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CLINICAL HEMATOLOGY AND BLOOD CHEMISTRY VALUES FOR THE COMMON BUZZARD (*Buteo buteo*)

MAURO HERNANDEZ, SONSOLES MARTIN AND PALOMA FORES
Departamento de Patología Animal II, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain

ABSTRACT.—Clinical hematology and serum chemistry values for 23 clinically normal Common Buzzards (*Buteo buteo*) have provided reference values for use in clinical pathology. Hematological values, including red and white blood cell counts, hematocrit, hemoglobin concentration, leukocyte differential count and red cell indices were established. Reference values of blood chemical parameters, including total plasma protein, total plasma solids, fibrinogen, glucose, aspartate aminotransferase (AST, SGOT), alanine aminotransferase (ALT, SGPT), gamma glutamyltransferase (γ-GT), creatine kinase (CK), lactate dehydrogenase (LDH), creatinine, uric acid, calcium and phosphorus were also established and compared to results obtained by other authors.

Valores de hematología y bioguímica sanguínea de ratoneros comunes (*Buteo buteo*)

EXTRACTO.—Los valores de hematología y bioquímica sanguínea de 23 ratoneros comunes (*Buteo buteo*) sanos proporcionaron valores de referencia para su uso rutinario en patología clínica. Los valores de hematología que se determinaron fueron: recuento de eritrocitos y leucocitos, hematócrito, concentración de hemoglobina, recuento diferencial de leucocitos e índices eritrocitarios. Los valores de bioquímica sanguínea determinados fueron: proteínas plasmáticas totales, sólidos plasmáticos totales, fibrinógeno, glucosa, aspartato aminotransferasa (AST, SGOT), alanino aminotransferasa (ALT, SGPT), gamma glutamil transferasa (γ-GT), creatina quinasa (CK), lactato deshidrogenasa (LDH), ácido úrico, creatinina, calcio y fósforo. Los resultados obtenidos se discuten con los de otros autores.

Clinical hematology and blood chemistry are useful diagnostic tools (Woerpel and Rosskopf 1984, Campbell and Dein 1984, Campbell 1988). In recent years, normal hematologic values for both pet and wild birds (Hawkey et al. 1983, 1985, Lumeij and de Bruijne 1985) and hematological changes in response to disease (Hawkey et al. 1984, O'Halloran et al. 1988) have been reported. Tissue enzyme profiles for some avian species have also been established (Lumeij and Wolfswinkel 1988, Lumeij et al. 1988a) and changes in plasma levels of selected enzymes as a result of disease have been reviewed (Lumeij et al. 1988b, Lumeij and Westerhof 1987).

Nevertheless, such tests are of limited clinical usefulness in raptors because of the lack of data for reference values in many species (Redig 1978) and the limited number of parameters and individuals for those species that have been tested (Cooper 1972, Elliot et al. 1974, Cooper 1975, Redig 1978, Kirkwood et al. 1979, Halliwell 1981, Ivins et al. 1985, Hawkey and Hart 1988).

The Common Buzzard (*Buteo buteo*) is one of the most frequently injured birds of prey in Spain. Data from physiological values of selected hematologic tests could be of great value for both diagnostic and prognostic purposes. Nevertheless, very few data have been found through the literature on hematologic values for this species (Veil 1978, Lepoutre 1982, Lepoutre et al. 1983) and no information is available on the normal plasma concentrations of enzymes commonly used in avian medicine. This paper deals with the normal clinical hematology and blood chemistry values for the Common Buzzard and their application as diagnostic aids in clinical practice.

METHODS

Blood samples were collected from 23 normal adult Common Buzzards housed in outdoor enclosures at the Centro de Especies Protegidas de Buitrago, belonging to the Comunidad Autonoma de Madrid.

Thirteen of the birds were imprinted adults of unknown age. Time spent in the Center ranged from 6 months to 2 years. Ten additional birds were illegally taken as nestlings, confiscated by the authorities and kept in the Center until their release into the wild some weeks after sampling Three of these birds were 1 year old and the remaining seven birds were 2 years old.

Birds were housed in 15 × 7 × 3 or 10 × 5 × 3 meter

Table 1. Hematological values for captive Common Buzzards. The number of samples (N), mean value ($\bar{X}$), standard deviation (SD) and observed range are given for each parameter.

PARAMETER	N	$\bar{X}$	SD	RANGE
TRBC ($\times 10^6/\mu l$)	22	2.94	0.82	5.44–2.06
TWBC ($\times 10^3/\mu l$)	23	8.04	1.77	10.6–4.6
PCV (%)	22	40.8	4.4	49–36
Hemoglobin (g/dl)	22	12.7	2.4	17.7–9.3
Heterophils (%)	23	63	13.1	75–45
Band (%)	23	0	0	—
Lymphocytes (%)	23	20	9.5	48–10
Eosinophils (%)	23	16	13.8	37–0
Monocytes (%)	23	0	1.0	4–0
Basophils (%)	23	0	0.7	3–0
Heterophils ($\times 10^3/\mu l$)	23	4.58	1.2	5.8–2.3
Band ($\times 10^3/\mu l$)	23	0	0	0
Lymphocytes ($\times 10^3/\mu l$)	23	1.4	0.73	2.9–0.2
Eosinophils ($\times 10^3/\mu l$)	23	1.2	1.1	3.4–0
Monocytes ($\times 10^3/\mu l$)	23	0.05	0.08	0.3–0
Basophils ($\times 10^3/\mu l$)	23	0.6	0.1	0.2–0
MCV (fl)	22	145.1	25.0	171.4–90.0
MCH (pg)	22	48.3	10.2	65.7–32.5
MCHC (g/dl)	22	32.4	6.7	45.3–22.6

pens with sides and top covered by wire netting and equipped with wooden perches. No birds were known to be sick or seriously injured during their stay in the Center. Birds with leg or wing injuries, skin wounds, bumblefoot and any other disease, or birds showing unusual behavior were not considered for the study. The buzzards were fed once a day either with chicken carcasses or day-old chicks and water was provided *ad libitum*.

Blood samples were taken in May, June and July 1989 at the same time each day (1100–1230 H) to eliminate diurnal fluctuations. Birds were always bled before being fed. Birds were physically restrained with the aid of a falconer's hood and a towel. Blood was taken by venipuncture from the basilic vein using disposable 23-gauge needles and 2 ml plastic syringes. After removing the needle, 1 ml of whole blood for hematology was placed in a commercially available plastic tube containing EDTA. The remaining blood for blood chemistry determinations was placed in another plastic tube containing 5% lithium heparin. Only one sample was taken per bird. The blood was tested within 3 hr of collection.

Total red and white blood cell counts (TRBC and TWBC) were performed with the Natt-Herrick solution (1:200 dilution) and Neubauer hemocytometer (Campbell 1988). The hematocrit value (PCV) was obtained by the standard microhematocrit method. Hemoglobin concentration was estimated in duplicate by the cyanomethemoglobin method and red cell indices (MCV, MCH and MCHC) were calculated by using the standard formulas (Campbell 1988). The leukocyte differential count was performed by the routine microscopic procedure in a smear stained with May-Grünwald Giemsa (Hawkey et al. 1983).

Polychromasia and anisocytosis were estimated by examining blood smears in order to assess erythropoietic activity. Biochemical methods used in blood chemistry determinations are summarized in the Appendix 1.

RESULTS

The values obtained are presented in Table 1 (hematology) and in Table 2 (blood chemistry). No distinction was made by sex, age, origin of the bird or the length of time in captivity. A slight anisocytosis and a certain number of polychromatic erythrocytes (usually a mean of 3% of the erythrocytes in one 100 × oil field) were regarded as normal. The tendency of thrombocytes to clump precluded the use of the Neubauer hemocytometer for counting them and thus their number, estimated in a smear, was reported as "decreased," "normal" (about a mean of 2 thrombocytes per 100X oil field of good cellularity) or "increased." White cell morphology in the Common Buzzard was similar to that described by Hawkey et al. (1983), Campbell (1988) and Hawkey and Dennet (1989) for other birds.

Normal plasma color varied from clear to yellow. No hemolysis due to the EDTA was found in the samples as has been reported in other bird species (Hawkey et al. 1983, Campbell 1988). Total plasma solids (TPS) values obtained by the refractometric

Table 2. Blood chemistry values for captive Common Buzzards.

PARAMETER	N	$\bar{X}$	SD	RANGE
TPS (g/dl)	21	4.6	0.8	5.89–2.3
TPP (g/dl)	21	3.1	1.5	5.3–1.1
Fibrinogen (g/dl)	21	1.5	0.8	2.9–0.3
Glucose (mg/dl)	20	301.1	53.1	370–173
AST (SGOT) (IU/liter)	21	227.7	155.5	365–66.9
ALT (SGPT) (IU/liter)	18	13.1	5.9	28.9–5.17
γ-GT (IU/liter)	18	3.5	0.7	5.1–<2.8
CK (IU/liter)	20	393.2	187.8	766–119
LDH (IU/liter)	14	631.5	153.0	820–300
Uric acid (mg/dl)	20	6.0	1.5	8.5–2.89
Creatinine (mg/dl)	19	0.9	0.3	1.4–<0.5
Calcium (mg/dl)	16	11.2	2.5	16.6–7.9
Phosphorus (mg/dl)	10	4.6	3.3	9.4–1.3

method were found higher than total plasma protein (TPP) values obtained by the Biuret method.

DISCUSSION

Total red blood cell counts (TRBC), hemoglobin concentration and PCV values were found similar to those described by other authors for the Common Buzzard (Leonard 1969, Veil 1978, Lepoutre 1982, Lepoutre et al. 1983), and for other raptor species (Cooper 1972, Balasch et al. 1973, Elliot et al. 1974, Cooper 1975, Redig 1978, Kirkwood et al. 1979, Gee et al. 1981).

The number of circulating erythrocytes, and thus values of TRBC, are influenced by a number of factors such as sex, age, and altitude (Leonard 1969, Stoskopf et al. 1983, Campbell and Dein 1984, Amand 1985). Nevertheless, in our study the wide range of variation of TRBC values may be due to the technical error inherent in the hemocytometer method (Steel et al. 1977, Smith and Lience 1977). The use of an electronic particle counter could minimize this error. PCV values are less subject to technical error and therefore are of greater clinical value.

Additionally, the PCV in conjunction with total plasma protein (TPP) was the easiest and least time-consuming means of assessing the hydration and anemic status of a bird (Campbell and Dein 1984, Jenkins 1987).

No reticulocyte counts were performed since significance of high reticulocyte numbers in bird circulating blood remains unknown (Hawkey et al. 1983). On the other hand, erythropoietic activity could be assessed estimating in the smear the number of polychromatic erythrocytes. These cells, unlike the mature erythrocytes, appear more rounded and have more basophilic cytoplasm (Campbell and Dein 1984).

The cyanomethemoglobin method is the most accurate method for estimating the hemoglobin concentration in both mammalian and avian blood (Smith and Lience 1977, Amand 1985). Since the red cell indices (MCV, MCH and MCHC) are calculated from the PCV, hemoglobin concentration and TRBC, their validity is influenced by the accuracy of the TRBC, the hemoglobin determination and PCV value (Amand 1985). MCV and MCH fall within the range of variation described for wild birds (Leonard 1969, Balasch et al. 1973, Hawkey et al. 1983). Nevertheless, the wide range of MCV and MCH values found in our study could be due to the inaccuracy of the hemocytometer method for red blood cell counts. The normal ranges for MCHC values are similar in all mammals and birds (Hawkey et al. 1983).

Several methods have been proposed for determining avian WBC counts (Campbell and Dein 1984, Amand 1985, Russo et al. 1986). Among them, the most accurate and widely used techniques are the eosinophil Unopette 5877 system (Becton-Dickinson and Co., Rutherford, New Jersey) and the Natt-Herrick solution. The method employed for the Unopette system is semi-indirect and involves the use of the eosinophil Unopette diluent, composed of phloxine and propylene glycol, and a Neubauer hemocytometer. The diluent phloxine only stains the granulocytes. Therefore, the count obtained must be corrected since mononuclear cells are not included (Campbell and Dein 1984). This is done by deter-

mining the ratio of granulocytes to mononuclear cells in a stained blood smear (Amand 1985). The Natt-Herrick solution stains only mononuclear cells, granulocytes, and thrombocytes, permitting direct count of these cells with a hemocytometer (Russo et al. 1986).

Direct hemocytometer counts are more accurate than WBC estimates determined by the Unopette system method because of the variation in differential counts (Russo et al. 1986). Both are subject to the technical error of the hemocytometer method, and changes in the WBC count may be caused by the variability inherent in the method of enumeration (Russo et al. 1986). Part of this error may be decreased by standardizing the technique (using the same chamber, coverslip and pipette and having the same person perform the counts) (Russo et al. 1986). In our study cell counts were performed by the same person and by the same procedure.

Total white cell counts were found to be similar to those described previously for the species (Leonard 1969, Veil 1978, Lepoutre et al. 1983) and within the range of variation described in pet and other wild birds (Hawkey et al. 1982, 1983, 1984, Woerpel and Rosskopf 1984, Calle and Stewart 1987).

No band heterophils have been found in the circulating blood of healthy buzzards. Thus, a certain number of these cells (left shift) should be considered as abnormal because they are indicative of a peripheral consumption of heterophils (Schalm et al. 1975).

High eosinophil counts have been found in buzzard blood (Veil 1978, Lepoutre 1982, Lepoutre et al. 1983). High counts are thought to be associated with intestinal parasitism (Hawkey et al. 1983). Nevertheless, Lepoutre et al. (1983) demonstrated that raptors, and the Common Buzzard in particular, are characterized by high numbers of eosinophils in circulating blood and this finding is not associated with a clinical condition. Blood chemistry values for a variety of species, including raptors, have been reported (Gee et al. 1981, Lepoutre 1982, Lepoutre et al. 1983, Ivins et al. 1985, Ferrer et al. 1987).

The refractometric method is being used for the determination of plasma protein concentration (total plasma solids) by avian practitioners. Nevertheless, Lumeij and de Bruijne (1985) demonstrated that the refractometric method is unreliable for determination of plasma protein concentration in avian blood since substances other than protein contribute substantially to the refractive index (Lumeij 1987). In this study, TPS were higher than TPP values obtained by the Biuret method. TPP values fell within the range described for both pet and wild birds (Gee et al. 1981, Woerpel and Rosskopf 1984, Ivins et al 1985).

Hawkey and Hart (1988) proved that fibrinogen level is one of the most useful tests for both confirming infection and other inflammatory diseases, and following the patient's progress. Nevertheless, data for only a limited number of raptor species are now available. Our results on the Common Buzzard are within the range described by these authors for raptors.

The glucose values obtained from buzzards in this study are within the normal range of variation observed in birds (Lepoutre et al. 1983). Blood glucose levels could be indicative of the general health status of the bird. Low glucose values result from hepatopathies, septicemia or endocrinopathies rather than from starvation since starved raptors do not show hypoglycemia and may even be hyperglycemic (Lumeij 1988).

No information is available on the normal range of variation of AST, LDH, CK, ALT and γ-GT values in the Common Buzzard (Gee et al. 1981, Halliwell 1981, Ivins et al. 1985). Aspartate aminotransferase (AST, formerly SGOT) is widely distributed in avian tissues and its relative distribution varies from one genus to another (Lumeij and Westerhof 1987). AST values in this study compare well with those described for pet and other birds (Gee et al. 1981, Woerpel and Rosskopf 1984, Ivins et al. 1985, Calle and Stewart 1987). Lactate dehydrogenase (LDH) also occurs in most avian tissues (Lumeij and Westerhof 1987) and the blood concentration found in the buzzard shows a wider range and a higher mean value than those described for psittaccines (Woerpel and Rosskopf 1984) but similar to those for raptors (Gee et al. 1981, Ivins et al. 1985). LDH values varied more than AST values. Creatine kinase (CK) is mainly found in muscle, and small amounts are found in kidney and duodenum (Lumeij and Wolfwinkel 1988, Lumeij et al. 1988). No information has been reported about normal levels of CK in raptor blood (Gee et al. 1981, Ivins et al. 1985). CK values in buzzards were higher than those observed for racing pigeons (Lumeij and de Bruijne 1985).

Alanine aminotransferase (ALT, formerly SGPT) is mainly found in liver and kidney in pigeons (Lumeij et al. 1988). Nevertheless, its low activity in

plasma, high activity in erythrocytes and seasonal variation (Lewandowski et al. 1986, Lumeij and Westerhof 1987) limits its use for diagnostic purposes. ALT values in this study were found lower than those observed in raptors (Gee et al. 1981) but similar to racing pigeons' plasma levels (Lumeij and de Bruijne 1985). Gamma glutamyl transferase (γ-GT) is almost entirely nephrospecific in birds (Lumeij and Wolfswinkel 1988). Enzymes occurring in the kidney might be of limited clinical value since these enzymes are largely eliminated via the urine after kidney damage (Lumeij and Wolfswinkel 1988). γ-GT values were found similar to those previously reported (Gee et al. 1981).

Plasma calcium and phosphorus levels were within the range of variation found for the species (Ferrer et al. 1987, Lepoutre 1982). Since there is an albumin-bound calcium fraction, it is necessary to consider calcium levels in conjunction with TPP levels (Ivins et al. 1985). Both calcium and phosphorus levels may be indicators of renal function and may help to evaluate nutritional deficiencies.

Uric acid is the major end product of deamination of amino acids in avian species and is excreted by the kidney mainly by tubular excretion (Lewandowski et al. 1986, Lumeij 1987). Since the rate of secretion is largely independent of the state of hydration, the measure of uric acid is the most reliable method to assess renal function in birds (Amand 1985, Lumeij and de Bruijne 1985, Lewandowski et al. 1986, Lumeij 1987). The Common Buzzard has lower values than other raptors and other birds (Gee et al. 1981, Lepoutre 1982, Woerpel and Rosskopf 1984, Ivins et al. 1985).

Plasma creatinine concentration is of questionable value in evaluating renal function in birds (Lewandowski et al. 1986, Lumeij 1987). The amount of creatinine formed from creatine is negligible and creatinine value measured by conventional methods includes pseudocreatinines, such as glucose, protein, ascorbic acid and pyruvic acid and may not reflect glomerular function (Lewandowski et al. 1986, Lumeij 1987). Creatinine levels in this study were within the range of variation found in raptors (Gee et al. 1981, Ivins et al. 1985) and considerably higher than values observed in pet birds (Woerpel and Rosskopf 1984).

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Appendix 1. Blood chemistry test methods used for analysis of Common Buzzard blood.

TEST	METHOD AND SOURCE
Total plasma solids (TPS)	Refractometer.
Total plasma protein (TPP)	Colorimetric Biuret Method. Weichselbaum, T.E. Amer. J. Clin. Path. 16 (1946): 40.[a]
Fibrinogen	Protein precipitated by the microhematocrit at 56° C.[b]
Glucose	GOD-POD Method. Trasch, H., P. Koller, W. Tritschler, Clin. Chem. 30 (1984):969.[a]
Aspartate aminotransferase (AST, SGOT)	GOT-POD Method. 25°. Deneke, V., W. Rittersdorf, W. Werner, Clin. Chem. 31 (1985):921.[a]
Alanine aminotransferase (ALT, SGPT)	GPT-POD Method. 25°. Deneke, V., W. Rittersdorf, Clin. Chem. 30 (1984): 1009.[a]
γ-Glutamyl transferase (γ-GT)	Reduction of $6[Fe(CN)_6]^{4-}$. 25°. Deneke, V., K. D. Willamowski, W. Tritschler. Clin. Chem. 30 (1984):1010.[a]
Lactic dehydrogenase (LDH)	Reduction of NAD. 30°. Z. Klin. Chem. u. Klin. Biochem. 10 (1972):182.[a]
Creatine kinase (CK)	Oxidation of 6-Phosphogluconate. 30°. Gruber, W. Clin. Chem. 24 (1978): 177.[a]
Uric acid	Uricase-POD Method. Merdes, H., W. Rittersdorf, W. Werner, J. Clin. Chem. Clin. Biochem. 28 (1985):608.[a]
Creatinine	Peroxidase Oxidation. Wahbfeld, A., G. Hozt and H. Bergmeyer. Page 1834 *in* H. Bergmeyer [Ed.], Methoden der Enzymatischen Analyse. 3rd ed. Vol. II. Verlag Chemie. Weinheim.[a]
Calcium	Methylthymol Blue Method. Gener, J., Vila, J. and Concustell, E. Lab. Knicherbocker S.A.E. (1971).[c]
Phosphorus	Phosphomolybdate Method. Drewes, P.A. Clin. Chim. Acta 39 (1972):81.[c]

[a] Boehringer Mannheim Gmbh Diagnostica, Mannheim, Germany.
[b] Hawkey et al. 1983.
[c] Knicherbocker Reagents, Knicherbocker Lab., Barcelona, Spain.