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Hematology, Morphology, Cytochemistry and Ultrastructure of Blood Cells in Painted Stork (*Mycteria leucocephala*)

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ABSTRACT

Blood samples of 12 (7 males and 5 females) painted storks (*Mycteria leucocephala*), at the Bangpra Waterbird Breeding and Research Center, were collected from the cutaneus ulna vein for basic hematologic, light microscopic and transmission electron microscopic studies of blood cells. There was no hematozoa detected in all painted storks. There was no significant difference in all hematological values between the male and female groups. Lymphocytes were the most commonly observed leukocytes in the male painted storks and average 6-8 μm in diameter. Heterophils were the most commonly observed leukocytes in the females and were the second most commonly observed leukocytes in the males. Heterophils contained many pleomorphic, dull eosinophilic granules, ribosomes and lobed nucleus. Heterophils were the medium-sized cells, average 8-12 μm in diameter. Heterophils stained strongly positive with α-naphthyl acetate esterase (ANAE). Eosinophils revealed many small round granules and usually were the largest leukocytes, average 9-14 μm in diameter. The number of eosinophils was quite high in both the male and female painted storks. Eosinophil granules stained intensely with β–glucuronidase (β–glu), moderately to strong stained with ANAE and also stained with new methylene blue. Basophils were as small as lymphocytes but revealed many small granules surrounded central round nucleus. Basophils stained moderately with β–glu. Transmission electron microscopic examination revealed organelles within erythrocytes, heterophils, eosinophils and basophils.

Key words: blood cell, cytochemistry, hematology, *Mycteria leucocephala*, painted stork, ultrastructure

INTRODUCTION

The painted storks (*Mycteria leucocephala*) are classified in the genus *Mycteria*, family *Ciconiidae* which includes 19 species in the world but 11 species in Asia. They have long, yellow, slightly decurved bill and pinkish head distinctive. White neck and mentle, wing converts and breast closely barred blackish; greater converts white producing white band across closed wing; scapular rose-pink. Their distribution were in South Asia, South-East Asia and southern part of East Asia (Sonobe and Usui, 1993).

Morphologic characteristics of avian blood cells are heterogeneous. Variations in cell characteristics and cell populations were exist among species within the class Aves (Fudge, 2000). Evaluation of avian hematologram has become a

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useful tool for the diagnosis of avian diseases (Campbell, 1995). However, basic hematological values, cytochemistry and ultrastructure of blood cells have not been described in these species. The purpose of this study was to obtain the hematologic data, cytochemistry and ultrastructure characterization of blood cells in the painted storks.

MATERIALS AND METHODS

In December 2001, blood samples were collected from 12 mature painted storks located at Bangpra Waterbird Breeding and Research Center. They were captive-bred and were housed freely in large enclosure. Each bird was manually restrained, and blood was collected from the cutaneus ulna vein using a 22-ga needle and disposable syringe. Blood smears were prepared immediately, then air-dried and stained with Wright's-Giemsa (WG) stains. The whole blood samples were kept in EDTA, stored at 4°C and processed within 2 hours. The complete hematology was performed using the same methods as studied in King cobra (Salakij *et al*., 2002).

Blood smears were fixed in methanol and stained with WG stain (Jain, 1986) for determination of differential leukocyte (WBC) count, identification of hematozoa infection and morphological evaluation of all blood cells. At least 200 WBCs were counted for differential WBC determination. For each parameter obtained, data from each group of cobra were calculated for means, variances and standard error (SE) using SPSS® for window™ (Norusis, 1993). Significant differences between means were determined using an independent sample T-test model.

Cytochemical staining characteristics of blood cells were evaluated using air-dried blood smears from 4 painted storks. Cells were stained with periodic acid-Schiff (PAS), Sudan black B (SBB), α-naphthyl acetate esterase (ANAE) as described by Jain (1986), and β–glucuronidase (β-glu) as described by Hayhoe and Quaglino (1980). Positive- and negative-stained cells were differentiated by counting 500 cells on each of the cytochemically-stained smears.

For transmission electron microscopy (TEM), buffy coats from 4 painted storks were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 for 24 hours, postfixed with 1% osmium tetroxide and embedded in Spurr's epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined using Jeol 1200Ex TEM. Identification of blood cells by TEM was based on the relative number, size, shape and distribution of granules and on nuclear appearance.

RESULTS

There was no hematozoa detected in all painted storks. There were no significant differences in all hematologic values between the male and the female painted storks (Table 1), so their values were pooled. There were no significant differences in nearly all cell diameter except for basophils and lymphocytes which those in the males were larger and smaller, respecitvely, than those in the female painted storks (Table 2). Cytochemical staining patterns of blood cells were summarized in Table 3. The morphological and cytochemical characteristics of individual blood cells were evaluated, as described below.

Erythrocytes

Erythrocytes (RBCs) were homogeneous in color but moderately heterogeneous in size and shape (Figure 1). Nuclei were oval to pleomorphic. The young RBCs were seldom shown (Figure 1e). Reticulocytes that contained distinct aggregated reticulum were aggregate reticulocytes (Figure 2b, arrow), whereas puntate reticulocytes contained a few dots (Figure 2b, arrowhead). The female painted storks had a higher number of aggregate reticulocytes but a lower number of puntate reticulocytes than the male painted storks. Fews

Table 1 Comparative hematology (mean ± SE) between the male and the female painted storks.

Parameter	Male	Female	All painted storks
Number	7	5	12
PCV (%)	48.9 ± 1.3	49.0 ± 1.9	47.4 ± 1.5
Hb (g/dL)	17.8 ± 0.2	17.7 ± 0.4	17.8 ± 0.4
RBC (x10^6/mL)	2.485 ± 0.238	2.214 ± 0.096	2.439 ± 0.113
MCV (fL)	211.5 ± 28.2	223.0 ± 13.3	213.0 ± 13.2
MCH (pg)	76.6 ± 8.9	80.4 ± 3.0	75.4 ± 3.8
MCHC (g/dL)	36.6 ± 1.0	36.4 ± 1.8	36.8 ± 1.0
WBC (×10^3/μL)	23.000 ± 2.193	20.900 ± 1.638	21.000 ± 1.206
Heterophils (×10^3/μL)	9.127 ± 1.334	10.234 ± 1.227	8.917 ± 0.687
Eosinophils (×10^3/μL)	2.540 ± 0.752	2.158 ± 0.629	2.279 ± 0.346
Basophils (×10^3/μL)	0 ± 0	0.019 ± 0.019	0.034 ± 0.029
Lymphocytes (×10^3/μL)	11.170 ± 1.534	8.296 ± 0.832	9.710 ± 0.917
Monocytes (×10^3/μL)	0.136 ± 0.060	0.191 ± 0.168	0.285 ± 0.091
Heterophils (%)	40.3 ± 5.8	49.9 ± 5.3	43.6 ± 3.2
Eosinophils (%)	10.4 ± 2.5	9.9 ± 2.6	10.3 ± 1.1
Basophils (%)	0 ± 0	0 ± 0	0.3 ± 0.3
Lymphocytes (%)	48.4.9 ± 4.5	40.0 ± 3.7	45.2 ± 2.9
Monocytes (%)	1.2 ± 0.4	1.0 ± 0.5	1.5 ± 0.5
Plasma protein (g/dL)	4.46 ± 0.21	4.88 ± 0.14	4.8 ± 0.1
Fibrinogen (mg/dL)	183.3 ± 16.6	150.0 ± 50.0	178.6 ± 11.4
Agg. Reticulocytes (%)	18.9 ± 3.2	20.8 ± 2.4	17.5 ± 1.6
Punct. Reticulocytes (%)	10.7 ± 2.7	8.1 ± 1.8	8.9 ± 1.2

Table 2 Comparative blood cell diameter in micrometer (mean ± SD) in the painted storks.

Parameter	Male	Female	All painted storks
Number of cells	40	40	80
RBC (width)	7.56 ± 0.52	7.53 ± 0.75	7.58 ± 0.69
RBC (length)	13.06 ± 0.80	13.20 ± 0.79	13.20 ± 0.80
Heterophils	10.91 ± 0.96	10.43 ± 1.58	10.66 ± 1.3
Eosinophils	11.03 ± 1.09	11.30 ± 1.43	11.15 ± 1.29
Basophils	7.72 ± 1.17[a]	7.13 ± 1.09[b]	7.39 ± 1.12
Lymphocytes	7.28 ± 1.14[a]	7.98 ± 0.95[b]	7.54 ± 1.11
Monocytes	11.53 ± 1.50	11.15 ± 0.92	11.22 ± 1.24

The figures on the same row with the same letter are not different ($p > 0.05$).

Table 3 Cytochemical staining patterns of blood cells from 4 painted storks.

Cell type	PAS	SBB	ANAE	β-glu
Heterophil	-	+	+++	±
Eosinophil	-	-	++	+++
Basophil	-	-	-	+
Lymphocyte	-	-	-	-/fine granular
Monocyte	NF	NF	NF	±
Thrombocyte	-	-	-	+
RBC	-	-/±	-	-

PAS indicates periodic acid-Schiff; SBB, Sudan black B; ANAE, a-naphthyl acetate esterase; and β-glu, β-glucuronidase. Staining was score as negative (-), weak (±, fews positve cells), moderate (+), moderate to strong (++), or strong (+++). NF indicates not found.

RBCs were positive with SBB staining (Table 3). Ultrastructurally, RBCs contained a nucleus, hemoglobin and some mitochondria (Figure 4a). Some hemolytic RBCs were also detected by TEM (Figure 4a).

Thrombocytes

Thrombocytes were elongate cells, approximately half the size of mature RBCs (Figure 1c, arrowhead). Nuclei were oval, with dense chromatin. When thrombocytes aggregated they turned into round cells. However, they were easily differentiated from lymphocytes by a characteristic perinuclear cytoplasmic vacuolation (Figure 1c).

Leukocytes

Leukocytes (WBCs) of the painted stork were categorized into 5 groups; heterophil, eosinophil, basophil, lymphocyte and monocyte.

Lymphocytes were the most prevalent circulating cells in the male painted storks (Table 1). They were small, well differentiated and average 6-8 mm in diameter (Figure 1c, 1e, Table 2). Lymphocytes had two patterns of cytochemical staining pattern with β-glu: negative and fine granular. The fine granular pattern consisted of many positive granules (Figure 3c, arrow).

Heterophils were the most commonly observed leukocytes in the female painted storks but were the second most commonly observed leukocytes in the males (Table 1). Heterophils contained lobed nuclei and oval to pleomorphic granules which had dull and eosinophilic staining with Wright's-Giemsa stain (Figure 1a). They were round and 8-12 μm in diameter (Table 2). Heterophils stained weakly or were negative with SBB and β-glu (Table 3) but strongly positive with ANAE (Figure 3a). Ultrastructurally, they contained numerous membrane-bound pleomorphic granules, some mitochondrias, rough endoplasmic reticulums and many ribosomes (Figure 4b). Monocytes were not frequently observed and their characters were similar to mammalian monocytes (Figure 1f).

Eosinophils were usually the largest WBCs in painted storks, average 9-19 μm in diameter (Table 2). Eosinophils contained lobed nuclei and many small round, bright eosinophilic granules (Figure 1b). These granules also stained with new methylene blue (NMB) on smear prepared for determination of reticulocytes (Figure 2c). Eosinophilic granules stained intensely with β-glu (Figure 3b). The number of eosinophils was quite high in both the male and female painted storks (Table 1). Ultrastructurally, eosinophil contained lobed nuclei, numerous membrane-bound round

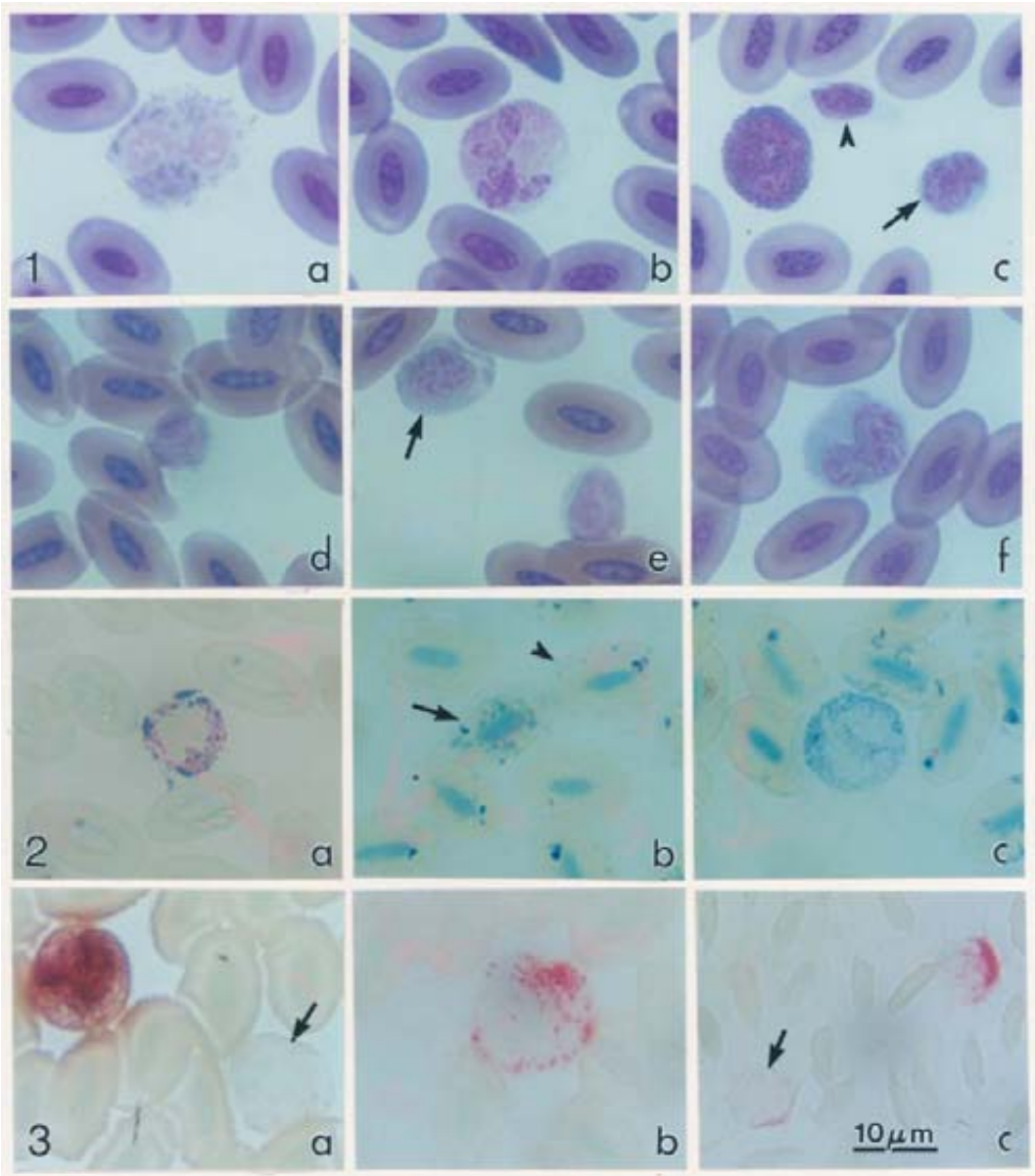

Figure 1 Wright's-Giemsa stained blood cells in the painted storks. (a) A 15 µm heterophil showing rod-shaped granules. (b) A 12 µm eosinophil with many small round granules and well-stained lobed nucleus. (c) A lymphocyte (arrow) compared with a thrombocyte (arrowhead) and an eosinophil. (d) A vacuolated basophil. (e) A young RBC (arrow) compared with a lymphocyte (lower left). (f) A 11 µm monocyte.

Figure 2 New methylene blue stained blood cells in the painted storks. (a) A 8 µm basophil. (b) An aggregate (arrowhead) and punctate reticulocyte (arrowhead). (c) An eosinophil.

Figure 3 Cytochemical stain of blood cells in the painted storks. (a) Heterophil granules strongly positive for α-napthyl acetate esterase (ANAE) compared with ANAE-negative eosinophil (arrow). (b) β-glucuronidase (β-glu)-positive eosinophil. (c) β-glu-positive basophil (upper right) and fine granular β-glu activity in lymphocyte (arrow).

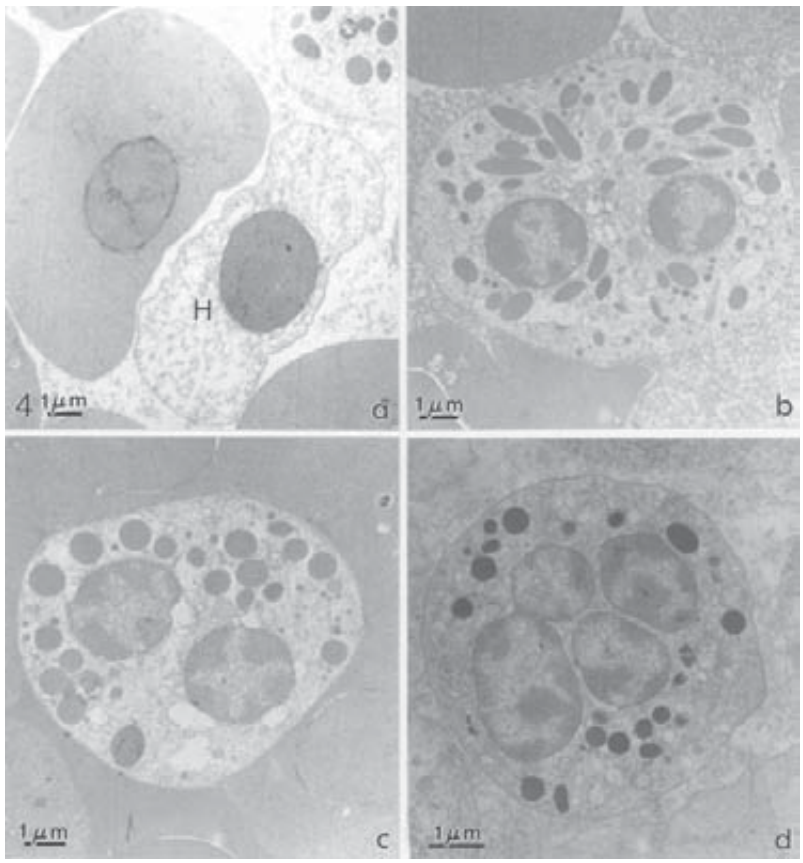

Figure 4 Transmission electron photomicrographs of painted stork blood cells. (a) Intact and hemolytic (H) erythrocytes. (b) Heterophil with two-lobed nucleus, ribosomes and many pleomorphic granules. (c) Eosinophil with two-lobed nucleus and round granules. (d) Eosinophil with four-lobed nucleus and round granules.

granules, some mitochondrias, rough endoplasmic reticulums and many ribosomes (Figure 4c, 4d).

Basophils were very small, average 6-9 μm in diameter (Table 2) which was smaller than heterophils or eosinophils. With Wright's-Giemsa stain, their granules were vacuolated or clear due to the bleaching effect of methanol fixation (Figure 1d). With NMB stain, basophil granules were easily identified by their numerous round, intensely dark blue cytoplasmic granules surrounded central round nucleus (Figure 2a). Cytochemically, basophils stained moderately to strongly with β-glu (Figure 3c). Ultrastructurally, basophils contained round nuclei, numerous membrane-bound pleomorphic granules, some mitochondrias and many ribosomes (Figure 5a, 5c). At a higher magnification, some granules were lamellar (Figure 5b, 5d). A vacuolated appearance surrounding some granules was the commonly observed feature (Figure 5a, 5c).

DISCUSSION

Painted stork RBCs were larger than chicken RBCs (6×12 μm; Bounous and Stedman, 2000) which corresponds with the MCV (Table 1).

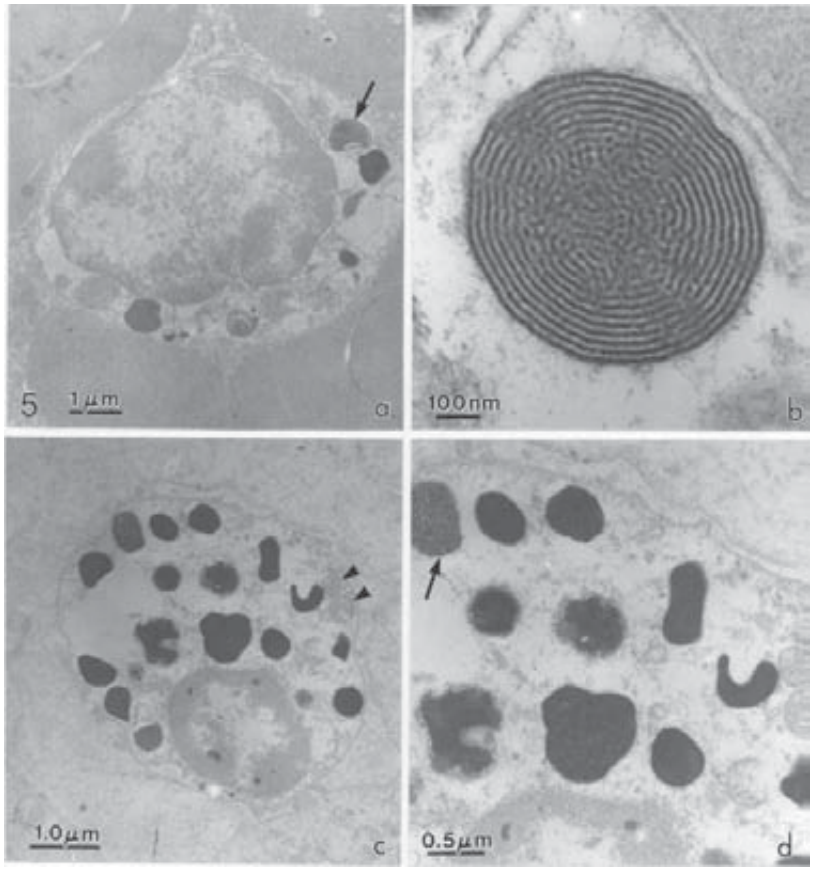

Figure 5 Transmission electron photomicrographs of painted stork blood cells. (a) A degranulated basophil containing large nucleus, some electron-dense granules and lamella granule (arrow). (b) Higher magnification of lamellar granules. (c) A basophil with many pleomorphic, electron-dense granules and well-differentiated mitochondria (arrowheads). (d) Higher magnification of granules in basophil in (c) showing lamella granule (arrow).

All RBC parameters in painted storks were higher than those of chickens except for RBC count which was slightly lower than those in chickens (Bounous and Stedman, 2000). Lymphocytes in the painted stork were the most prevalent circulating cells like those in chickens (Bounous and Stedman, 2000).

Heterophils in the painted storks were the predominant granulocyte like those in chickens and turkeys (Bounous and Stedman, 2000). They were moderately positive with SBB staining and intensely stained with ANAE (Table 3) while those of chicken were negative for SBB (Anderson and Latimer, 1990). Both heterophils and eosinophils in the painted storks contained lobed nuclei, so ultrastructurally, they were identified by the shape of the granules which were ovoid or elongate in heterophil and round in eosinophils.

The small round eosinophil granule characteristic in the painted stork was similar to most of avian (which may be rod-shape in some avian species; Campbell, 1995). The high number of eosinophils in the painted storks (Table 1) without parasites detected may be species

characteristics which is similar to those in raptors (Latimer and Bienzle, 2000). Chicken eosinophils were positive for SBB (Anderson and Latimer, 1990) but those of the painted storks were negative for SBB (Table 3).

It was quite difficult to differentiate basophils in WG stained smears because of vacuolated or clear granules that might make it be misidentified as lymphocytes. But they were identified more easily on Wright's stained preparation or in new methylene blue stain. The bleaching effect of basophil granules in painted storks were resembled those of brown boobies (Work, 1999) and chickens (Bounous and Stedman, 2000). Ultrastructurally, basophil granules appeared homogeneously at a low magnification. When examined at a higher magnification, some granules contained lamellae components. A vacuolated appearance surrounding some of basophil granules (Figure 5) reflected the water solubility of some granule components (Steffens III, 2000). At a low magnification, the granules of heterophils, eosinophils and basophils were homogeneuos but the basophil contained a round nucleus and were smaller than both other cells. Basophils in the painted stork stained only with β-glu whereas basophils from King cobras stained positively with PAS, SBB, ANAE and β-glu (Salakij *et al*., 2002).

These results provide comparative hematological data and a guide for identification of blood cells in painted storks. This may be beneficial for further study and related research.

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