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# Hematology, Cytochemistry and Ultrastructure of Blood Cells in Common Palm Civet (*Paradoxurus hermaphroditus*)

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## ABSTRACT

Hematology and blood cells from 4 adult common palm civets (*Paradoxurus hermaphroditus*) in Khaokeaw Open Zoo were prepared for morphology, cytochemistry and ultrastructure observations. There was no blood parasite in all civets. The red blood cells showed relatively uniform in size, with 4.3 $\mu$m mean diameter. Neutrophils had tight constricted, multilobed nuclei and contained many specific granules which clearly detected by transmission electron microscope (TEM). Neutrophils stained strongly positive with Sudan black B (SBB) and peroxidase (PO) but negative with alpha-naphthyl acetate esterase (ANAE) and β-glucuronidase (βG). By scanning electron microscope (SEM), they showed many microvilli and some micropores. Eosinophils contained numerous large round red refractive granules and stained strongly positive with SBB and PO, but negative with ANAE and βG. By SEM, the granule contour of eosinophil was most easily delineated from the other granulocytes. Ultrastructurally, they contained large round granules with homogenous content without crystalloid-core. Basophils had variable numbers of dull grey, pleomorphic granules and were negative for SBB and PO, but strongly positive for ANAE and moderately positive for βG. By SEM, the surface of basophil was smooth with small granule contour. Ultrastructurally, they contained smaller, homogenous and less electron-dense granules than those in the eosinophils. Lymphocytes were negative for SBB and PO but had 2 patterns of reactivity for ANAE and βG, including focal dot and fine granular staining. Monocytes were negative for SBB, PO and βG but moderately positive for ANAE. The large round eosinophil granules were the characteristic of leukocytes in common palm civet.

**Key words:** blood cells, common palm civet, cytochemistry, hematology, ultrastructure

## INTRODUCTION

The common palm civets (*Paradoxurus hermaphroditus*) are wildly spread civets. Their color are vary from olive-gray to cream with three dark stripes on back and additional dark spots on flanks, sometimes forming indistinct lines. They are found from India through southern China

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(Francis, 2001). Blood smear examinations provide more information on morphology of red blood cells (RBCs), white blood cells (WBCs) and platelets which is useful for clinical diagnosis (Mills, 1998). Cytochemistry is useful for characterizing undifferentiated and acute leukemia in humans (Hayhoe and Quaglino, 1980; Apibal, 1987) and animals (Facklam and Kociba, 1986; Jain, 1986). Ultrastructural examination of leukocytes may be beneficial to the identification of higher resolution of small organelles (Steffens III, 2000). The ultrastructure of blood cells and platelets of common palm civets had been studied (Navephap and Navephap, 1998). But the morphology, cytochemistry and scanning electron microscopic examination of blood cells have not been reported. The aim of this work was to contribute to the knowledge concerning the basic hematological values, the light morphology, cytochemical reaction and ultrastructural characteristics of blood cells in common palm civets.

## MATERIALS AND METHODS

On February 2005, 4 adult common palm civets (2 males and 2 females) in Khaokeaw Open Zoo were chemically restrained with xylazine (Rompun®, 5 mg in all civets) and ketamine (90 mg in males, 60 mg in females). Two milliliters of blood samples were collected from jugular vein and transferred to tubes containing ethylenediamine tetraacetic acid (EDTA, 2 mg/ml). They were clinically healthy, with normal body temperature and normal appetites. Some of the blood without anticoagulant was directly smeared on the slides. Two directed blood smears from each civet were stained with Wright's stain. A minimum of 200 leukocytes were counted for differential leukocyte determination. The complete blood cell count was performed within 24 hours using the Baker 9110 (Biochem Immuno System, [U.S.] Inc. Allentown, PA) at Kamphaeng Saen Animal Hospital. The feline preprogrammed threshold was selected to analyze common palm civet's blood. Blood cell diameters were randomly measured from Wright's stained smears under light microscope. For each hematological parameter, means and standard deviations were calculated using Microsoft Excel.

Cytochemical staining characteristics of blood cells were evaluated using air-dried blood smears without anticoagulant from all civets. Cells were stained with Sudan black B (SBB), peroxidase (PO), $\alpha$-naphthyl acetate esterase (ANAE) as described by Jain (1986) and $\beta$-glucuronidase ($\beta$G) 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 scanning electron microscopy (SEM) and transmission electron microscopy (TEM), blood cells from all civets were processed as described by Salakij *et al*. (2005a). Identification of blood cells by SEM and TEM was based on the relative number, size, shape and distribution of granules and on nuclear appearance.

## RESULTS

There was no blood parasite in all common palm civets collected. The complete hematological values and differential count for WBCs are summarized in Table 1 and Table 2, respectively. The cytochemical staining patterns of white blood cells were tabulated (Table 3). The morphological under light microscope, SEM, TEM and cytochemical characteristics of individual blood cells were evaluated.

### Red blood cells (RBCs)

The RBCs showed slightly biconcave and slightly central pallor (Figure 1a, 1i, 1m, 2a). They were 4.3 ± 0.4 µm in mean diameter (N= 60) and highly anisocytosis with high values of red cell distribution width (RDW) (Table 1). SEM

**Table 1** Hematological data (mean ± SD) of four common palm civets.

| Parameter | Male (N = 2) | | Female (N = 2) | | All civets |
|---|---|---|---|---|---|
| PCV (%) | 43.3 | 46.5 | 41 | 36 | 41.7 ± 4.4 |
| Hemoglobin (g/l) | 147 | 154 | 137 | 117 | 139 ± 16 |
| RBC (X$10^{12}$/l) | 15.43 | 16.64 | 11.1 | 9.9 | 13.3 ± 3.2 |
| RDW (%) | 31 | 32 | 28 | 29.7 | 30.2 ± 1.7 |
| MCV (fl) | 28 | 28 | 36.9 | 36.3 | 32.3 ± 5.0 |
| MCH (pg) | 9.5 | 9.3 | 12.3 | 11.8 | 10.7 ± 1.5 |
| MCHC (g/dl) | 34 | 33.1 | 33.4 | 32.5 | 33.3 ± 0.6 |
| WBC (X$10^9$/l) | 4.000 | 7.70 | 7.10 | 6.25 | 6.262 ± 1.621 |
| Neutrophils (X$10^9$/l) | 0.800 | 5.621 | 2.414 | 2.500 | 2.710 ± 2.058 |
| Band neutrophils (X$10^9$/l) | 0 | 0 | 0.071 | 0.062 | 0.033 ± 0.038 |
| Eosinophils (X$10^9$/l) | 0.640 | 0.847 | 0.213 | 0.375 | 0.518 ± 0.280 |
| Basophils (X$10^9$/l) | 0 | 0 | 0.071 | 0.062 | 0.033 ± 0.038 |
| Lymphocytes (X$10^9$/l) | 3.120 | 0.847 | 0.834 | 2.750 | 2.637 ± 1.275 |
| Monocytes (X$10^9$/l) | 0.240 | 0.385 | 0.497 | 0.500 | 0.405 ± 0.122 |
| Platelets (X$10^{11}$/l) | 3.19 | 5.15 | 3.27 | 4.53 | 4.035 ± 0.964 |
| MPV (fl) | 15.9 | 15.7 | 14.7 | 15.2 | 15.4 ± 0.54 |
| Plasma protein (g/l) | 76 | 76 | 66 | 64 | 70.5 ± 6.4 |
| Aggregate reticulocytes X$10^9$/l | 0 | 45.3 | 0 | 144.0 | 47.3 ± 43.7 |
| Punctate reticulocytes X$10^9$/l | 15.43 | 16.4 | 0 | 0 | 8.02 ± 5.6 |

**Table 2** Differential count (%) of white blood cells (mean ± SD) in four common palm civets.

| Cell type | Male (N = 2) | | Female (N = 2) | | All civets |
|---|---|---|---|---|---|
| Neutrophils | 20 | 73 | 34 | 40 | 41.8 ± 22.5 |
| Band neutrophils | 0 | 0 | 1 | 1 | 0.5 ± 0.5 |
| Eosinophils | 16 | 11 | 3 | 6 | 9 ± 5.7 |
| Basophils | 0 | 0 | 1 | 1 | 0.5 ± 0.5 |
| Lymphocytes | 78 | 11 | 54 | 44 | 46.8 ± 27.8 |
| Monocytes | 6 | 5 | 7 | 8 | 6.5 ± 1.3 |

**Table 3** Cytochemical staining patterns of white blood cells in common palm civets.

| Cell type | SBB | PO | ANAE | βG |
|---|---|---|---|---|
| Neutrophils | + + + | + + + | - | - |
| Eosinophils | +++ | +++ | - | - |
| Basophils | - | - | + ++ | ++ |
| Lymphocytes | - | - | - /focal dot / fine granular | - /focal dot / fine granular |
| Monocytes | ± | ± | ± | ± |

Sudan black B (SBB), peroxidase (PO), periodic acid Schiff (PAS), α-naphthyl acetate esterase (ANAE), β-glucuronidase (βG). Staining was scored as negative (-), weak (±), moderate (+), moderate to strong (++), or strong (+++).

seldom-detected a few defective RBCs such as cytoplasmic knob (Figure 2b), apple stem cell process (Figure 2c) or defective cytoplasmic holes (Figure 2c, d). Under TEM, mature RBCs contained only hemoglobin (Figure 2e) and some RBCs contained Heinz bodies (Figure 2e, f). The apple stem cell process was also detected by TEM (Figure 2e, f).

**Platelets**

The platelets in common palm civets were approximately 1/5 to 1/2 of RBC in size and had prominent reddish-purple granules which were easily seen in modified stained smear (Figure 1e). The mean platelet volume (MPV) was very large when compared with mean corpuscular volume (MCV) (Table 1). Platelets were easily aggregated (Figure 3a). They contained dense granules and alpha-granules (Figure 3b) when observed with TEM.

**Neutrophils**

Neutrophils were the second most common leukocytes in the civets except in one male civet (Tables 1 and 2). They were the smallest granulocytes with 12.0 ± 0.9 μm in mean diameter (N=30). Neutrophils had tight constricted, multilobed nuclei (Figure 1a) and contained many specific granules which detected by TEM (Figure 5a, b). Neutrophils stained strongly positive with SBB (Figure 1b) and PO, but negative with ANAE (Figure 1c) and βG (Figure 1d). By SEM, neutrophils showed several microvilli and some micropores (Figure 4a). Ultrastructurally, neutrophils contained many electron-dense specific granules, some mitochondria and ribosomes (Figure 5a, b).

**Eosinophils**

Eosinophils contained numerous large round red refractive granules (Figure 1e). They were the largest granulocytes with 13.8 ± 1.2 μm in mean diameter (N=30). Eosinophils stained strongly positive with SBB (Figure 1f) and PO (Table 3), but negative with ANAE (Figure 1g) and βG (Figure 1d). By SEM, the large round granular contour of eosinophils were most easily delineated from the other granulocytes (Figure 4b, c). Ultrastructurally, eosinophils contained large round granules with homogenous content without lamellar component (Figure 5c, d).

**Basophils**

Basophils had variable numbers of dull grey, pleomorphic granules (Figure 1i). They were 13.0 ± 1.2 μm in mean diameter (N=30). Basophils were negative for SBB (Table 3) and PO (Figure 1j), but weakly positive for ANAE (Figure 1k) and moderately positive for βG (Figure 1h, l). By SEM, the surface of basophil showed rod-shaped granule contour (Figure 4d). Ultrastructurally, they contained smaller, homogenous but less electron-dense granules than those found in the eosinophils (Figure 5e, 5f). However, the number of granules in basophils was more than those found in eosinophils.

**Lymphocytes**

Lymphocytes were the most common leukocytes in civets except in one male civet (Tables 1 and 2). Most lymphocytes were small lymphocytes (diameter < 9 μm, 8.9 ± 0.9 μm in mean diameter (N=30) (Figure 1m). Some lymphocytes contained small azurophilic granules in the cytoplasm (Figure 1m). Lymphocytes were negative for SBB (Table 3) and PO (Figure 1n), but had 2 patterns of reactivity for ANAE and βG including focal dot and fine granular staining (Figure 1o, 1p). By SEM, lymphocytes were small round bulking of nuclear with some cytoplasmic blebs (Figure 4e). Ultrastructurally, nuclei of lymphocytes contained heterochromatin which was highly marginated at the nuclear envelope (Figure 6a).

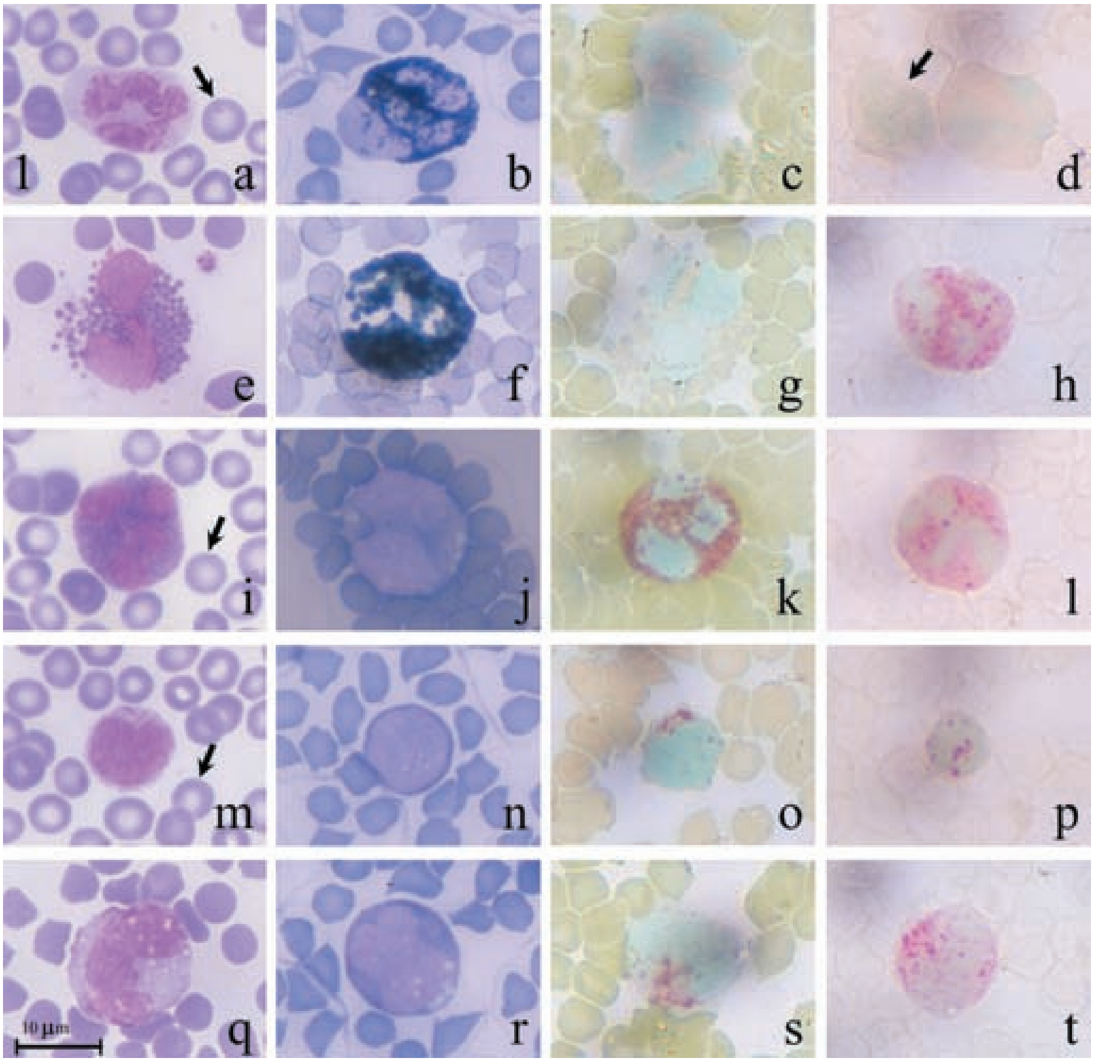


**Figure 1** Morphology and cytochemical staining of common palm civet white blood cells (WBCs). Figures a, e, i, m and q were Wright's stain. Figures b and f were Sudan Black B (SBB) stain. Figures j, n and r were peroxidase (PO) stain. Figures c, g, k, o and s were α-naphthyl acetate esterase (ANAE) stain. Figures d, h, l, p and t were β-glucuronidase (βG) stain. a. A segment neutrophil. RBC with slightly central pallor (arrow), b. SBB-positive neutrophil, c. Two ANAE-negative neutrophils, d. βG-negative neutrophil (arrow) next to a βG-negative eosinophil, e. An eosinophil, f. SBB-positive eosinophil, g. ANAE-negative eosinophil, h. βG-positive basophil, i. A basophil. RBC with slightly central pallor (arrow), j. PO-negative basophil, k. ANAE-positive basophil, l. βG-positive basophil, m. A lymphocyte. RBC with slightly central pallor (arrow), n. PO-negative lymphocyte, o. Fine-granular ANAE-positive lymphocyte, p. Fine-granular βG-positive lymphocyte, q. A monocyte, r. PO-negative monocyte, s. ANAE-positive monocyte, t. βG-positive monocyte.

**Monocytes**

Monocytes were the largest agranulocytes with 13.5 ± 1.0 μm in mean diameter (N=30). They contained variable-shaped nuclei with occasional vacuolation (Figure 1q). These cells were negative for SBB, and PO (Figure

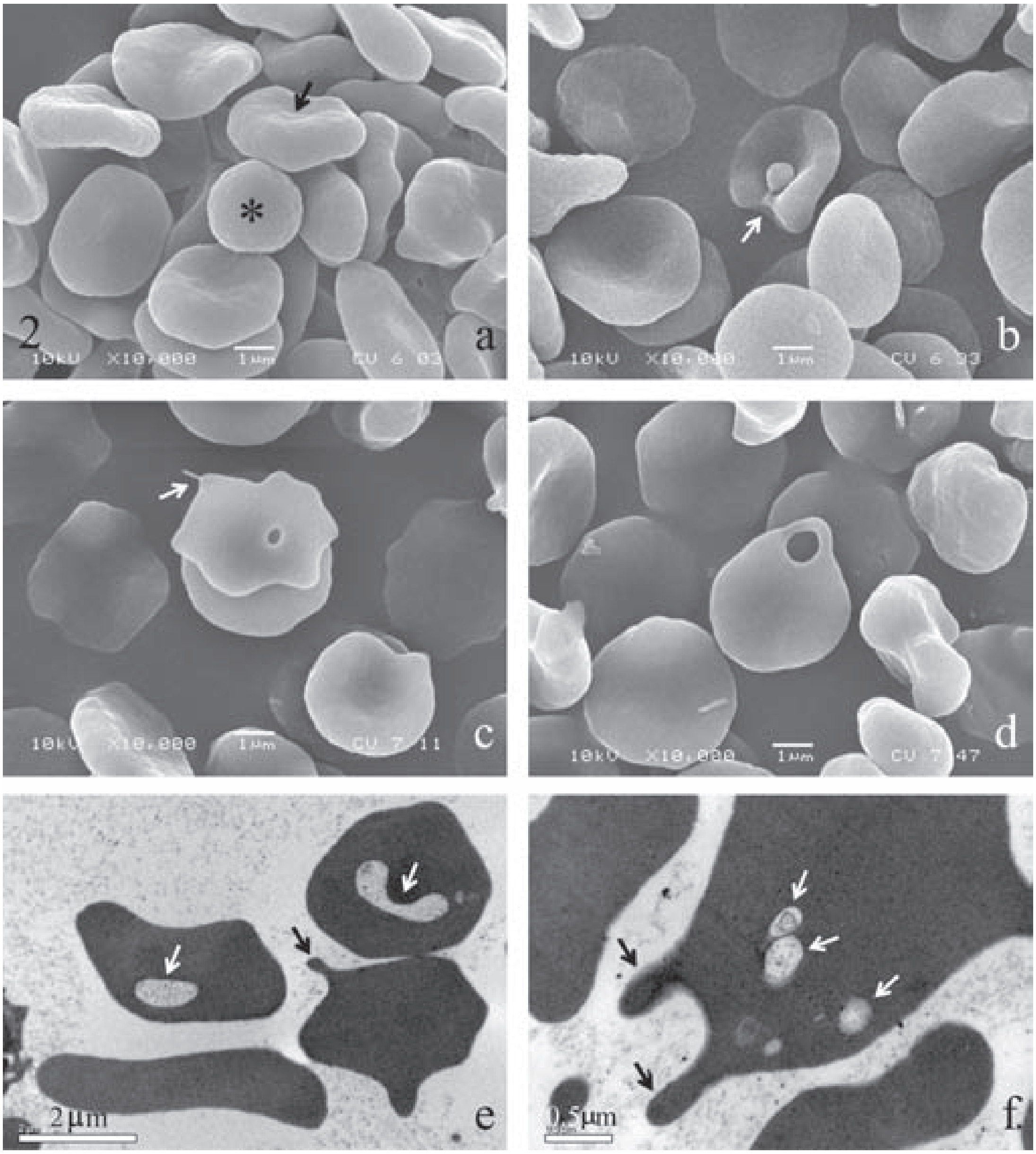


**Figure 2** Electron micrographs of common palm civet red blood cell (RBC). a. Scanning electron micrograph (SEM) of RBCs and one lymphocyte (*). RBC showed slightly central concave (arrow), b. Defective RBCs with cytoplamic knob (arrow), c. A blunt-end crenation RBC with small defective hole and apple stem cell process (arrow), d. RBC with defective hole, e., f. Transmission electron micrograph (TEM) of RBCs showing apple stem cell process (black arrows). Some RBCs contained Heinz bodies (white arrows).

1r) but moderately positive for ANAE (Figure 1s) and βG (Figure 1t). Monocytes showed many cytoplasmic pseudopodia when observed by SEM (Figure 4f). Ultrastructurally, monocytes contained many mitochondria, ribosomes and cytoplasmic pseudopodia (Figure 6b).

## DISCUSSION

Hematological parameter (PCV, Hb and RBC count) of female civets indicated the inferior in quality as compare to those of the male counterpart (Table 1). The mean diameter of RBCs was less than those of cat (Jain, 1993) and large Indian civets (Salakij *et al*., 2006), but a little larger than goat RBCs which had the smallest RBCs among mammals (Jain, 1993). The small sized RBCs (low MCV, MCH) reflected the large number of RBCs. The anisocytosis of RBCs was correlated with the high percentage of RDW. The high percentage of RDW was common findings in cow, horse, sheep and goat (Jain, 1993). The Heinz bodies found in RBCs of common palm civets were observed in normal healthy cat (Jain, 1993) and large Indian civets (Salakij *et al*., 2006).

The mean platelet volume (MPV) of common palm civets was large which were similar to those of large Indian civets (Salakij *et al*., 2006) and domestic cat (Jain, 1993). The large platelets of common palm civets may cause false high value of the RBC count by automate cell counter as also suggested by Duncan *et al.,* (1994). Both aggregate and punctate reticulocytes were found in common palm civets similar to large Indian civets (Salakij *et al*., 2006), dogs, cats and pigs (Jain, 1993, Duncan *et al.,* 1994). The diameters of all blood cells in this study were larger than those studied by Navephap and Navephap (1998) which may due to the different sample measurement. In this study, we measured on blood smear under light microscope while Navephap and Navephap (1998) measured under TEM which blood cells were much shrinkage during processing.

The ultrastructure of platelets were not different from those of previous study of common palm civets (Navephap and Navephap, 1998), bovine (Fern, 2000), African elephant (DuPlessis and Stevens, 2002), Asian elephant (Salakij *et al*., 2005) and Asiatic black bear (Salakij *et al*., 2005a). The ultrastructure of lymphocytes and monocytes of common palm civets were the same as those previous studies of common palm civets (Navephap and Navephap, 1998), Asiatic black bear (Salakij *et al*., 2005a), large Indian civet (Salakij *et al*., 2006), reindeer (Henkel *et al*., 1999), sun bear (Salakij *et al*., 2005b), avian and porcine (Steffens III, 2000). The cytochemial pattern of lymphocytes and monocytes of common

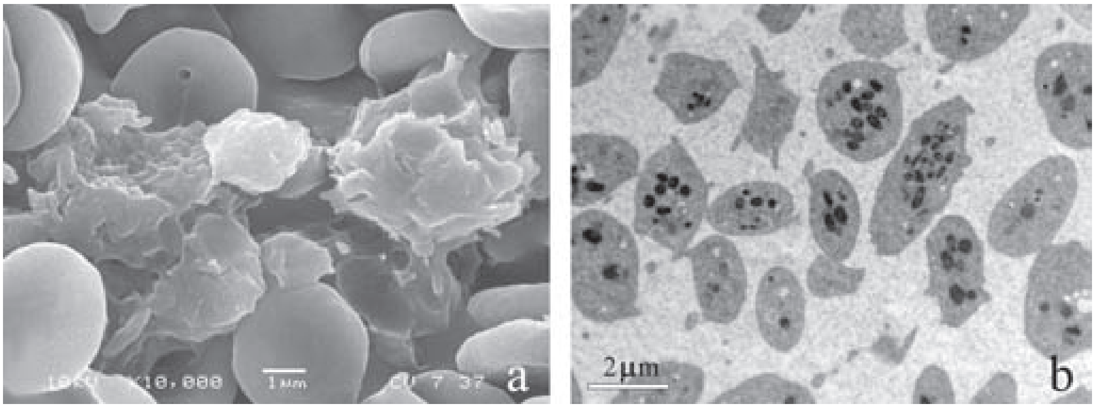


**Figure 3** Electron micrographs of common palm civet platelets. a. SEM of aggregate platelets, b. TEM of platelets showing clearly defined dense granules.

palm civets were similar to those of humans (Apibal, 1987) and other mammals (Henkel *et al*., 1999; Raskin and Valenciano, 2000; Salakij *et al*., 2000; 2005a-d; 2006).

The numbers of white blood cells was quite fluctuate and therefore could not be properly interpreted due to the low number of collected samples. The high number of neutrophils in one

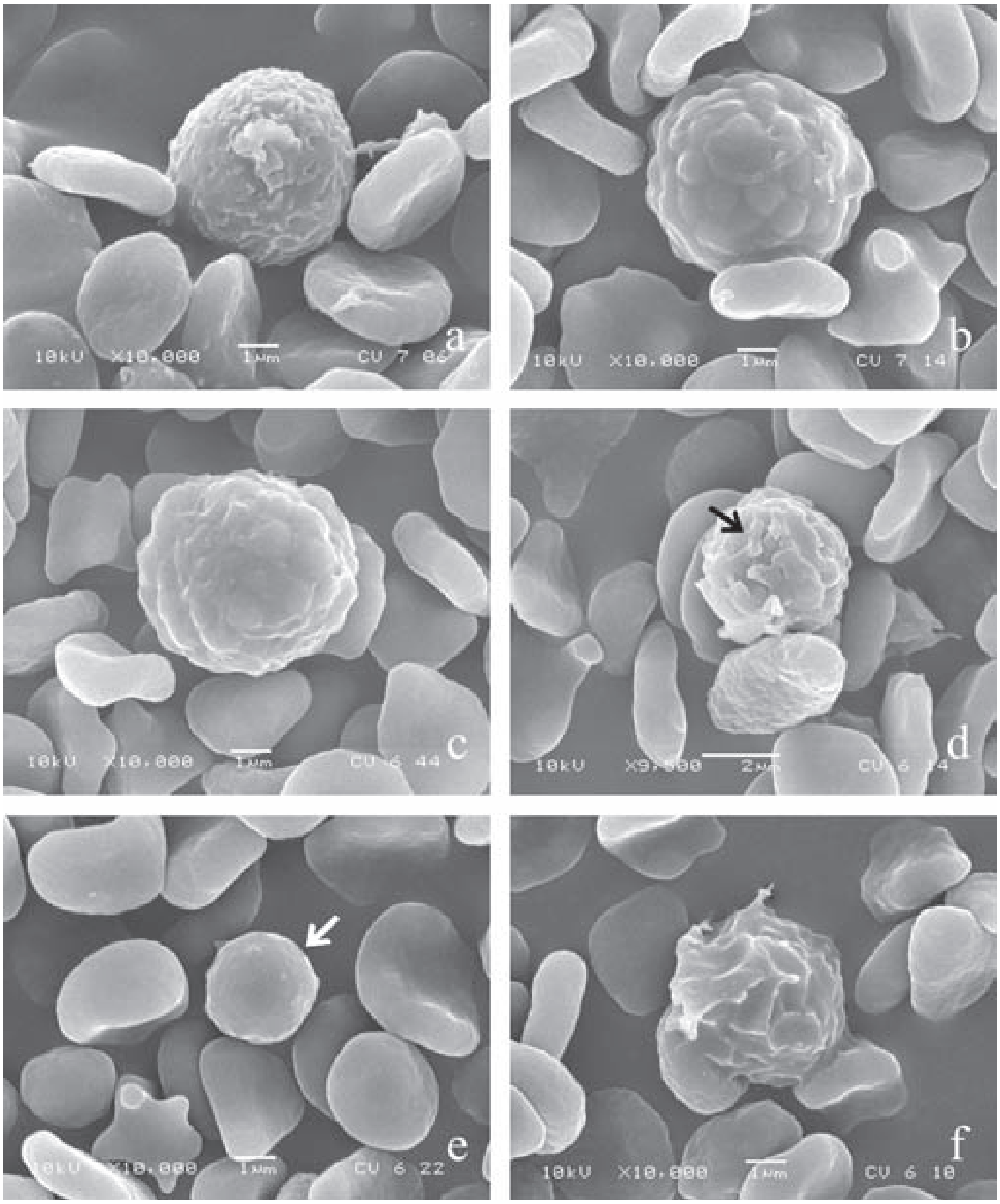


**Figure 4** SEM of WBCs in common palm civet. a, A neutrophil, b., c. Eosinophils showing large round bulging granular contour, d. A basophil with rod-shaped granular surface (arrow), e. A lymphocyte (arrow), f. A monocyte.

male civet may due to the physiologic neutrophilia from the excitement as also reported by Duncan *et al*. (1994). The ultrastructure of neutrophils in common palm civets were similar to those in reindeer (Henkel *et al*., 1999), Asian elephant (Salakij *et al*., 2005d), Douc langur (Salakij *et al*.,

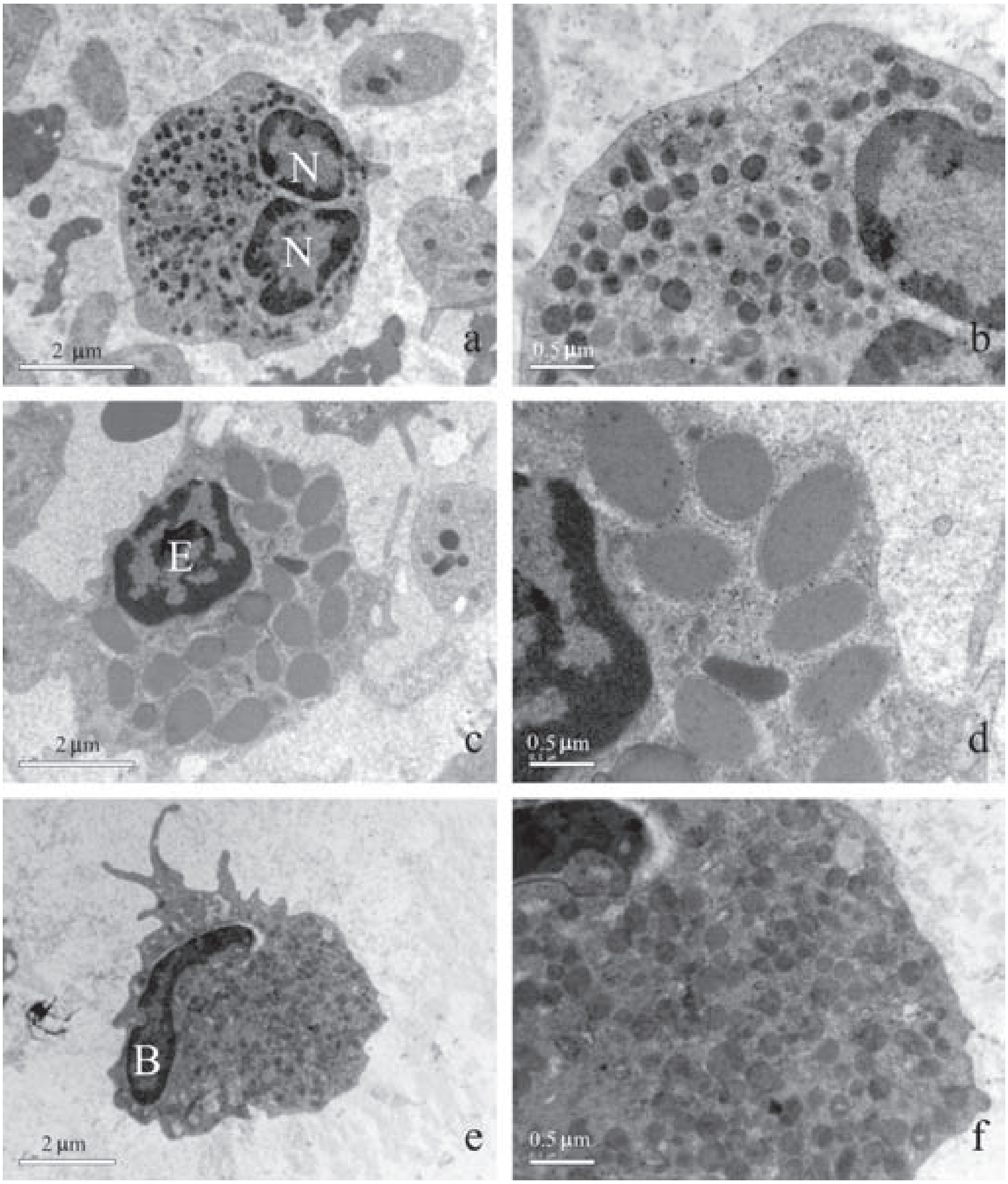


**Figure 5** TEM of common palm civet neutrophils and basophils. a. Two-lobed nucleus neutrophil (N), b. Higher magnification of a, showing electron-dense granules and many ribosomes, c. An eosinophil (E), d. Higher magnification of granules in c, showing oval, homogeneous electron-dense granules, e. Lobed-nucleus basophil (B), f. Higher magnification of e, showing less electron density granules than those of neutrophils.

2005c), large toothed ferret-badger (Narkkong *et al.*, 2005), but was different from those of Asiatic black bears (Salakij *et al.*, 2005a) which contained both large and small granules.

The large round granules in eosinophil of common palm civets were different from those in large Indian civets (Salakij *et al.*, 2006). The granules of eosinophils seen in common palm civets under TEM were homogenous without lamellar component which was also found in large Indian civet (Salakij *et al.*, 2006). The homogeneous electron-dense of specific granules in the eosinophils of this study was similar to those of previous study (Navephap and Navephap, 1998). Interestingly, the surface contour of basophils in common palm civet could be easily differentiates from eosinophils while those of large Indian civets were quite similar to the eosinophils (Salakij *et al.*, 2006).

As for ultrastructure, basophils in common palm civets were different from those of large Indian civets whose granules were larger and contained crystalloid component (Salakij *et al.*, 2006). Basophils in this study contained smaller granules than those of basophils of common palm civets studied by Navephap and Navephap (1998). The basophils in Asian wild dog (Salakij *et al.*, 2000) were strongly positive for βG while those in sun bear (Salakij *et al.*, 2005b) and common palm civets were negative for SBB.

The PO activities in all WBCs were the same as SBB activities (Table 3). So the SBB staining of WBCs in common palm civets may serve as demonstration of endogenous myeloperoxidase similar to those in humans (Hayhoe and Quaglino, 1980) and domestic animals (Raskin and Valenciano, 2000). The SBB and PO positive eosinophils of common palm civets were similar to those in reindeer (Henkel *et al.*, 1999) and Asian wild dog (Salakij *et al.*, 2000). But the SBB and PO negative eosinophils were found in large Indian civet (Salakij *et al.*, 2006) and domestic cat (Jain, 1993). This data may support the heterogeneity of enzyme content in granules of eosinophils among civets and other mammals.

## CONCLUSIONS

The large round eosinophil granules were the characteristic of white blood cells in common palm civets. The SBB and PO positive eosinophils of common palm civets were different from those of large Indian civet. The ultrastucture of granules in eosinophils in common palm civets were homogeneous without any lamellar component.

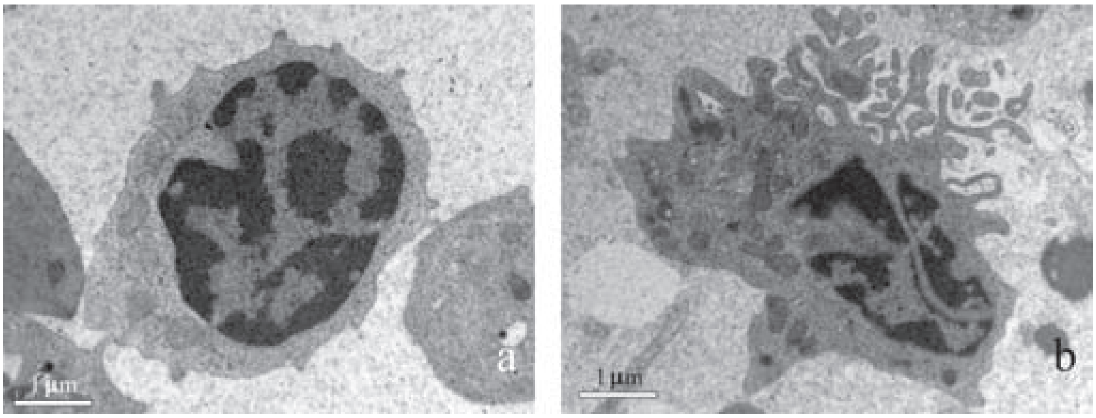


**Figure 6** TEM of common palm civet lymphocyte and monocyte. a. A lymphocyte, b. Monocyte with many rough endoplasmic reticulum and ruffle membrane (upper side).

The surface contour of blood cells studied by SEM can differentiate white blood cells in common palm civets. This study provides more available information on morphology, cytochemical staining and ultrastructure of blood cells in common palm civet which may be useful for civet health management.

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