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Changes in haematology, plasma biochemistry and erythrocyte osmotic fragility of the Nigerian laughing dove (*Streptopelia senegalensis*) in captivity

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Summary: The haematology, plasma biochemistry and erythrocyte osmotic fragility of the Nigerian laughing dove (*Streptopelia senegalensis*) were studied after 4 and 8 weeks in captivity. At 8 weeks, there was a normocytic hypochromic anaemia characterized by reduced values for packed cell volume (PCV), red blood cell count (RBC), haemoglobin (Hb) concentration, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), but the mean corpuscular volume (MCV) was unaltered compared with the corresponding values at 4 weeks. The platelet count, total white blood cell count, heterophil, lymphocyte and monocyte counts were also lower at 8 weeks than those of the birds sampled at 4 weeks in captivity. There was also a stress induced increased heterophil/lymphocyte ratio and the erythrocytes were more fragile in hypotonic solution in birds sampled at 8 weeks. Plasma aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) increased at 8 weeks, though non-significantly, which might have been due to muscle wasting consequent upon decreased muscular activities associated with prolonged captivity. The results suggest that maintaining wild birds in captivity for a prolonged period could be stressful as shown by the heterophil/lymphocytes ratio and reduced erythrocyte osmotic resistance, and could lead to decreases in erythrocyte parameters and muscle wasting.

Keywords*:* Haematological parameters, erythrocyte osmotic fragility, laughing dove, captivity

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INTRODUCTION

Several attempts have been made by livestock farmers and researchers to raise under domestic conditions animals and birds captured in the wild, either to allow healing of diseases or to rear fledglings or cubs which have lost their parents or, to attempt breeding of animal species that are considered to be endangered (Brossy et al., 1999). Some of these animals are even domesticated as pets while some are reared for economic purposes especially in developing countries.

One of the major challenges encountered by the attempts to domesticate wild animals is their inability to survive in captivity. This may be due to the change in their environmental conditions or to subclinical infections in the wild becoming aggravated in captivity. For example, Brossy et al. (1999) reported a mortality of about 50% among 2,000 African penguins (*Spheniscus demersus*) reared annually in South Africa as a result of *Plasmodium relictum* and *Babesia peircei* infection. The poor ability to survive may also follow disturbances in haematological and biochemical parameters as a result of stress induced by captivity (Martinez-Perez 2003, Lashev et al., 2005). The stress factors on the other hand may reduce immunity in affected animals due to increased corticosterone and altered neutrophil/lymphocytes ratio (Gross and Siegel 1983, Newman et al., 2006). Martinez-Perez (2003) reported significant decreases in the values of haematocrit, haemoglobin, heterophils, lymphocytes, monocytes and the red blood cell (RBC) count in captive scarlet macaws (*Ara macao*). These parameters however increased to normal levels after re-introduction into the wild. The levels of total protein, calcium, phosphorous and creatine phosphokinase were also found to increase significantly while that of alanine transaminase (ALT) decreased after reintroduction of these animals into their natural habitat. This indicates that captivity imposes some form of stress on the animals, hence the improvement in these haematological and biochemical parameters after re-introduction to the wild. Similarly, Lashev et al. (2005) reported that captivity is the source of stress in white storks (*Ciconia ciconia*) having a higher lympho-cyte/heterophil ratio, lower haemoglobin concentration, lower heterophil and monocyte counts

and higher lymphocyte count compared with data obtained in the free living species of the birds. RBC count, haematocrit and haemoglobin values and neutrophil/heterophil ratio of the dusky-footed wood mouse (*Neotoma* fuscipes) had also been reportedly modified by stress associated with one week of habituation and captivity (Weber et al., 2002).

The laughing dove is a common wild bird in the hot humid Nigerian environment. The birds are generally found in association with domestic pigeons (*Columbia livia*) and around poultry houses, but are not domesticated. They are however commonly captured and kept by the rural communities as pets. The present study was undertaken to determine the possible changes in the haematological and plasma biochemical parameters and erythrocyte osmotic fragility of the Nigerian laughing dove (*Streptopelia senegalensis*) in captivity and to identify the challenges that may be associated with domestication of this wild bird.

MATERIALS AND METHODS

Twenty apparently healthy, freshly caught, adult laughing doves were obtained from a local market in Ibadan, Nigeria. They were kept in cages at the experimental animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria to acclimatize for a period of four weeks. The birds were dewormed immediately on arrival with Piperazine dihydrate by Alfasan, Holland (0.1g/L of drinking water), followed by prophylactic treatment with antibiotics, NCO Mix® by Kepro B.V, Holland (containing neomycin, chloramphenicol and oxytetracycline) at a dose rate of 0.25g/L of drinking water for 5 days. Grower mash feed (Capsfeed Ltd, Ibadan, Nigeria) and guinea corn (*Sorghum bicolour*) and water were provided *ad libitum.*

Blood sampling and analysis

Blood samples were collected from 5 birds each by decapitation after 4 and 8 weeks in captivity into bottles containing ethylene diamine tetra-acetic acid (2mg/ml), as an anticoagulant. From the blood samples, the packed cell volume (PCV) was determined by microhaematocrit method. Red blood cells (RBC) and white blood cells (WBC) were counted using the haemocytometer method. Haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the PCV, RBC and Hb values (Jain, 1986). Fresh smear of each blood sample was fixed with methanol and stained with Giemsa for differential leucocyte count. Erythrocyte osmotic fragility was measured as described by Oyewale (1992). Plasma was then obtained from the remaining samples by centrifugation at 3000g for 10 min and stored at 4^{o}C until analyzed.

Plasma Biochemistry

Plasma aspartate transaminase (AST) and alanine aminotransferase (ALT) levels were determined as described by Reitman and Frankel (1957), alkaline phosphatase (ALP) by hydrolysis of *p*-nitrophenylphosphate according to the method described by Wenger et al. (1984). Total protein was determined by Biuret method (Keller et al. 1984) while plasma albumin was obtained by manual dye method described by Tietz and Shuey (1986). Globulin was calculated by subtracting albumin from the total protein. Plasma bicarbonate, chloride and inorganic phosphate were determined according to the methods of Van Slyke and Aullen (1977), Schales and Schales (1971) and Delsal and Manhouri (1958), respectively. Plasma sodium and potassium were determined by flame photometry while the plasma urea and creatinine were determined by the methods described by Harrison (1947).

Statistical analysis

All results were compared by Student's t-test using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The value of $P<0.05$ was considered statistically significant.

RESULTS

Table 1 shows the haematology of the laughing dove after 4 and 8 weeks in captivity. There were decreases in RBC ($P<0.001$), PCV ($P<0.01$), Hb ($P<0.001$) and platelet values ($P<0.001$) in the birds sampled at 8 weeks when compared with those at 4 weeks in captivity. Although at 8 weeks the values of MCH and MCHC were significantly lower ($P<0.05$ and $P<0.01$, respectively), the MCV was similar to that obtained at 4 weeks. The total WBC, lymphocyte, heterophil and monocyte counts were significantly lower ($P<0.001$) at 8 weeks. The heterophil/lymphocyte ratio of the laughing doves at 4 weeks was higher (2.36 ± 0.09) than that of the birds sampled at 8 weeks of captivity (1.95 ± 0.16).

Table 2 shows the plasma biochemical parameters of the laughing dove after 4 and 8 weeks in captivity. No significant differences were apparent between the two periods of captivity in the plasma levels of ALP, AST, ALT, total protein, albumin, globulin, creatinine, urea, sodium, potassium, chloride, bicarbonate and phosphate.

Table 1. Haematological parameters of the Nigerian laughing dove (*Streptopelia senegalensis*) after 4 and 8 weeks in captivity. Values are means ± SEM.

Parameters	4 weeks (n=5)	8 weeks (n=5)
PCV (%)	42.60 ± 0.86	34.60 ± 1.47**
HB (g/dl)	14.04 ± 0.25	11.26 ± 0.48***
RBC ($x10^6/\mu L$)	3.76 ± 0.012	3.01 ± 0.11***
MCV (fl)	112.7 ± 1.92	113.0 ± 1.56
MCH (pg)	39.18 ± 0.55	36.77 ± 0.49*
MCHC (g/dl)	33.09 ± 0.12	32.58 ± 0.09**
Platelets ($x10^3/\mu L$)	144.60 ± 2.68	119.20 ± 2.1***
WBC ($x10^3/\mu L$)	17.68 ± 0.12	10.32 ± 2.39***
Lymphocytes($x10^3/\mu L$)	11.68 ± 0.55	6.46 ± 1.12***
Heterophils ($x10^3/\mu L$)	4.95 ± 0.053	3.32 ± 0.072***
Monocytes ($x10^3/\mu L$)	0.28 ± 0.010	0.19 ± 0.011***
Eosinophils ($x10^3/\mu L$)	0.21 ± 0.08	0.25 ± 0.20
Heterophil/Lymphocyte ratio	2.36 ± 0.09	1.95 ± 0.16**

Asterisks indicate significant differences between 4 and 8 weeks in captivity: * P<0.05, ** P<0.01, *** P < 0.001
n = Number of birds.

Table 2. Plasma biochemical parameters of the laughing dove at 4 and 8 weeks of in captivity. Values are means ± SEM.

Parameters	4 weeks (n=5)	8 weeks (n=5)
ALP (i.u/l)	549.30 ± 92.79	580.60 ± 43.31
AST (i.u/l)	405.50 ± 143.50	524.60 ± 89.73
ALT (i.u/l)	60.75 ± 25.71	96.80 ± 22.25
Total Protein (g/dl)	2.40 ± 0.80	3.00 ± 1.12
Albumin (g/dl)	0.85 ± 0.45	1.65 ± 0.33
Globulin (g/dl)	1.55 ± 0.56	1.35 ± 0.85
Creatinine (i.u/l)	1.08 ± 0.46	2.32 ± 0.85
Urea (mmol/l)	14.50 ± 2.10	12.54 ± 3.20
Sodium (mmol/L	115.80 ± 11.83	104.65 ± 7.72
Potassium (mmol/L)	4.37 ± 2.33	4.89 ± 3.22
Chloride (mmol/L)	100.00 ± 8.35	123 ± 12.35
Bicarbonate (mmol/L)	13.00 ± 1.96	9.45 ± 3.10
Phosphate (mg/dl)	8.20 ± 5.50	11.65 ± 4.20

n= number of birds

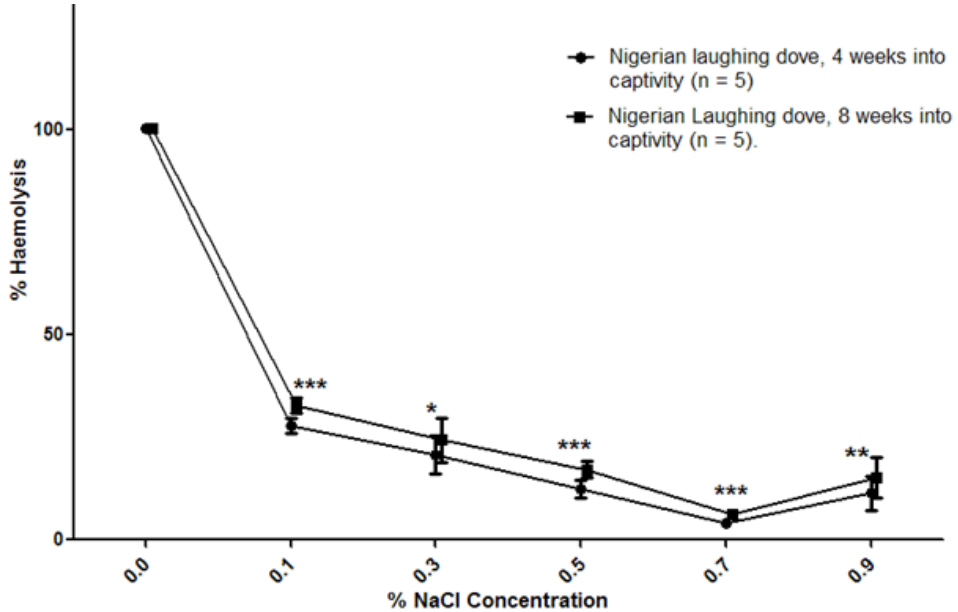

Figure 1. Erythrocyte osmotic fragility of the Nigerian Laughing dove (*Streptopelia senegalensis*) in captivity at for 4 and 8 weeks. Values are means while vertical bars represent SEM. n = number of birds.*P<0.05, **P<0.01, ***P<0.001

As shown in Fig. 1, the fragility of erythrocytes of the laughing dove at 8 weeks in captivity was significantly higher than at 4 weeks at NaCl concentrations of 0.2% ($P<0.001$), 0.3% ($P<0.05$), 0.5% ($P<0.001$), 0.7% ($P<0.001$) and 0.9% ($P<0.01$).

DISCUSSION

Rearing of the Nigerian laughing dove in captivity in this study resulted in normocytic hypochromic anaemia after 8 weeks. Although the MCV was similar at 4 and 8 weeks in captivity, the lower values of RBC, PCV, HB, MCH and MCHC at 8 weeks suggest that prolonged captivity is devastating to wild animals. This is in agreement with the decreases in RBC, PCV and Hb values in captivity reported in the wild cunner fish, *Tautogolabrus adspersus* (Hickey, 1982), dusky-footed wood rats, *Neotoma fuscipes* (Weber et al., 2002) beluga whales, *Delphinapterus leucas* (St Aubin and Geraci, 1989) and white storks, *Ciconis ciconia* (Lashev et al., 2005). The decreases in red cell values are attributable to reduced oxygen demand by the animals in captivity as a result of reduced physical activities (St Aubin and Geraci, 1989).

The laughing dove in the present study had similar Hb, RBC and WBC values to those reported by Lashev et al. (2009) in the domestic pigeons (*Columbia livia*), domesticated African collard doves (*Streptopelia roseogrisea*) and free living collard doves (*Streptopelia decaocto*). In most cases however, the values of RBC, PCV, Hb and WBC observed in the present study in the Nigerian laughing dove were lower than those found in other avian species (Oyewale and Ogwuegbu, 1986, Oyewale and Durotoye, 1988, Oyewale, 1994, Pierman et al., 2000, Miller et al., 2001, Villegas et al., 2004, Lashev et al., 2009). It could be assumed that our present values for the Nigerian laughing dove are in the lower limits for the avian range. However, a more definite conclusion can only be drawn after studying a larger number of individual laughing doves.

The total WBC counts observed in our study with the laughing doves decreased in captivity as a result of reductions in the heterophil, lymphocyte and monocyte counts. This indicates that captivity imposes immunological challenges different from those seen in free living laughing doves. The heterophil/lymphocyte (H/L) ratio, considered by several authors (Gross and Siegel, 1983, McFarlane et al., 1989, Maxwell, 1993, Vleck et al., 2000) as providing important information for immune system tension, was higher at 4 weeks (2.36 ± 0.09) than at 8 weeks (1.95 ± 0.16) in captivity. The H/L ratio of *Streptopelia senegalensis* being considerably higher in this study than the H/L ratios of 0.673 ± 0.015, 0.76 ± 0.023 and 1.05 ± 0.038 reported in *Columbia livia*, *Streptopelia decaocto* and *Streptopelia roseogrisea* respectively (Lashev et al., 2009), could probably reflect the prolonged effect of restraint stress.

The finding that the levels of plasma AST, total protein, albumin, creatinine, urea and phosphorus in the laughing dove did not differ between 4 and 8 weeks in captivity (Table 2) is not unexpected. This is in view of the similarity of diet and physical activity of the two groups of birds in captivity. However, in the adult black vulture, *Aegypius monachus* (Villegas et al., 2002) and adult Egyptian vulture, *Neophron percnopterus* (Dobado-Berrios et al., 1998), the levels of total protein, AST, creatinine and phosphorus have been shown to be higher in captive birds than in free living birds. Whether the levels of biochemical parameters obtained in our captive doves differ from those of the freeliving doves is yet to be determined. When compared with previously reported values in some birds of prey, the plasma ALP, AST and ALT values in *Streptopelia senegalensis* in this study are higher than those reported in imperial eagle (*Aquila adalberti*), golden eagle (*Aquila chrysaeto*), Egyptian vulture (*Neophron pernopterus*) and Griffon vulture (*Gyps fulvus*) reported by Polo et al., (1992).

The observed increase in erythrocyte osmotic fragility in the laughing dove at 8 weeks in captivity (Fig 1) suggests that the anaemia seen in the wild bird at this time may have been due to increased susceptibility of the cells to osmotic lysis. Similarly, increased erythrocyte fragility was seen in American plaice (*Pleuronectes platessa*) held captive for 8 weeks (Sidall et al., 1994). The exact mechanism by which captivity increases erythrocyte fragility in the laughing dove is not known at this time, although erythrocyte osmotic fragility has been shown to be influenced by prolonged exercise stress (Senturk et al., 2001), temperature and pH (Oyewale, 1994), age of animals (Oyewale and Durotoye, 1988) and age of erythrocytes (Tilfert et al., 2007). However, our laboratory is initiating studies to determine the composition of the erythrocyte membrane, function of the nucleus in maintaining osmotic fragility and the influence of such environmental factors as diet, season and restraint stress on the laughing dove, which are all necessary to understand the physiological and adaptive mechanism in this wild bird.

The study has shown from the preliminary data that prolonged captivity is stressful to the laughing dove. This must therefore be taken into consideration when these birds are captured for experimental studies or as pet and necessary steps taken to reduce stress. The effects of antioxidants on the markers of

oxidative stress such as glutathione, hydroxyl radical generation is currently being investigated in these birds in our laboratory.

REFERENCES

Brossy, J.J., Plös, A.L. Blackbeard, J. M. and Kline, A. (1999) Diseases acquired by captive penguins: What happens when they are released into the wild. Marine Ornithology 27: 185-187.

Delsal, J. L and Manhouri, H. (1958). Bull. Soc. Chim. Biol., Paris, 40, 1623.

Dobado-Berrios, P. M., Tella, . L., Ceballos, O and Donazar, J. A. (1998). Effects of Age and Captivity on Plasma Chemistry Values of the Egyptian Vulture. The Condor, Vol. 100, No. 4: 719-725.

Gross, W. B and Siegel, H. S. (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27: 972-979.

Hickey, C. R. (1982): Comparative Hematology of Wild and Captive Cunners (*Tautogolabrus adspersus*). Transactions of the American Fisheries Society 111: 242-249.

Jain, N.C (1986): Schalm's Veterinary Haematology 4th ed. Lea and Febiger, Philadelphia.

Keller, A. (1984): Total Serum protein. In: Kaplan, L. A and A. J. Pesce (Ed.) Clinical Chemistry, Theory, Analysis, and Correlation. St. Lious, Mosby Company, USA.

Lashev, L., Habenov, H., Nikolov, Y., Lasheva, V and Mihailov, R. (2009). Comparison of some haematological parameters between three species from the *Columbidae* family. Vet. Arhiv 79: 409-414.

Lashev, L., Lasheva, V., Daskalov, Y. and Chakarova, V (2005). Values of some haematological indices in White Storks (*Ciconia ciconia*), reared in captivity. Bulgarian Journal of Veterinary Medicine, 8, (4): 265-267.

Maxwell, M. H. (1993). Avian blood leucocycte responses to stress. World's Poultry Sci. J. 49: 34-43.

Mayer, S. (1998). A Review of the Scientific Justifications for Maintaining Cetaceans in Captivity. A Report for the Whale and Dolphin Conservation Society (WDCS), UK. Pp 1-45.

McFarlane, J. M and Curtis, S. E. (1989). Multiple concurrent stressors in chicks. Effects on plasma corticosterone and the heterophil:lymphocyte ratio. Poultry Sci. 68: 522-527.

Miller, M. J., Wayland, M. E and Bortolotti, G. R (2001). Haemogram for and nutritional condition of migrant bald eagles tested for exposure to lead. J. Wildlife Dis. 37: 481-488.

Newman, S. H,. Carter, H. R., Whitworth, D. l and Zinkl, J. G. (2006). Health assessments and stress response of xantus's Murrelets to capture, handling and radio-marking. Marine Ornithology 33: 147-154.

Oyewale, J. O. (1992). Effects of temperature and pH on osmotic fragility of erythrocytes of the domestic fowl (*Gallus domesticus*) and guinea fowl (*Numida maleagridis*). Res. Vet. Sci. 52: 1-4.

Oyewale, J. O. (1994): Further studies on osmotic resistance of nucleated erythrocytes: Observations with pigeon, peafowl, lizard and toad erythrocytes during changes in temperature and pH. J. Vet. Med. A 41, 62-71.

Oyewale, J. O. and Durotoye, L. A. (1988): Osmotic fragility of erythrocytes of two breeds off domestic fowl in the warm humid tropics. Laboratory Animals, 22: 250-254.

Oyewale, J. O. and Ogwuegbu, S.O. (1986): Haematological studies on the guinea-fowl (*Numida maleagris*). Bull. Animal Health Prod. Africa, 34:61-65.

Piersman, T., Koohass, A., Decunga, A and Gwinner, E. (2000). Red blood cell and white blood cell counts in sandpipers (*Philomachus pugnas, Calidris cantus*): Effects of captivity, season, nutritional status and frequent bleeding. Can. J. Zoo., 78: 1349-1355.

Polo, F. J., Celdran, J. F., Peinado, V. I. Viscor, G. and Palomeque, J (1992): Hematological values for four species of birds of prey. The condor 94:1007-1013.

Reitman, S. and Frankel, S. (1957): A colorimetric methods for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Amer. J. Clin. Pathology 28: 6-63.

Senturk, U. K., Gunduz, F., Kuru, O., Aktein, M. R., Kipmewn, D., Yalcin, O., Bor- Kucukatay, M., Yesilkaya, A. and Baskurt, O. K. (2001): Exercise induced oxidative stress affects erythrocytes in sedentary rats but not exercise trained rats. J. Appl. Physiology 91: 1999-2004.

Schales, O and Schales, S. (1971): Determination of chloride in Laboratory. J. Biol. Chem. 140: 879.

Siddall, M. E. Measures, L. N. Desser, S. S. (1994): Seasonal changes in erythrocyte osmotic fragility and haematocrit in American plaice infected with *Haemohormidium terranovae* Journal of Fish Biology Volume 47, (1): 1 – 6.

St. Aubin, D.J. and Geraci, J.R. (1989): Adaptive changes in haematologic and plasma chemical constituents in captive beluga whales, Delphinapterus leucas. Canadian Journal of Fisheries and Aquatic Science 46: 796-803.

Tietz, N. W and Shuey, D. F. (1986): Reference intervals for alkaline phosphatase activity determined by the IFCC and AACC Reference Methods. Clin. Chem., 32: 1593-1594.

Tiffert, T., Daw, N., Etzion, Z., Bookchin, R.M. and Lew, V.L., 2007: Age decline in the activities of the Ca^{2+} sensitive K^{+} channels in human red blood cells. The J. Gen. Physiol. 129 (5): 429-436.

Van Slyke, W. and Aulle, H. S. (1977): Textbook of Clinical Chemistry. Philadelphia W. S. Co.: 112-197.

Villegas, A., Saucher, J. M., Corbacho, C., Corbacho, P and Vargas, J. M. (2004). Blood values of bald ibis (*Geronticus eremita*) in captivity: Comparative ranges and variability with age, sex and physical condition. J. Ornithol. 5: 98 - 104.

Vleck C. M., Vertalino, N., Vleck, D and Bucher, T. l. (2000). Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adklie penguins. The Condor 102: 392-400.

Weber, D. K., Danielson, K., Wright, S and Foley, J. E (2002). Hematology and serum biochemistry values of dusky-footed wood rat (*Neotoma fuscipes*). Journal of Wildlife Diseases, 38 (3): 576-582.

Wenger C. et al. (1984). Alkaline phosphatase: In Kaplan A et al. Clin. Chem. The C.V. Mosby Co. St Louis. Princeton, Toronto. 1094-1098.