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HEMATOLOGICAL VALUES FOR FOUR SPECIES OF BIRDS OF PREY[1]

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Hematological and blood chemistry values can be obtained easily and are useful in determining the health or general condition of birds (Cooper et al. 1986). To rehabilitate individual raptors or to breed and release endangered birds-of-prey, a knowledge of their normal concentrations of blood constituents is of paramount importance (Lepoutre et al. 1983).

While a few hematological values in raptors have been published (Elliot et al. 1974, Cooper 1975, Balasch et al. 1976, Smith and Bush 1978, Hunter and Powers 1980, Gee et al. 1981, Leonard 1982, Lepoutre et al. 1983, Ferrer et al. 1987), data for many parameters such as white blood cells (WBC) counts, proteins and most of the other blood chemistries are scarce or nonexistent for many birds-of-prey. Such parameters are clinically important for diagnosing and monitoring avian medical problems (Leonard 1982, Cooper et al. 1986). Our objective was to provide baseline data on the blood characteristics of four species of captive and protected European raptors. They include *Aquila adalberti* (Spanish Imperial Eagle), *Aquila chrysaetos* (Golden Eagle), *Neophron pernopterus* (Egyptian Vulture), and *Gyps fulvus* (Griffon Vulture). The Spanish Imperial Eagle inhabits the Iberian Peninsula exclusively and is in acute danger of extinction. Fewer than 40 pairs exist. The Golden Eagle and the Griffon Vulture are rare, and the Egyptian Vulture is a vulnerable species that could become endangered if real protection is not afforded by the Government Agency for the Conservation of Nature (I.C.O.N.A. 1986). Due to the precarious status of these species, raptor recuperation centers and zoological institutions are trying to save them from extinction. A hematological and clinical chemical "screening" is recommended in veterinary diagnosis for all captive-breeding projects and, when possible, for the populations of vulnerable species (Cooper et al. 1986).

METHODS AND MATERIALS

Blood samples of Golden Eagle (*Aquila chrysaetos*), Egyptian Vulture (*Neophron percnopterus*), and Griffon Vulture (*Gyps fulvus*), as well as three blood samples of the Spanish Imperial Eagle (*Aquila adalberti*) were obtained from birds at the Barcelona Zoo (Spain). Nine Imperial Eagles came from the Raptor Recuperation Center in Madrid (Spain). All animals were adults, between 4 and 20 years old, with no clinical signs of disease. The gender of all the species was unknown, with the exception of the Imperial Eagle, with three females and nine males. Due to the low number of females, all animals were grouped together. The diet of the eagles and vultures was freshly killed rabbits. Vultures were also fed some horse meat. To obtain blood samples, the birds were anesthetized with isofluorane by means of a mask and then were placed on their back and their feathers removed from the medial aspect of the elbow to expose the brachial vein. Sampling was done during November and December 1990, at the same hour (10:00 to 11:00) to reduce diurnal variation in blood parameters (Dolnik 1973), and always at least 12 hours after the last feeding. Blood samples were drawn with a syringe containing lithium heparin. A small blood sample was separated and hematological analyses were completed within 2 hr after blood withdrawal. The cellular portion of the blood was removed by centrifugation and the plasma was immediately frozen at −28°C. Biochemical analyses were subsequently conducted. All analyses were done in duplicate.

Hematocrit was determined by centrifuging a heparinized capillary tube of blood for 6 min at 11,500 rpm. Hemoglobin concentration was measured by adding 10 μl of well-mixed blood to 5 ml of Drabkin's reagent (Drabkin and Austin 1935). After 10 min, this sample was centrifuged at 2,500 rpm for 5 min to avoid interference of the lysed nuclei during determination of optical density, which was measured in a spectrophotometer at 540 nm. The erythrocyte number (RBC) was counted in a Thoma's hemocytometer after the sample was diluted (1:200) in saline solution. The leukocyte count (WBC) was determined on 1:50 diluted blood by means of a Neubauer's hemocytometer using Natt and Herrick dilution fluid (1952). We kept fresh blood on ice (2 hr old at the most) to avoid aggregation of white cells. The hematimetric indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the RBC count, hematocrit and hemoglobin concentration. Average RBC and nucleus length and width were measured from selected smears. These were fixed with methanol and stained with May-Grünwald and Giemsa solutions, as described by Lucas and Jamroz (1961), and then used for a differential count of the white blood cells. Between 400 and 500 white cells were counted in each sample. The red blood cells were examined at 1,000× and their dimensions estimated with a calibrated eyepiece. Fifty erythrocytes were measured on

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TABLE 1. Measurements of erythrocytes in the Spanish Imperial Eagle, Golden Eagle, Egyptian Vulture and Griffon Vulture (mean ± standard deviation and range). C: Cell; N: Nucleus.

	Imperial Eagle	Golden Eagle	Griffon Vulture	Egyptian Vulture
Number	12	5	12	5
C. length (μm)	14.89 ± 0.98	14.69 ± 0.88	14.08 ± 1.14	15.07 ± 1.04
Range	12.6–16.7	12.8–17.0	11.0–16.5	13.0–17.5
C. width (μm)	7.96 ± 0.56	8.18 ± 0.77	7.69 ± 0.78	7.93 ± 0.57
Range	7.0–9.5	6.6–9.8	6.2–9.0	7.0–9.5
C. length/C. width	1.88 ± 0.18	1.81 ± 0.20	1.84 ± 0.13	1.91 ± 0.19
Range	1.5–2.3	1.5–2.3	1.6–2.1	1.6–2.3
N. length (μm)	7.38 ± 0.83	6.76 ± 0.75	6.82 ± 0.45	6.72 ± 0.80
Range	6.1–9.7	5.0–8.6	5.9–7.5	5.0–8.6
N. width (μm)	2.64 ± 0.33	2.68 ± 0.24	2.55 ± 0.33	2.45 ± 0.41
Range	2.0–3.5	2.3–3.3	2.0–3.3	1.7–3.5
N. length/N. width	2.84 ± 0.52	2.55 ± 0.39	2.71 ± 0.38	2.81 ± 0.54
Range	1.9–4.0	1.7–3.3	2.1–3.7	2.0–4.5

smears selected for excellence of staining and cell morphology. Ratios of maximum length to width were calculated as an index of the deviation of RBCs from a spherical shape.

Glucose (Glucofix Menagent, Menarini, Italia), urea (Urea HF Menagent, Menarini), uric acid (Uric acid HF Menagent, Menarini), cholesterol (Cholesterol HF Menagent, Menarini), creatinine (3385 Merckotest, Merck, Federal Republic of Germany), serum aspartate aminotransferase (AST) (3397 Merckotest, Merck), serum alanine aminotransferase (ALT) (3398 Merckotest, Merck), alkaline phosphatase (AP) (Alkaline Phosphatase Menagent, Menarini), creatinine phosphokinase (CPK) (14328 Merck-1-Test, Merck), lactic dehydrogenase (LDH) (3349 Merck-1-Test, Merck), and gamma glutamyl transpeptidase (γ-GT) (12189 Merck-1-test, Merck) and chloride (Chlorofix Menagent, Menarini), were determined using specific commercial kits. Total plasma proteins (TP) were measured using the Lowry technique (Lowry et al. 1951) and the different plasma proteins by cellulose acetate electrophoresis. The fractions were estimated quantitatively by means of densitometric curves (Cellogel; using automatic lector Cellomatic C.G.A. Atom). Sodium and potassium were analyzed by flame emission spectrophotometry and calcium, magnesium and total phosphorus by inductively coupled plasma (ICP spectrophotometer Jobin Yvon-JY-38 VHR).

Mean values were compared using Student's *t*-test. Statistical significance was always considered to be $P < 0.05$.

RESULTS

Tables 1, 2, 3 and 4 summarize the mean, standard deviation and range of hematologic and biochemical values of the Imperial and Golden Eagle and the Griffon and Egyptian Vultures. The small number of Golden Eagles and Egyptian Vultures analyzed is not sufficient to draw conclusions but the data are included because of (1) the rarity of the animals, (2) the impossibility of obtaining more samples and (3) the interest in these hematologic and biochemical values among people working for the protection of these species.

There was a considerable range in the values of some parameters including the white blood cells, especially lymphocyte and heterophil numbers, urea, uric acid, LDH, CPK, AP, AST and ALT. However, significant differences ($P < 0.05$) were found between the two more numerous species, the Imperial Eagle and the Griffon Vulture, only for monocytes, percentage of eosinophils, glucose, triglycerides, albumin, γ-globulin, calcium, phosphorus and magnesium.

DISCUSSION

The levels of hemoglobin, hematocrit, red blood cells and the hematimetric indices were similar among the four species studied and compared to those levels reported for others birds-of-prey (Elliot et al. 1974, Cooper 1975, Balasch et al. 1976, Smith and Bush 1978, Gee et al. 1981, Leonard 1982, Lepoutre et al. 1983, Ferrer et al. 1987). For example, the hematocrit was within the range of 39–44% reported for healthy raptors (Rehder et al. 1982). Some authors described higher hematocrits for raptors which, according to them, could be due to differences in age (Gessaman et al. 1986) or season (Hunter and Powers 1980). Also, the higher hematocrit may be a normal value for strong fliers (Carpenter 1975).

Erythrocyte dimensions were uniform in the four species analyzed, but were slightly larger than those of most other avian species (Hartman and Lessler 1963; Palomeque and Planas 1977, 1981). Other authors (Elliot et al. 1974) observed that the number of erythrocytes in falconiforms is at the lower end of the range of 1.9 to 5.0 (10^{12}/l) for birds in general (Jones and Johansen, 1972) and concluded that with a smaller number of red cells and similar hematocrits, the erythrocyte size of birds-of-prey must be larger. Although erythrocyte dimensions are very homogeneous among birds, particularly when compared with other verte-

TABLE 2. Hematologic values in the Spanish Imperial Eagle, Golden Eagle, Egyptian Vulture and Griffon Vulture (mean ± standard deviation and range).

Test	Imperial Eagle $n = 12$		Golden Eagle $n = 5$		Griffon Vulture $n = 12$		Egyptian Vulture $n = 5$	
	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range
PCV (%)	43.7 ± 3.6	37.0–49.5	42.1 ± 3.7	38.0–46.0	45.5 ± 3.4	40.0–51.8	42.5 ± 3.6	38.0–48.0
Hb (g/100 ml)	13.6 ± 1.4	13.5–16.2	13.8 ± 1.3	12.5–15.2	15.1 ± 1.9	12.4–18.2	13.7 ± 1.2	12.4–15.5
RBC (10^{12}/l)	2.41 ± 0.15	2.20–2.74	2.56 ± 0.54	1.96–3.22	2.63 ± 0.32	2.19–2.87	2.30 ± 0.44	1.94–2.94
MCV (μm^3)	179.5 ± 10.5	157.0–190.7	168.2 ± 18.6	150.9–187.8	170.0 ± 15.5	150.9–189.3	187.7 ± 21.7	163.3–212.6
MCH (pg/cell)	56.7 ± 4.2	49.0–63.2	44.0 ± 12.7	47.1–63.7	55.3 ± 6.7	46.8–66.6	60.2 ± 6.3	52.6–67.3
MCHC (g/100 ml)	31.3 ± 1.4	29.3–33.3	32.8 ± 0.3	32.7–33.1	32.3 ± 2.5	29.7–36.6	32.2 ± 0.4	31.6–32.6
WBC (10^9/l)	14.87 ± 6.30	3.38–25.13	12.32 ± 7.95	5.87–24.00	13.19 ± 7.32	5.00–24.00	16.03 ± 4.19	10.63–21.88
Heterophils (10^9/l)	10.57 ± 5.03	6.14–18.59	3.87 ± 1.98	2.88–5.99	7.04 ± 5.02	1.95–16.56	11.95 ± 2.20	9.79–14.88
Heterophils (%)	60.57 ± 13.10	37.96–82.61	58.83 ± 9.75	49.00–68.50	57.83 ± 9.87	51.00–72.00	69.75 ± 13.50	58.00–64.00
Lymphocytes (10^9/l)	4.80 ± 2.07	2.93–9.17	2.29 ± 0.11	2.22–2.41	5.02 ± 2.70	1.92–9.00	4.90 ± 2.47	1.24–6.41
Lymphocytes (%)	30.79 ± 11.27	13.04–52.78	34.17 ± 5.80	27.5–38.00	40.67 ± 10.52	24.00–49.00	27.25 ± 12.70	9.00–38.00
Monocytes (10^9/l)	0.29 ± 0.17	0.00–0.67	0.27 ± 0.23	0.09–0.53	0.06 ± 0.07	0.00–0.20	0.08 ± 0.09	0.00–0.17
Monocytes (%)	1.28 ± 0.71	0.00–2.65	4.34 ± 4.16	0.00–9.00	0.57 ± 0.53	0.00–1.00	0.50 ± 0.58	0.00–1.00
Eosinophils (10^9/l)	1.08 ± 0.36	0.60–1.69	0.17 ± 0.10	0.06–0.26	0.51 ± 1.19	0.00–3.20	0.36 ± 0.16	0.14–0.51
Eosinophils (%)	6.94 ± 2.92	3.48–11.29	2.33 ± 1.15	1.00–3.00	0.86 ± 1.57	0.00–4.00	2.00 ± 0.82	1.00–3.00
Basophils (10^9/l)	0.00	0.00	0.20 ± 0.03	0.00–0.06	0.00	0.00	0.09 ± 0.11	0.00–0.22
Basophils (%)	0.00	0.00	0.20 ± 0.45	0.00–1.00	0.00	0.00	0.40 ± 0.54	0.00–1.00

TABLE 3. Blood chemistry values in the Spanish Imperial Eagle, Golden Eagle, Egyptian Vulture and Griffon Vulture (mean ± standard deviation and range).

Test	Imperial Eagle $n = 12$		Golden Eagle $n = 5$		Griffon Vulture $n = 12$		Egyptian Vulture $n = 5$	
	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range
Glucose (mmol/l)	19.5 ± 3.1	13.6–25.6	16.8 ± 1.6	14.5–18.4	16.2 ± 2.5	14.2–22.2	17.7 ± 4.2	14.2–24.9
Urea (μmol/l)	1,663 ± 618	813–3130	1,800 ± 1,100	650–2,983	1,315 ± 755	535–2,825	1,117 ± 717	717–1,967
Uric acid (μmol/l)	223 ± 114	96–451	345 ± 147	161–518	328 ± 167	167–678	230 ± 159	159–405
Cholesterol (mmol/l)	5.8 ± 0.7	4.7–6.8	4.8 ± 0.3	4.3–5.0	5.1 ± 1.1	3.1–7.1	7.9 ± 0.8	6.4–8.7
Triglycerides (mmol/l)	0.75 ± 0.18	0.51–1.19	0.64 ± 0.17	0.55–0.84	1.10 ± 0.47	0.56–1.90	1.33 ± 0.43	0.75–1.86
Creatinine (μmol/l)	81.4 ± 29.2	35.4–107.1	61.9 ± 16.8	37.2–83.2	65.5 ± 40.7	30.9–86.7	44.3 ± 26.6	19.5–88.5
LDH (IU/l)	394 ± 113	271–586	471 ± 132	374–683	254 ± 153	103–586	629 ± 398	505–1,073
AST (IU/l)	93.1 ± 32.8	47.3–159.7	62.6 ± 7.2	55.3–71.4	118.5 ± 25.7	93.5–156.8	64.9 ± 7.2	58.9–77.1
ALT (IU/l)	12.1 ± 3.6	6.0–17.1	13.9 ± 4.4	9.1–19.0	11.4 ± 6.1	3.9–19.8	6.6 ± 4.9	1.97–14.6
CPK (IU/l)	213 ± 116	99–449	150 ± 73	62–259	274 ± 251	113–774	401 ± 47	346–464
AP (IU/l)	91.7 ± 54.3	45.3–237.2	86.2 ± 62.7	24.9–178.6	108.1 ± 114.3	30–354.4	65.8 ± 24.5	47.8–108.9
γ-GT (IU/l)	0		0		0		0	

TABLE 4. Proteins, total phosphorus and inorganic chemicals in blood plasma of Spanish Imperial Eagle, Golden Eagle, Egyptian Vulture and Griffon Vulture (mean ± standard deviation and range).

Test	Imperial Eagle $n = 12$		Golden Eagle $n = 5$		Griffon Vulture $n = 7$		Egyptian Vulture $n = 5$	
	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range
Proteins (g/l)	38 ± 4	33–49	33 ± 2	32–36	39 ± 8	30–51	39 ± 7	31–48
Pre-albumin (g/l)	7.1 ± 1.8	3.7–8.9	5.1 ± 0.7	4.5–5.8	1.7 ± 2.4	0.0–5.3	4.5 ± 1.5	3.3–6.5
Albumin (g/l)	10.7 ± 2.5	7.4–13.9	9.5 ± 0.8	8.6–10.0	17.7 ± 4.4	13.0–22.3	15.3 ± 1.4	9.9–19.7
α-Globulin (g/l)	7.1 ± 3.1	4.3–13.8	4.5 ± 1.2	3.6–5.9	8.3 ± 2.5	5.8–12.5	8.9 ± 3.6	5.0–13.7
β-Globulin (g/l)	9.2 ± 3.0	6.4–14.9	10.3 ± 1.39	9.0–11.5	11.7 ± 4.0	8.3–18.3	5.5 ± 1.4	4.0–7.3
γ-Globulin (g/l)	2.9 ± 0.4	2.3–3.0	2.3 ± 0.4	2.0–2.7	5.8 ± 3.4	1.8–9.9	6.0 ± 1.4	4.3–7.7
Albumin/Globulin	0.97 ± 0.28	0.56–1.39	0.84 ± 0.08	0.78–0.89	0.76 ± 0.29	0.52–1.16	0.87 ± 0.29	0.61–1.29
Sodium (mmol/l)	159.5 ± 4.6	155–166	162 ± 7.8	153–170	154.9 ± 6.7	144–167	151.6 ± 4.7	148–157
Potassium (mmol/l)	1.8 ± 0.4	1.2–2.9	2.2 ± 0.9	1.4–3.2	2.1 ± 0.9	0.8–3.4	1.6 ± 0.3	1.5–2.00
Chloride (mmol/l)	104.3 ± 5.5	96.6–111.5	107.4 ± 4.0	102.4–112.0	98.2 ± 12.2	85.5–114.3	100.5 ± 4.5	97.0–108.3
Calcium (mmol/l)	2.2 ± 0.1	2.0–2.4	2.7 ± 0.2	2.6–2.9	3.5 ± 0.9	2.4–4.7	2.7 ± 0.4	2.5–3.2
Phosphorus (mmol/l)	5.8 ± 0.5	4.1–6.4	4.7 ± 0.4	4.3–5.1	4.3 ± 1.1	2.8–6.4	7.3 ± 1.6	4.9–8.5
Magnesium (mmol/l)	0.67 ± 0.04	0.63–0.79	0.88 ± 0.21	0.67–1.13	0.79 ± 0.13	0.71–1.08	0.75 ± 0.08	0.63–0.79
Osmolality (mOsm/Kg)	317 ± 11	299–327	309 ± 5	302–314	307 ± 4	302–313	306 ± 6	296–311

brate classes (Hartman and Lessler, 1964), it is improbable that the range of differences in size would confer any physiological advantage for gas exchange.

Total number of leukocytes in the Golden Eagle and the Egyptian and Griffon Vultures were lower than reported by Leonard (1982) in these birds and in others birds of prey (Elliot et al. 1974, Leonard 1982, Lepoutre et al. 1983), although some authors (Smith and Bush 1978, Cooper et al. 1986) report values similar to ours in other species of falconiforms. Nevertheless, there is considerable variation in the leukocyte count (Elliot et al. 1974, Leonard 1982, Lepoutre et al. 1983) that may be due to different methodologies or to disease. The proportion of heterophils to lymphocytes was higher in the four species we studied and also in two species of vultures and the Golden Eagle (Leonard 1982) than in other species. Although the same type of predominant leukocyte does not always appear in raptors, some authors described the heterophils as the more numerous leukocytes in some species and not in others (Leonard 1982, Lepoutre et al. 1983, Cooper et al. 1986). Leonard (1982) and Lepoutre et al. (1983) reported that about half of the birds-of-prey considered had a higher percentage of lymphocytes or physiological lymphocytosis. The other types of leukocytes had slightly lower values in percentage and absolute number than for some other birds of prey, although the variation is also very wide (Leonard 1982).

Total protein levels were within the normal range of 3 to 5 g/dl for most birds (Elliot et al. 1974, Smith and Bush 1978, Gee et al. 1981, Lepoutre et al. 1983, Garcia-Rodriguez et al. 1987, Ferrer et al. 1987, Coleman et al. 1988). However, Cooper et al. (1986) found lower values in the Mauritius Kestrels (*Falco punctatus*). An albumin/globulin ratio of less than one indicates that globulins are the largest protein. This is true in other raptors (Balasch et al. 1974, Gee et al. 1981, Lepoutre et al. 1983, Ferrer et al. 1987). Since age, seasonal changes, diet hydration and captive status affect plasma proteins (Lewandowski 1986), it is very important to consider the physiological status or condition of the bird at the time of sampling.

Glucose concentrations in the Imperial Eagle and Griffon Vulture were similar to those previously published (Ferrer et al. 1987) and was in the general range described for raptors (Gee et al. 1981, Ferrer et al. 1987, Garcia-Rodriguez et al. 1987, Minick and Duke 1991). However, it was higher than in many other types of birds (Gee et al. 1981, Leonard 1982). Wild birds tend to have lower plasma glucose than captive birds (Lewandowski et al. 1986).

Blood urea values in the Imperial Eagle and Griffon Vulture were similar to those reported by Ferrer et al. (1987) and values of all the species analyzed were similar to those of other birds-of-prey (Balasch et al. 1976, Gee et al. 1981; Cooper et al. 1986, Ferrer et al. 1987, Lumeij and Remple 1991). However, raptors in general show higher values than other orders of birds (Gee et al. 1981). High blood urea levels may result from ingesting large quantities of animal protein, which is the main component of the raptor's diet (Sturkie 1965).

The uric acid levels of the animals in this study were lower (Gee et al. 1981, Garcia-Rodriguez et al. 1987) or similar (Lumeij and Remple 1991) to published levels of other birds of prey but were in the same range as other types of birds (Leonard 1982, Lewandowski et al. 1986). Our findings do not support Bell's and Sturkie's (1965) hypothesis that high levels of uric acid occur in birds on rich protein diets.

Cholesterol and creatinine concentrations were similar to the average values in other falconiforms and other types of birds (Gee et al. 1981, Ferrer et al. 1987, Garcia-Rodriguez et al. 1987, Gee et al. 1981). However, triglyceride concentrations in this study were lower than the values for other raptors (Garcia-Rodriguez et al. 1987, Gee et al. 1981). All these variables are influenced by diet. Serum cholesterol increases in birds fed a diet rich in fat and low in protein (Perry et al. 1986, Peinado et al. 1992).

LDH, AST, ALT, CPK and AP plasma enzymes were within the range of activity of other birds-of-prey (Gee et al. 1981, Cooper et al. 1986), although with a very wide range of variation. Interpretation of the interspecific variations in the activity values of the plasma enzymes is difficult since their distribution in avian tissues varies among the species (Lewandowski et al. 1986). Some of these enzymes usually increase in sick birds, especially AST, ALT, AP, γ-GT and CPK and are commonly used in veterinary diagnosis (Lewandowski et al. 1986). Nevertheless, there are some other methodological factors which can influence the enzymatic values such as AST in skeletal muscle injury (Woerpel and Rosskopf 1984), CPK by capture and restraint of the animal, LDH with hemolisis, AP with fracture repair, etc. Contrary to Gee et al. (1981), who found a low enzymatic activity in gamma glutamyl transpeptidase in blood, no significant quantities of this plasma enzyme were detected in all the birds analyzed.

Sodium, potassium and chloride concentrations also fell within the range for other species of falconiform birds (Gee et al. 1981, Lepoutre et al. 1983). Although some potassium values reported by others are higher than ours, this could be the consequence of a slight hemolysis or failure to remove plasma from the red blood cells because erythrocytes contain 23 times as much potassium as plasma (Lewandowski et al. 1986). Calcium and magnesium also are similar to values of other raptors (Gee et al. 1981, Lepoutre et al. 1983, Cooper et al. 1986, Ferrer et al. 1987, Garcia-Rodriguez et al. 1987, Viñuela et al. 1991, Rehder et al. 1982). No values have been previously published for total phosphorus.

Many blood values determined in our study were within the range of variation reported for different species of raptors in the literature (e.g., Gee et al. 1981, Leonard 1982). Some of those values are also similar to birds in general (Leonard 1982, Lewandowski et al. 1986), although for some parameters such as urea and erythrocyte size, the birds-of-prey differ from other birds.

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TEMPORAL RELATIONSHIPS IN WHITE-THROATED SPARROW SONG

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Bird song has been likened to human music for centuries (e.g., Anonymous 1717, Hartshorne 1973). The analogy between bird song and music is perpetrated to some degree by the use of musical terms to describe avian vocalizations: individual sound elements are called "notes," and strings of notes are called "songs." In most modern studies of song this analogy has fallen out of favor (e.g., Dobson and Lemon 1977). However, recent studies have shown that fixed relationships between the frequencies of adjacent notes, a defining feature of human music, occur in the songs of several species, and the birds seem to use this feature to recognize their songs (e.g., see Weary et al. 1991).

The aim of the present study was to determine if there are other predictable relationships between notes. In particular we investigated whether the duration of song notes sung by White-throated Sparrows (*Zonotrichia albicollis*) are correlated. When an individual produces a relatively long first note, do the subsequent notes tend to be longer? These temporal relationships between notes are termed rhythm when describing music. The familiar tune is still recognized when it is sped up or slowed down (changes in tempo), because the temporal relationships between the notes (and internote intervals) are maintained.

The only formal study of rhythm perception in nonhuman animals was done in the laboratory on European Starlings (*Sturnus vulgaris*) (Hulse et al. 1984). This study showed that birds can learn to discriminate rhythmic from arrhythmic sound patterns, and can generalize this discrimination over changes in tempo. How important are the temporal relationships among sounds in nature? For birds, there is some evidence that these relationships are important. Becker's (1982) review pointed to several temporal features of song that are important in species recognition. For example, the manipulation of the intervals between notes in a song affects recognition in several species, including White-throated Sparrows (Falls 1963). Also, interspecific comparisons have demonstrated that birds which sing longer songs also tend to use greater intervals to separate them (Dobson and Lemon 1975, Weary and Lemon 1988). Thus, certain aspects of timing do seem important in bird song.

Other evidence is harder to interpret. For example, Lambrechts and Dhondt (1987) found that during the course of a song, Great Tits (*Parus major*) tend to sing with progressively longer intervals between notes. The rhythmic aspect of this behavior is that when Great Tits produce longer inter-note intervals, they also produce longer inter-phrase intervals (phrases consist of groups of notes). The arrhythmic aspect is that there is no corresponding increase in the duration of the notes themselves. Thus, in one respect Great Tits maintain rhythm over a change in tempo, but in another they do not.

METHODS

White-throated Sparrows sing two types of songs, each composed of, on average, five notes (Fig. 1). Most common is the ascending song in which there is an increase in frequency between the first and second notes. Less common is the descending song where the major change in frequency is a decrease between the second and third notes (Borror and Gunn 1965). Each of the songs we considered fell unambiguously into one of these two categories. For ascending (56 birds) and descending (20 birds) songs, we measured the duration of the first three notes, as well as the duration of the silent interval between the first and second notes and between the second and third notes. Because some subjects sang songs composed of only three notes, this was the maximum number which we could consider in our analysis. Three songs were recorded from each bird. Values from the three songs per bird were averaged, and this mean value was then used in all analyses. Birds were recorded

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