

SEX, AGE, AND SEASONAL DIFFERENCES IN THE BLOOD PROFILE OF BLACK BEARS CAPTURED IN NORTHEASTERN PENNSYLVANIA¹

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Abstract: Sixty-six blood samples were collected from 44 live-trapped black bears (*Ursus americanus*) for 23 blood chemistry and hematology determinations. Statistical factorial experiments for analysis of variance revealed significant ($P < 0.25$) individual variability for 19 of 23 blood characteristics. Male bears had significantly ($P < 0.05$) higher serum calcium, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) than females. Cholesterol and MCH increased significantly with age; total protein and globulin were both significantly higher in adults than in yearlings or cubs, and total bilirubin in cubs and yearlings was significantly higher than in adults. Preening samples (1 July-31 December) had significantly higher glucose, packed cell volume (PCV), and mean corpuscular volume (MCV) but lower MCHC than postdenning (1 January-30 June) samples. Leukocyte differentials were comparable to findings by others. Of 48 serum samples submitted for brucellosis and 1 suspicious and 1 positive reaction were observed for leptospirosis; a female cub had 50 percent or more cell agglutination at a 1:1,600 dilution for *Leptospira pomona*. Examination of approximately 250 blood smears resulted in no observations of blood parasites.

Fundamental to an understanding of the effects of various factors on the health and welfare of a species is the establishment of base-line data for physiological parameters such as blood chemistry and hematology. The potential of blood analysis for determining effects of disease, immobilization, nutrition, stress, habitat quality, and population density on individuals or populations has been demonstrated or suggested for several species (Packer 1968, Franzmann and Thorne 1970, Franzmann 1971, Seal et al. 1972a, Presidente et al. 1973, Seal et al. 1975). Some blood values for black bears have been reported; however, most data have been for captive animals or have involved small sample sizes (Svihla et al. 1955; Jacobs 1957; Youatt and Erickson 1958; King et al. 1960; Erickson and Youatt 1961; Hock 1966; Seal et al. 1967; Brown et al. 1968, 1971; Halikas and Bowers 1972; Halloran and Pearson 1972; Pearson and Halloran 1972; Nelson et al. 1973; Eubanks et al. 1976).

The purpose of the present study was to establish base-line physiological data for continuing black bear research in northeastern Pennsylvania and to investigate possible effects of sex, age, or season on these data.

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METHODS

Wild black bears were captured with trailer-mounted culvert traps (Erickson 1957) and Aldrich foot snares (Bacus 1964). Captured animals were immobilized with M-99 (Etorphine, American Cyanamid Company, Agriculture Division, Princeton, N.J.) (Wallach et al. 1967). Blood samples were obtained from a femoral artery or vein in 3 15-cc clot tubes and 1 10-cc tube containing ethylenediamine tetraacetate (dipotassium) (EDTA) anticoagulant, with a B-D Vacutainer Aspirating Syringe (Becton, Dickinson, and Company, Rutherford, N.J.). Clotted samples were usually centrifuged within 1 hour of collection, with a standard clinical centrifuge for 10 minutes, to obtain the serum.

Blood chemistry and hematology analyses were contracted to Automated Analytical Laboratories, Ventura, California, which provided air mailers and serum vials containing stabilizers for enzymes and glucose. We selected the Multi-26 Health Screening Panel; using this program, each sample (3 cc of serum and 2 cc of whole blood) provided information on 23 blood chemistry and hematology parameters.

Eight thin blood smears for leukocyte differential counts and erythrocyte morphologies were prepared from each whole blood sample, usually within 1 hour of collection. Four smears were mailed to the Animal Diagnostic Laboratory at The Pennsylvania State University for staining with Wright's and Giemsa's stains, and the remainder were stored unstained. Leukocyte differentials were determined by examining a minimum of 200 cells and reporting the results as percentage composition.

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To establish base-line data, we used single samples from each bear to calculate mean, standard error of the means, and maximum-minimum values for each parameter.

To determine possible influences of sex, age, and season, and to obtain an indication of the sensitivity of blood analysis, factorial experiments for analysis of variance were performed on single samples from each bear. Multiple samples from individuals recaptured 1 or more times were included in the analysis if they were collected during the same season and year. Analysis was based on the model

$$Y(IJKL) = S(I) + T(J) + R(K) + ST(IJ) + SR(IK) + TR(JK) + STR(IJK) + B(IJKL) + E$$

where

- S = sex,
 T = season (1 = predenning, 1 July-31 December, and 2 = postdenning, 1 January-30 June),
 R = age (1 = cubs, 2 = yearlings, and 3 = adults and unknowns),
 B = individual variability, and
 E = random error term.

The model states that the blood parameter being considered, $Y(IJKL)$, is a function of the I th sex, the J th season, the K th age, and the L th individual.

Multiple samples were first tested for significant ($P < 0.25$) individual variability. If significance was indicated, analysis was based on the above model; if no significance was indicated, analysis was based on the model

$$Y = S + T + R + ST + SR + TR + STR + E.$$

We further tested parameters demonstrating significant ($P < 0.05$) age effects, using the Student-Newman-Keuls' multiple range test to determine differences between age groups.

Arc sine transformations were performed on all percentage and ratio data, including albumin/globulin (A/G) ratios, packed cell volume (PCV), and mean corpuscular hemoglobin concentrations (MCHC), before analysis of variance was performed.

Seasonal categories were selected on the basis of date distribution. No animals were captured during December through February or during July and most of August. The traditional division into 4 seasons was

therefore impractical and predenning and postdenning classifications were used.

Blood samples were also taken from 2 captive male black bears, retained at The Pennsylvania State University, for comparison with wild bears.

Although a study of disease in black bears was not a primary consideration of our overall study, the availability of extra sera and blood smears afforded an opportunity to conduct a limited disease study. Frozen sera were submitted to the Animal Diagnostic Laboratory, The Pennsylvania State University, for brucellosis and leptospirosis screening. Sera were screened for brucellosis by plate agglutination card tests. Reagents used in the screening were supplied by the Animal Health Division of the U.S. Department of Agriculture.

Leptospirosis determinations were by plate agglutination using killed *Leptospira* antigens from Fort Dodge Laboratories, Fort Dodge, Iowa. The sera were tested for the following leptospiral species antigens: *pomona*, *grippotyphosa*, *hardjo*, *autumnalis*, *icterohaemorrhagiae*, *wolfii*, *canicola*, *ballum*, *australis*, and *hyos*. Sera demonstrating positive or suspicious results were shipped to Veterinary Services Laboratory, Ames, Iowa, for testing by the agglutination lysis method with live antigens.

Blood smears used for leukocyte differential counts were also completely scanned for microfilariae, nematode larvae, and intracellular parasites.

RESULTS AND DISCUSSION

Sixty-six blood samples were obtained from 44 black bears (24 males, 20 females) captured between October 1972 and November 1973 (Table 1). Statistical analysis revealed significant ($P < 0.25$) individual variability for all but 4 parameters, indicating that individual variability is an important factor and should be included in the statistical model (Table 2). Significant ($P < 0.05$) sex, seasonal, and age differences were also observed for several parameters (Table 2).

Sex Differences

Female bears had significantly lower serum calcium, MCH, and MCHC than males (Table 2, Fig. 1). These differences may reflect long-term effects of pregnancy and lactation. The low MCH and MCHC levels indicate that female bears may be suffering from mild anemia that could be due in part to iron deficiency caused by high iron demands during pregnancy and lactation and low dietary replacement. Black bear milk has been reported to contain high levels of iron (Hock and Larson 1966).

Table 1. Base-line blood values obtained from 44 black bears captured in northeastern Pennsylvania.

	Mean	SE of mean (N)	Maximum	Minimum
Sodium (mEq/l)	142	<1 (44)	155	131
Potassium (mEq/l)	4.3	0.1 (44)	6.7	3.2
Calcium (mg/100 ml)	9.0	0.1 (44)	10.5	4.4
Inorganic phosphorus (mg/100 ml)	6.3	0.2 (44)	9.0	2.8
Glucose (mg/100 ml)	173	8 (44)	325	95
Urea nitrogen (mg/100 ml)	13	1 (44)	60	2
Uric acid (mg/100 ml)	2.3	0.1 (44)	4.5	1.3
Cholesterol (mg/100 ml)	323	13 (44)	690	170
Total protein (g/100 ml)	7.0	0.1 (44)	8.2	4.0
Albumin (g/100 ml)	2.7	0.1 (42)	3.6	1.4
Globulin (g/100 ml)	4.3	0.1 (42)	5.6	2.4
Albumin/globulin ratio	0.7	<0.1 (42)	0.9	0.3
Total bilirubin (mg/100 ml)	0.2	<0.1 (44)	0.8	0.1
Alkaline phosphatase (IU/l)	69	5 (44)	140	25
Lactic dehydrogenase (IU/l)	787	101 (43)	3,750	330
Serum glutamic oxalacetic transaminase (IU/l)	154	44 (44)	1,650	30
White blood cells ($\times 10^3/\text{mm}^3$)	10.2	0.5 (44)	22.8	6.9
Red blood cells ($\times 10^6/\text{mm}^3$)	8.08	0.1 (44)	9.99	6.51
Hemoglobin (g %)	16.3	0.3 (44)	20.3	13.2
Packed cell volume (%)	48.4	0.8 (44)	61.6	38.6
Mean corpuscular volume (μ^3)	61	<1 (44)	66	56
Mean corpuscular hemoglobin ($\mu\mu\text{g}$)	20.2	0.2 (44)	23.3	18.1
Mean corpuscular hemoglobin concentration (%)	33.5	0.2 (44)	37.0	31.0
Nonsegmented neutrophils (%)	1.0	0.3 (44)	10.0	0.0
Segmented neutrophils (%)	77.0	1.4 (44)	94.0	50.0
Lymphocytes (%)	16.0	1.3 (44)	42.5	2.0
Monocytes (%)	3.0	0.2 (44)	7.5	0.5
Eosinophils (%)	2.0	0.4 (44)	1.2	0.0
Basophils (%)	0.0	0.0 (44)	1.0	0.0
Nucleated red blood cells/100 white blood cells	1.0	0.4 (44)	17.5	0.0

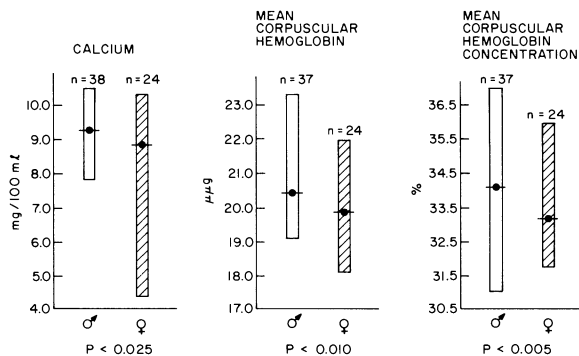


Fig. 1. Means and ranges of blood values that were significantly different between sexes of black bears in northeastern Pennsylvania.

Significantly lower serum calcium levels have also been reported for female black bears in Tennessee (Eubanks et al. 1976) but not for brown bears (*Ursus arctos*) from Yukon Territory, Canada (Halloran and Pearson 1972). Hypocalcemic tendencies in human females during gestation are well recognized (Searcy 1969) and lactating human females may lose 2-3 g of calcium phosphate each day (Guyton 1971). Seasonal

variations and lower calcium levels in female white-tailed deer (*Odocoileus virginianus*) have also been attributed to effects of lactation (Sauer 1973). The calcium demand of lactation in bears may be more dramatic because black bear milk contains relatively high levels of calcium (Hock and Larson 1966, Jenness et al. 1972) and the lactation period includes a time of dormancy with no dietary intake of calcium. The probable source of calcium during dormancy is resorption from bone storage areas or reserves. The impact, if any, of this calcium loss on the formation of cemental annuli needs to be evaluated. This evaluation may be particularly necessary in Pennsylvania, where variability in the levels of winter activity has been observed for female bears in differing reproductive conditions (Alt et al. 1979).

If data obtained by Hock and Larson (1966) and Butterworth (1969) hold approximately true for black bears in Pennsylvania, then one can estimate that a dormant female bear nursing 3 cubs (not unusual in Pennsylvania) would lose about 301 g of calcium during the first 12 weeks of cub development. If calcium con-

Table 2. Summary of significance for analysis of variance of blood parameters related to individual variability, sex, season, and age of black bears in northeastern Pennsylvania. Where no values are given, the parameters were not significant ($P < 0.25$ for B , $P < 0.05$ for all other parameters). B = individual variability; S = sex; T = season (predenning 1 July - 31 December and postdenning 1 January - 30 June); R = age (1 for cubs, 2 for yearlings, 3 for adults and unknowns); ST = sex-season interaction; TR = season-age interaction; SR = sex-age interaction; STR = sex-season-age interaction.

Test	Probability less than							
	B	S	T	R	ST	TR	SR	STR
Sodium	0.0250							
Potassium	0.2500							
Calcium		0.025						
Inorganic phosphorus	0.0500							
Glucose	0.0250		0.0050				0.025	
Blood urea nitrogen	0.0010							
Uric acid	0.0005							
Cholesterol	0.0050			0.0250				
Total protein	0.0250			0.0010				
Albumin								
Globulin	0.2500			0.0005				
Albumin/globulin ratio								
Total bilirubin	0.0250			0.0500				
Alkaline phosphatase	0.0010							
Lactic dehydrogenase	0.0005							
Serum glutamic oxalacetic transaminase	0.0005							
White blood cells	0.2500							
Red blood cells	0.0500							
Hemoglobin	0.1000							
Packed cell volume	0.0250		0.0250					
Mean corpuscular volume	0.0050		0.0100	0.050				
Mean corpuscular hemoglobin	0.0010	0.010		0.025				
Mean corpuscular hemoglobin concentration		0.005	0.0005			0.025		

centrations presented by Jenness et al. (1972) are used, this value would be 957 g. The human body is reported to contain 22 g of calcium per kg of fat-free body weight; 99 percent of this calcium is stored in bone (Pike and Brown 1975). The average total weight (not fat-free) of female bears 2 years of age or older in this study was approximately 95 kg (Matula 1976). On the basis of the above estimates and assumptions, it is conceivable that these bears could lose 5-50 percent or more of their total calcium during dormancy. The problem with this estimate is that we have to assume that the calcium storage ability of female black bears approximates that of humans. Nevertheless, the loss may be significant, and therefore the possible calcium and iron deficiencies observed in the present study may have an impact on the dietary requirements and food habits of these animals.

Age Differences

Serum cholesterol increased significantly with age (Table 2, Fig. 2). This increase is similar to responses reported for humans (Searcy 1969) and may be associated with changes in diet. Seal et al. (1975) considered low cholesterol, blood urea nitrogen (BUN), and uric acid in wolf (*Canis lupus*) pups from different years as indicative of diets low in protein and animal flesh.

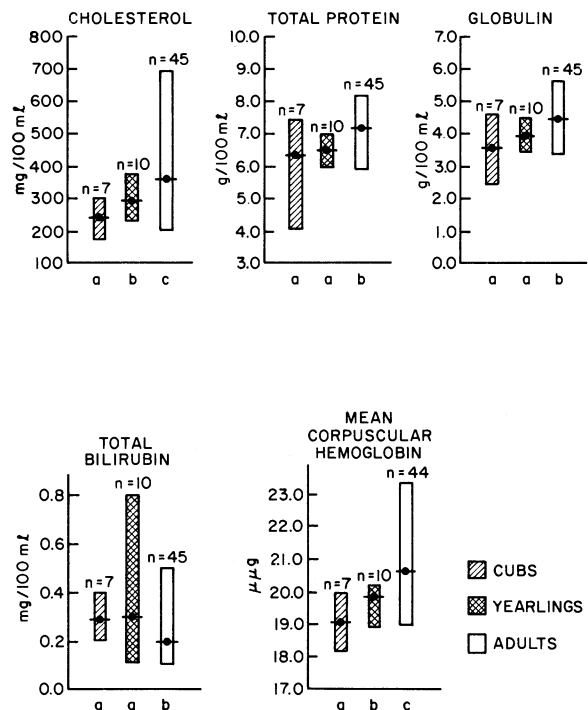


Fig. 2. Means and ranges of blood values that were significantly different among black bears of different ages in northeastern Pennsylvania. Unlike letters indicate significant difference ($P < 0.05$).

Searcy (1969) indicated that an excessive caloric intake without a corresponding increase in energy expenditure or a diet rich in saturated fats would result in increased serum cholesterol levels in humans. It is possible, then, that the increase in black bear cholesterol levels with age might be associated with an increased consumption of high-energy foods and/or animal flesh or may be related to regulatory mechanisms not yet understood.

Total protein and globulin were both significantly higher in adults than in yearlings or cubs (Table 2, Fig. 2). The globulin differences may reflect changes in alpha, beta, gamma, or all fractions due to dietary changes, to development of immunoglobulins, or to a combination of these and other factors (Latner 1975, Henry 1969). However, electrophoretic fractionation of serum proteins was not done, and inferences as to the specific proteins reflecting these differences cannot be made. It should be noted that albumin and globulin values reported here are relative values, due to the dye-binding technique used in the albumin determinations.

Total bilirubin levels in cubs and yearling bears were significantly higher than in adults (Table 2, Fig. 2). Younger animals may be more susceptible to erythrocyte damage or hepatic alterations due to immobilization and handling techniques, or they may demonstrate differences in erythrocyte physiology, rate of red blood cell production and destruction, or hepatic function. The possibility of erythrocyte destruction due to hemolytic, toxic, infective, and/or parasitic factors may also be considered. It is also possible that, although statistical significance is indicated, the differences may be physiologically insignificant.

MCH increased significantly with increasing age of the bears (Table 2, Fig. 2). Similar, although not statistically significant, trends were also noted for MCHC and hemoglobin, which indicates that younger bears may have hypochromic microcytic anemia. Anemia in young bears would be similar to hypochromic anemia observed in human infants and children, which is attributed to iron deficiency due to demands of growth (Wintrobe 1967). The endoparasite and ectoparasite load is also a factor to be considered. Pearson and Halloran (1972) noted that young brown bears had lower red blood cell counts, hematocrits, and hemoglobin concentrations than older animals.

Seasonal Differences

Predenning glucose levels were significantly higher than in postdenning samples (Table 2, Fig. 3). The higher predenning levels may be a function of

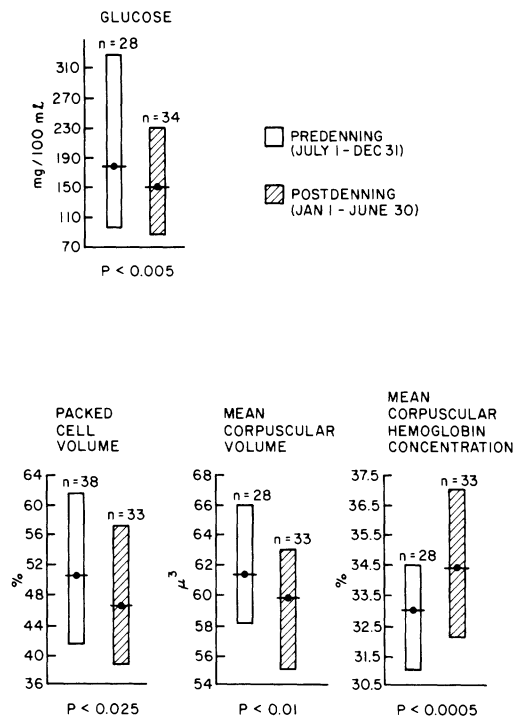


Fig. 3. Means and ranges of blood values that were significantly different between seasons for black bears in northeastern Pennsylvania.

physiological responses to preparation for denning or to changes in diet and levels of food consumption. Similar spring-to-summer trends have been reported for brown bears (Halloran and Pearson 1972). Erickson and Youatt (1961) noted that some black bears had increasing blood sugar levels during torpor and others had decreasing values.

Predenning samples in the present study also demonstrated significantly higher PCVs and MCVs but lower MCHC levels than postdenning samples (Table 2, Fig. 3). Predenning samples also tended to have higher (but not significantly higher) circulating erythrocyte levels. These findings suggest that black bear erythrocytes increase in size without a corresponding increase in hemoglobin content during the predenning period, which would result in higher PCVs and depressed MCHC levels.

Black bears in general have smaller-sized but greater numbers of circulating red blood cells than do humans (Wintrobe 1967), dogs (*Canis familiaris*) (Schalm et al. 1975), or wolf pups (Seal et al. 1975) (Table 1). This condition appears to be an advantage to a hibernating species because it increases erythrocyte surface area, which in turn provides a more efficient exchange of oxygen and carbon dioxide. However, why would the same species have an increase in MCV before dormancy? The larger erythrocyte size and lower hemoglo-

bin concentration may be a result of increased hematopoietic activity and a release of younger or immature cells into the circulatory system. The release of immature cells into the circulation could be verified by reticulocyte counts. An increase in circulating erythrocytes would not be reflected in the red cell count or PCV if plasma volume proportionally increased. An increase in the total blood volume in black bears between post-denning and predenning would be expected as a result of the large weight gains observed in bears during this period (Matula 1976). Pearson and Halloran (1972) similarly noted an increase in MCV in brown bears between spring and summer, but observed significant decreases in erythrocyte counts and PCVs.

Other Results

Significant interactions were noted for 2 blood parameters: a sex-age interaction for glucose and a season-age interaction for MCHC (Table 2). The sex-age interaction for glucose indicated that male cubs had markedly higher glucose levels than female cubs, but sex differences between yearlings and adults were much less pronounced. The season-age interaction for MCHC demonstrated diverging trends from comparable MCHC values in younger animals to seasonally different MCHC values in adults. Physiological explanations for these 2 trends are difficult to ascertain. We feel that a much larger sample size is needed to verify these results and to help clarify any trends.

Comparisons of blood values from 2 captive bears with those of wild bears indicated that the most important differences were consistently lower levels of glucose, alkaline phosphatase, lactic dehydrogenase (LDH), and serum glutamic oxalacetic transaminase (SGOT) in the captive animals, which probably reflects a lower level of stress and excitability as well as a continuous feeding regimen in captive animals (Matula 1976). The 2 captive bears also had slightly higher MCV and probably normochromic red cells.

Three bears captured with snares each had elevated sodium, total protein, albumin, globulin, red cell count, hemoglobin, and PCV values. An increase in these parameters probably indicates dehydration. They also demonstrated increased levels of glucose, alkaline phosphatase, LDH, SGOT, and a higher white cell count; elevation of these blood parameters is probably associated with increased stress and physical activity. One of the snared bears had previously been captured in a culvert trap, and at that time his blood was judged to be in the normal range (Matula 1976).

Leukocyte differential counts for wild black bears in

this study (Table 1) were generally comparable to those reported in other studies (Musacchia et al. 1955, Jacobs 1957, King et al. 1960, Pearson and Halloran 1972). Statistical analyses for sex, season, and age differences were not completed due to the complexity of the analyses, which was compounded by the possibility of large sample-size errors (Davidsohn and Nelson 1969). The data were described, however, according to age, sex, and season to reveal any possible trends. The differentials appeared to be fairly stable for sex and season but cubs may tend to have higher neutrophil and lower lymphocyte levels than older bears (Matula 1976).

Of 48 serum samples submitted for brucellosis and leptospirosis determinations, all were negative for brucellosis; 1 suspicious and 1 positive reaction were observed for leptospirosis. Sera from a 2-year-old female obtained on 2 separate occasions (14 May and 5 June 1973) demonstrated 50 percent or more cell agglutination for dilutions of 1:400 and 1:200, respectively, for *Leptospira pomona* and a possible cross-reaction of 1:200 and 1:100, respectively, for *L. autumnalis*.

Sera from a female cub agglutinated at 1:1,600 dilution for *L. pomona*, 1:400 dilution for *L. icterohaemorrhagiae*, and 1:200 dilution for *L. autumnalis*. Reactions for *L. icterohaemorrhagiae* and *L. autumnalis* are probably results of cross-reaction.

Caution must be employed when interpreting these results because an absolute diagnosis of leptospirosis can be made only if *Leptospira* has been isolated from specimens of the infected host (NADL 205 Form (Revised May 1973), Veterinary Services Laboratory, Ames, Iowa). However, blood from the female cub had the lowest MCH and SGOT levels recorded in this study and also had a low MCHC and a slightly increased total bilirubin value. Icterus and anemia are 2 common clinical signs observed in leptospirosis when clinical manifestations are present (Roth 1970). Because the bear was so young, it is tempting to speculate that she was actively infected at the time of sampling.

Examination of approximately 250 blood smears resulted in no observations of blood parasites. Apparently, black bears in northeastern Pennsylvania are relatively free of blood parasites within the limits of our techniques for detecting them. These results are difficult to explain in light of results reported by King et al. (1960) for bears in New York.

Care must be exercised when evaluating blood measurements, for many factors can influence the results. For example, feeding time and quantity and quality of food ingested before blood is sampled can affect blood

chemistries in monogastric animals. It is for this reason that human subjects fast for 12 hours before blood profiles are done. Other important factors include capture, handling, and immobilization methods (Franzmann and Thorne 1970, Franzmann 1971, Seal et al. 1972b); the length of time the animal spent in the trap; excitability of the animal (Geraci and Medway 1973); weather conditions; physical condition of the animal; blood collection, handling, storage, and analysis techniques (Wintrobe 1967, Lampasso 1968, Cohen 1969; Davidsohn and Henry 1969, Searcy 1969, Medway and Geraci 1972, Geraci and Medway 1973, Geraci and Engelhardt 1974); and general nutritional status of the animal (Seal et al. 1972a). All of these factors, and others, may influence 1 or several physiological parameters and therefore should be noted and, where possible, standardized.

Another important consideration involves the use of automated blood analyzers for hematology determinations. Some models measure red blood cell counts, hemoglobin, and MCV values and then calculate the PCV, MCH, and MCHC from these values. The analyzer used in this study was the Coulter Counter Model S, Coulter Electronics Inc., Hialeah, Florida. This analyzer had a present red cell size threshold of $30 \mu^3$, and smaller cells are not counted or averaged into the MCV calculation (personal communication with Coulter consultants). The relatively low mean MCV of $61 \mu^3$

for bears in this study indicates that an appreciable number of red cells may have been below the $30\text{-}\mu^3$ threshold. If so, then MCV values reported in this study may be inflated and red cell counts may be underestimated.

Although means and standard error of the means presented in Table 1 may be considered as "normals" for black bears in northeastern Pennsylvania, they are not conclusive. Adequate midwinter and midsummer samples are needed to provide a better understanding of seasonal changes and, in general, larger samples sizes are needed for better representation of all age- and sex-classes. Also, most of the sampling reported here occurred during 1 year, which may not have been a "normal" year, as heavy defoliation of the study area by gypsy moth (*Porthetria dispar*) and mast failure during that year may have affected the food habits and behavior of the bears.

Future studies of blood profiles of black bears should be very critical of the techniques to be used in collecting, handling, and analyzing the samples. In particular, the large number of blood parameters demonstrating individual variability in this study (Table 2) suggests that a concerted effort should be made to obtain repetitive samples from the same animal, including multiple samples taken during a single handling. The resulting variability should then be considered when evaluating the data for sex, age, seasonal, or other effects.

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