 2 to 4 times the upper reference limit (107 U/L). There was no evidence of impaired hepatic function, as AST and ALP activities were not increased, and further tests that could have confirmed a reduction in hepatic function were not performed.

Notably, there was no evidence of progressive impairment of renal function among dogs in the present study, as plasma urea concentration did not increase significantly with advancing age. Although age-related changes in results of organ-specific biochemical tests, such as urea and creatinine concentrations and ALT activity, have previously been identified in Beagles, these occurred at around 12 years of age.⁸ Few of the dogs in the present study were > 12 years old, which may have limited the power of this study to examine this feature of longevity.

To our knowledge, this is the first study to evaluate hematologic and plasma biochemical test results over the lifetimes of 2 breeds of dogs and identify significant age-breed interactions. Our results were consistent with previous observation of differences in PCV, total and differential WBC counts, and plasma protein concentrations in Basenjis, compared with published reference ranges for Beagles and dogs selected at random.¹⁴ It is also reported that certain breeds have unique hematologic features, with PCVs > 50% reported for Poodles, German Shepherd Dogs, Boxers, Beagles, Dachshunds, Chihuahuas, and Greyhounds.²¹ However, it is not clear whether these represent true breed differences or, as the author suggests, clinical observations that these dogs experience splenic contraction because of nervousness.

Despite the evidence of breed effects in the present study, there were no substantial differences between breeds for calculated reference ranges. From these limited data, it appears that data derived from Beagles, the breed studied almost exclusively to date, may be applied to other breeds, although studies with more breeds are needed to confirm this. There was 1 exception to this lack of breed effect: upper reference limits for WBC counts in Beagle puppies < 1 year old were 1.5 to 2 times the upper reference limits for Labrador Retrievers. With this exception, breed did not appear to be as important as age and does not warrant particular attention in the interpretation of clinical data.

Whether this is true for other breeds, for instance those at the extremes of size (ie, toy and giant breeds), remains open to question.

The effects of sex and neutering were not assessed in this study because of imbalances in the ratios of males to females and the age at neutering between breeds. Earlier studies have shown small but statistically significant differences between male and female Beagles in regard to PCV; Hb concentration; differential WBC counts⁹; cholesterol concentration^{10,11}; ALP activity¹⁰; and concentrations of glucose, α -1 and γ -globulins, calcium, sodium, potassium, and phospholipids.¹¹ Despite these differences, reference ranges were similar for male and female Beagles for all analytes except glucose, in which the upper reference limit was higher in females than in males,¹¹ suggesting that sex was unlikely to be clinically important in evaluating results of hematologic and plasma biochemical testing in individual dogs. To our knowledge, there have been no longitudinal studies of the effects of neutering on results of hematologic or plasma biochemical tests.

In summary, this study documented age-related changes in hematologic and plasma biochemical test results in 2 breeds of dogs. These changes were evident during the first year of life, reflecting growth and maturation of the puppies. In some instances, notably WBC and RBC counts; Hct; ALP activity; and Hb, calcium, protein, globulin, and phosphorus concentrations, values diverged markedly from those of adults, necessitating the use of age-specific reference ranges for the interpretation of clinical data.

^aMasterfoods, Melton Mowbray, UK.

^bBaker System 9000, Serno-Baker Diagnostics Inc, Allentown, Pa.

^cCobas MIRA Plus, Roche Diagnostic Systems, Branchburg, NJ.

^dSPSS for Windows, release 11.0.0, SPSS Inc, Chicago, Ill.

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