The Thai Journal of Veterinary Medicine

Volume 38
Issue 3 *September, 2008*

Article 1

9-1-2008

Ultrastructures of Red Blood Cells, White Blood Cells and Platelets in Four Species of Macaques in Thailand

Kamol Sakulwira

Wimon Pothiwong

Pakorn Prachammuang

Siripen Vethchagarun

Original Article

Ultrastructures of Red Blood Cells, White Blood Cells and Platelets in Four Species of Macaques in Thailand

Kamol Sakulwira[1*] Wimon Pothiwong[1] Pakorn Prachammuang[1]
Siripen Vethchagarun[2]

Abstract

The structure of blood cells and platelets in 11 macaques (2 stump-tailed macaques, 5 long-tailed macaques, 1 rhesus macaque and 3 pig-tailed macaques) were studied using light and transmission electron microscopy (TEM). Erythrocytes were non-nucleated, biconcave disks without cytoplasmic organelles. Neutrophils had numerous small specific granules. Eosinophils contained specific granules with a dense matrix and a pale crystalloid body. Basophils revealed large round specific granules. Lymphocytes possessed scant cytoplasm with some mitochondria and azurophilic granules. Monocytes contained a large number of mitochondria, azurophilic granules and endoplasmic reticulum. Platelets were represented by anucleation and they had several different organelles in the cytoplasm. The structure of erythrocytes, neutrophils, basophils, lymphocytes, monocytes and platelets of macaques were similar to those of other mammals whereas eosinophils differed among several species.

Keywords : blood, macaque, monkey, primate, TEM

[1]**Department of Veterinary Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330.**
[2]**Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok, 10330.**
[*]**Corresponding author**

Thai J. Vet. Med., 2008. 38(3): 19-25

บทคัดย่อ

โครงสร้างของเซลล์เม็ดเลือดแดง เม็ดเลือดขาวและเกล็ดเลือดในลิงสกุลแมคแคค 4 ชนิด ในประเทศไทย

กมล สกุลวิระ[1*] วิมล โพธิวงศ์[1] ปกรณ์ ประจำเมือง[1] ศิริเพ็ญ เวชชการัณย์[2]

ศึกษาเซลล์เม็ดเลือดและเกล็ดเลือดในลิงสกุลแมคแคค 11 ตัว ประกอบด้วยลิงเสน 2 ตัว ลิงแสม 5 ตัว ลิงวอก 1 ตัวและลิงกัง 3 ตัว โดยใช้กล้องจุลทรรศน์ชนิดแสงสว่างและกล้องจุลทรรศน์อิเล็กตรอนชนิดลำแสงผ่าน เซลล์เม็ดเลือดแดงมีรูปร่างกลมไม่พบนิวเคลียสหรือออร์กาเนลใดๆ นิวโทรฟิลมีแกรนูลจำเพาะขนาดเล็กเป็นจำนวนมาก อีโอซิโนฟิลมีแกรนูลจำเพาะทึบแสงอิเล็กตรอน ซึ่งภายในแกรนูลมีคริสตัลลอยด์ติดสีจาง เบโซฟิลมีแกรนูลจำเพาะกลมขนาดใหญ่ ลิมโฟไซต์มีไซโตพลาสมจำนวนน้อยภายในมีไมโตครอนเดรียและอะซูโรฟิลิคแกรนูล พบไมโตครอนเดรียจำนวนมาก แกรนูลที่ทึบแสงและเอ็นโดพลาสมิกเรติคิวลั่มภายในเซลล์โมโนไซต์ เกล็ดเลือดไม่มีนิวเคลียส ภายในมีออร์กาเนลหลายชนิด โครงสร้างของเซลล์เม็ดเลือดแดง เม็ดเลือดขาวและเกล็ดเลือดในลิงสกุลแมคแคคมีความคล้ายคลึงกับของสัตว์เลี้ยงลูกด้วยน้ำนมโดยทั่วไป แต่จะแตกต่างกันในเซลล์อีโอซิโนฟิล

คำสำคัญ : เซลล์เม็ดเลือด แมคแคค ลิง ไพรเมท กล้องจุลทรรศน์อิเล็กตรอนชนิดลำแสงผ่าน

[1]ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330
[2]ศูนย์เครื่องมือวิจัยและเทคโนโลยี จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330
[*]ผู้รับผิดชอบบทความ

Introduction

The macaques constitute a genus of Old World monkeys of the subfamily *Ceropithecinae*. Twenty-two macaque species are currently recognised (Groves, 2005). They are well known to science owing to their relatively easy upkeep in captivity, and have been used extensively in medical and biological research because of their evolutionary proximity to human beings.

The blood cells can be categorized into red blood cells, white blood cells and platelets. The red blood cells are round and have neither nucleus nor organelles. The white blood cells are divided into two group; granulocytes and agranulocytes (Jain, 1993). The granulocytes (neutrophils, eosinophils and basophils) are round and their nuclei are lobulated. Specific granules of various sizes are distributed in the cytoplasm. The agranulocytes (lymphocytes and monocytes) have a round nucleus and little cytoplasm.

The ultrastructural morphology of blood cells have been documented in humans (Sokol et al., 1988; Tanaka and Goodman, 1972), dogs (Shively et al., 1969), domestic cats (Ward et al., 1972), wild cats (Pothiwong et al., 2006), swamp buffaloes (Pothiwong et al., 2002), sheep (Yamada, 1970), goats (Anosa, 1993), pigs (Nafstad and Nafstad, 1968), horses (Sonoda and Kobayashi, 1966), donkeys (Pothiwong and Vetchagarun, 1986), camels (Johnson et al., 1999), crocodiles (Sakulwira et al., 2004), tortoises (Alleman et al., 1992) and king cobras (Salakij et al., 2002). To date, non-human primates have rarely been studied (Dvorak et al., 1989; Taylor et al., 1980). The purpose of this study was to observe the structure of blood cells and platelets in 4 Thai species of macaques *(Macaca arctoides, M. fascicularis, M. mulatta and M. nemestrina)* using light and transmission electron microscopy.

Materials and Method

Animals: Two adult female stump-tailed macaques *(Macaca arctoides)*, five adult male long-tailed macaques *(M. fasicularis)*, one adult male rhesus macaque *(M. mulatta)*, two adult female and one adult male pig-tailed macaques *(M. nemestrina)*, held captive at Dusit Zoo, Bangkok, Thailand, were used for this study. Blood samples were obtained via femoral venipuncture, during a brief period of anesthesia induced by ketamine hydrochoride (Calypsol®, Gedeon Richter Ltd).

Light microscopic specimen preparation: Blood smears were fixed in methanol and stained with Wright-Giemsa

stain for morphological evaluation of all types of blood cells. Cell size was measured by MicroPublisher 5.0 camera, Q Imaging Corporation, Canada and software from Media Cybernetics.

Transmission electron microscopic specimen preparation: Blood samples were anticoagulated with EDTA and centrifuged at 2,000 rpm for 10 minutes. The plasma was removed and 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB) was layered on top of the buffy coat. After 15-30 minutes, the hardened buffy coat layer was collected with a wooden dowel, placed into fresh glutaraldehyde, cut into small pieces, prior to being washed 3 times with 0.1M PB, post-fixed with 1% osmium tetroxide in 0.1M PB, dehydrated in graduated concentrations of alcohol, and embedded in Spurrís resin. Ultrathin sections were cut using a diamond knife on an ultramicrotome, placed on a mesh grid, stained with uranyl acetate and lead citrate, before examined under a TEM (JEM-2100, 120 KV, JEOL, Japan). Photomicrographs were taken at 5,000 to 20,000 times of magnification.

Results

The structure of red blood cells, white blood cells and platelets were similar to each species of macaques. Macaque erythrocytes were non-nucleated, biconcave disks with 7.0±0.5 μm in diameter (n=40). They contained no cytoplasmic organelles (Fig. 1). Macaque neutrophils had diameter of 12.0±1.3 μm (n=12) and their nuclei were divided into 2-5 lobes connected by a fine nuclear strand or filament (Fig. 2). There was an appendage like a small drumstick (Barr body) in the nucleus of the neutrophil from female macaques. Ultrastructurally, neutrophils contained numerous small round or oval specific granules and some mitochondria. Macaque eosinophils had diameter of 12.0±1.3 μm (n=8) and their nuclei were divided into 2-3 lobes (Fig. 3). The specific granules of the eosinophil had a brighter purple-red color than those of the neutrophil, and might be superimposed on the nucleus. Ultrastructurally, the heterochromatin was generally distributed in the periphery of the nucleus, whereas the loosely arranged euchromatin was found in the center. Eosinophilic granules were mostly round, 1.0±0.5 μm in diameter (n=10) and contained a dense matrix and a pale core or crystalloid body. Macaque basophils had diameter of 11.5±1.5 μm (n=5) and their cytoplasm were rich in round specific granules. The nucleus was bi-or tri-lobed although it was difficult to locate because of a large number of granules (Fig. 4). Electron micrographs of a basophil revealed large round homogenous matrix and electron-dense, membrane-bounded specific granules. Specific granules were 1.25±0.5 μm in diameter (n=10).

Macaque lymphocytes had 11.0±1.5 μm in diameter (n=15) (Fig. 5) and their cytoplasm were transparent. The nucleus was round and large in comparison to the cell and it occupied most of it. Ultrastructurally, lymphocyte contained scant cytoplasm with some mitochondria and few azurophilic granules. The nucleus was round with peripheral clumps of heterochromatin. Macaque monocytes were 14.0±1.5 μm in diameter (n=10) and contained a large cuneiform or horseshoe-shaped nucleus. The cytoplasm was transparent, but with an appearance of “ground glass” (Fig. 6). Under the electron microscope, the nucleus appeared irregular and showed dense chromatin condensation along the nuclear membrane. The cytoplasm contained mitochondria, azurophilic granules and endoplasmic reticulum. Macaque platelets were 2.5±1.0 μm in diameter (n=15) (Fig. 7). They were found either separated from each other or aggregated together. Electron micrographs of platelet showed anucleation and a mass of cytoplasm with a few thin projections or spicules extending from the surface. The cytoplasm possessed several different organelles, including various granules, glycogens, mitochondria and microtubules.

Discussion

In the present study, the erythrocytes of macaques are non-nucleated, biconcave disks, similar to those of other mammals (Nafstad and Nafstad, 1968; Shively et al., 1969; Tanaka and Goodman, 1972; Pothiwong and Vetchagarun, 1986; Pothiwong et al., 2002; Pothiwong et al., 2006) while erythrocytes of reptiles are nucleated and oval-shaped (Alleman et al., 1992; Salakij et al., 2002; Sakulwira et al., 2004). Erythrocytes are extremely flexible and could be distorted in response to mechanical forces, but are also elastic and quickly regain their biconcave disc form after removal of the distorting force. This is particularly helpful in easing their flow through

small capillaries (Skalak and Branemark, 1969).

The leukocytes fall into two groups; one with specific granules and the other without. The ones with granules are neutrophils, eosinophils and basophils. Macaque neutrophils are the most common granulocytes. There is an appendage like a small drumstick (Barr body) emerging from the nucleus of the neutrophil from female macaques, similar to those of other domestic mammals.

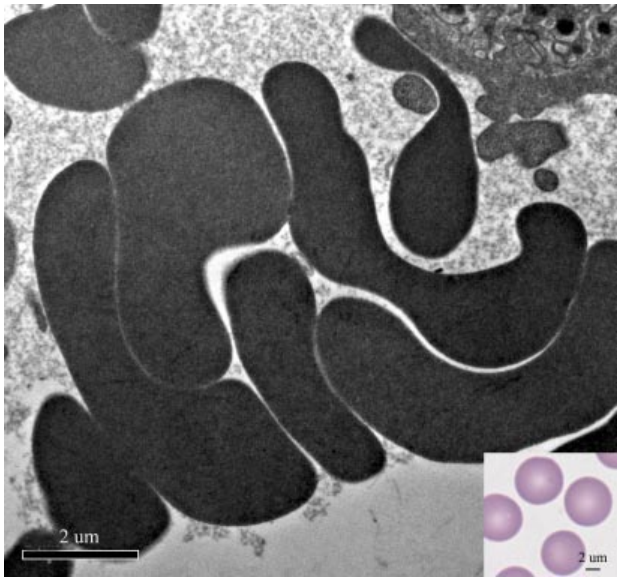

Figure 1 An electron micrograph of a pig-tailed macaque's red blood cells showing non-nucleated, biconcave disk. A light micrograph of red blood cells is shown in the right bottom inset.

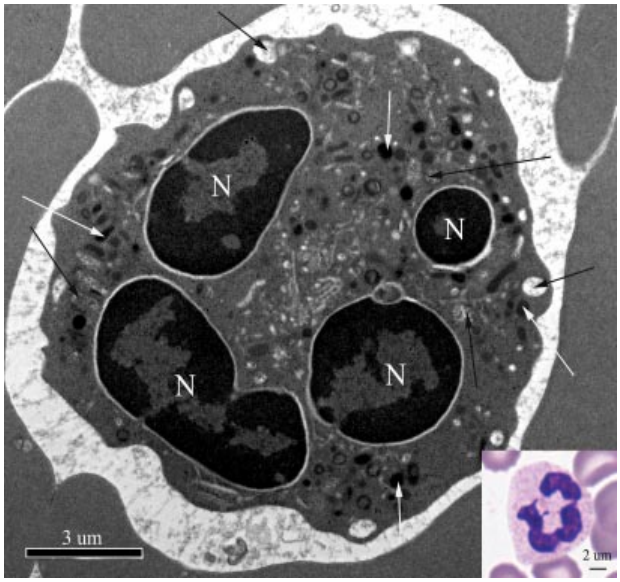

Figure 2 An electron micrograph of a rhesus macaque's neutrophil showing nucleus (N), mitochondria (black arrows) and specific granules (white arrows). A light micrograph of a neutrophil (inset) demonstrates the Barr body.

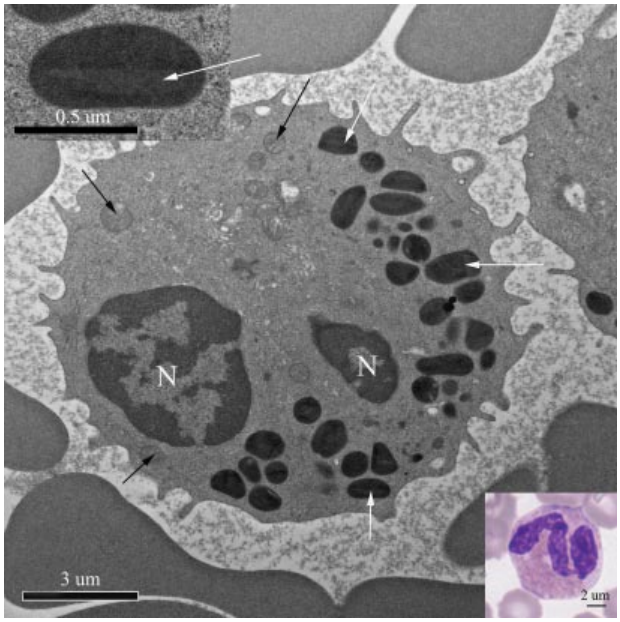

Figure 3 An electron micrograph of a pig-tailed macaque's eosinophil showing nucleus (N), mitochondria (black arrows) and specific granules (white arrows). The left top inset shows a crystalloid body (white arrow) in a specific granule. A light micrograph of an eosinophil is shown in the right bottom inset.

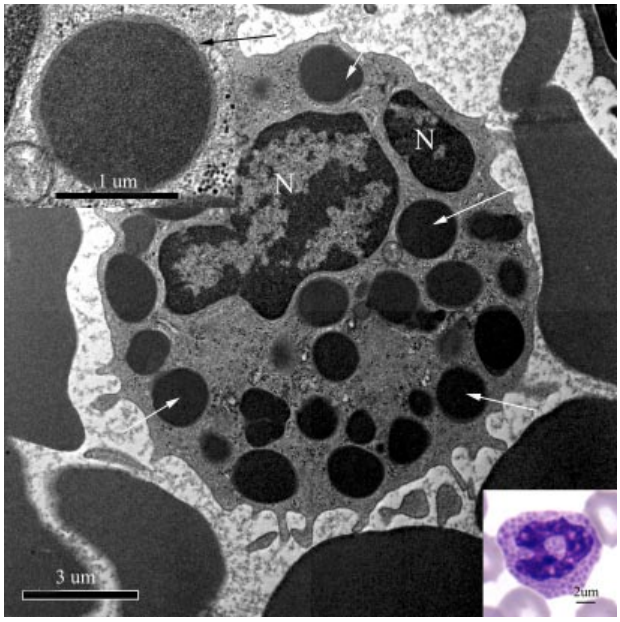

Figure 4 An electron micrograph of a rhesus macaque's basophil showing nucleus (N), specific granules (white arrows). The left top inset shows a space and membrane-bounded specific granule (black arrow). A light micrograph of a basophil is shown in the right bottom inset.

Specifc granules of neutrophils are relatively small and well dispersed. The eosinophilic granules of humans, dogs, goats, guinea pigs, mice, rats and camels consist of a peripheral matrix and a central electron-dense crystalloid core (McEwen, 1992) while in some human eosinophils and macaque eosinophils in this study have a dense matrix and a pale core or crystalloid body. In contrast, eosinophilic granules of horses (Jain, 1986), donkeys (Pothiwong and Vetchagarun, 1986), cows, mink and gorillas (McEwen, 1992) are homogenous, lacking a defined core. Crystalloid structures unique for eosinophilic granules are observed in various species, including man, rabbit, horse, monkey, rat and mouse. In cats, there are concentrically arranged lamellated structures (Osaka, 1959). Macaque basophils are the rarest leukocytes. Electron microscopic studies on the human basophils, presented by several investigators (Anderson, 1966; Watanabe et al., 1967; Zucker-Franklin, 1967), show a lobulated nucleus in the middle of the cytoplasm which contains Golgi apparatus, endoplasmic reticulum, and glycogen particles. In this study, the basophilic granules of macaques are more numerous than those of other mammals.

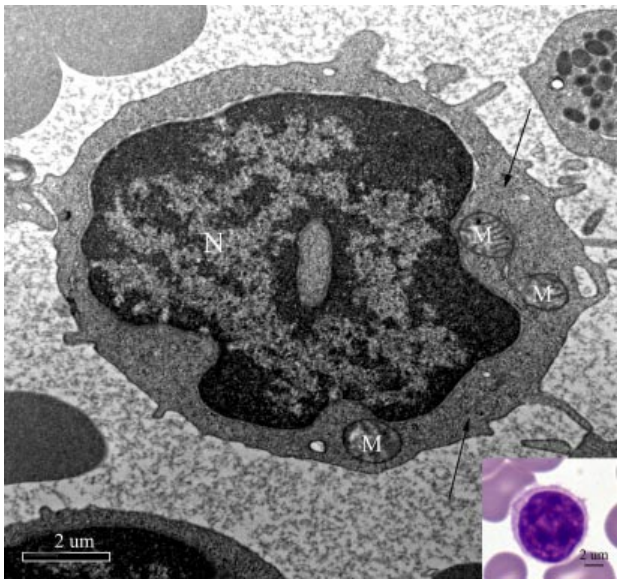

Figure 5 An electron micrograph of a pig-tailed macaque's lymphocyte showing nucleus (N), mitochondria (M) and azurophilic granules (arrows). A light micrograph of a lymphocyte is shown in the right bottom inset.

The agranulocytes are lymphocytes and monocytes. Macaque lymphocytes are the most common agranulocytes. Their nuclei are generally round. The narrow rim of the cytoplasm has a few mitochondria and the endoplasmic

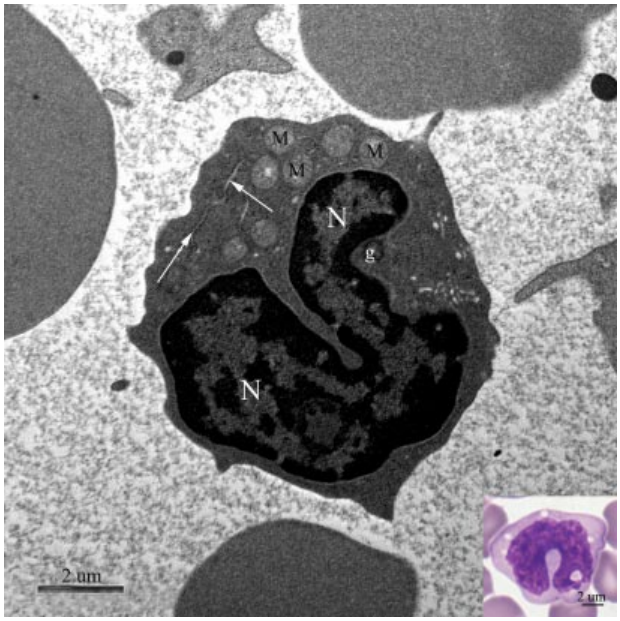

Figure 6 An electron micrograph of a long-tailed macaque's monocyte showing nucleus (N), mitochondria (M), azurophilic granule (g) and endoplasmic reticulum (arrows). A light micrograph of a monocyte is shown in the right bottom inset.

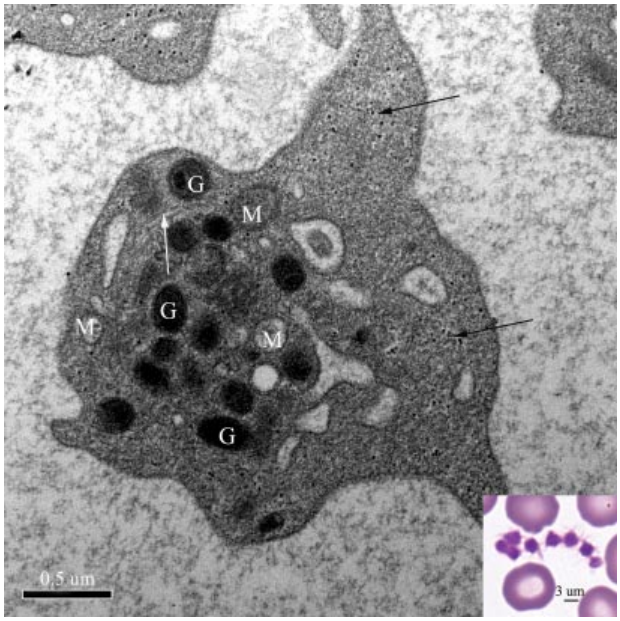

Figure 7 An electron micrograph of a stump-tailed macaque's platelet showing mitochondria (M), granules (G), glycogens (black arrows) and microtubule (white arrow). A light micrograph of platelets is shown in the right bottom inset.

reticulum is rarely seen. Ribosomes are generally small while glycogen granules and cytoplasmic inclusions can be infrequently observed in the cytoplasm. However, intranuclear fibrils (Stefani and Tonaki, 1970) and peculiar cytoplasmic inclusions (Hovig et al., 1968[a]) have also been reported. In this study, the cytoplasm of lymphocytes contains mitochondria and azurophilic granules. Macaque monocytes are the biggest agranulocytes. They appear relatively similar to that of most common domestic species (Nichols et al., 1971; Sonoda and Kobayashi, 1970). Ultrastructurally, the cytoplasm of monocytes in this study contains azurophilic granules, similar to those found in human cytoplasm (Tanaka and Goodman, 1972). The cytoplasm of platelets, in this investigation, has several organelles including granules, glycogens, mitochondria and microtubules. The granules have been thought to contain serotonin (Tranzer et al., 1966). Hovig et al. (1968b) reported that microtubules may be related to thromblasthenin, a contractile protein which is involved in clot retraction.

Conclusion

In this study, the structure of erythrocytes, neutrophils, basophils, lymphocytes, monocytes and platelets of macaques are similar to those of other mammals whereas eosinophils differ among several species. These findings are useful for identifying the blood cells of macaques and can assist the diagnoses of certain diseases, such as herpes-B virus (Mitsunaga et al., 2007) and human immunodeficiency virus (Robinson et al., 2007). Electron micrography is a valuable tool for studying cell cycle-related morphological changes in macaque blood cells in both normal and pathological conditions.

Acknowledgements

Financial support was provided by a research grant (RG 2/2549) from the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. The authors thank Dr. Somchai Chotapisitkul, Dusit Zoo, Bangkok, Thailand for his assistance in sample collection and the Department of Veterinary Anatomy, Faculty of Veterinary Science, Chulalongkorn University for their invaluable cooperation in this project. We also thank Assoc. Prof. Dr. Weerapong Koykul for reviewing the manuscript.

References

Alleman, A.R., Jacobson, E.R. and Raskin, R.E. 1992. Morphological and cytochemical characteristics of blood cells from the desert tortoise *(Gopherus agassizii)*. Am. J. Vet. Res. 53: 1645-1651.

Anderson, D.R. 1966. Ultrastructure of normal and leukemic leukocytes in human peripheral blood. J. Ultrastruct. Res. 9: 1-42.

Anosa, V.O. 1993. Ultrastructure of developing and mature caprine leukocytes. Anat. Histol. Embryol. 22: 328-341.

Dvorak, A.M., Monahan-Earley, R.A., Estrella, P., Kissell, S. and Donahue, R.E. 1989. Ultrastructure of monkey peripheral blood basophils stimulated to develop in vivo by recombinant human interleukin 3. Lab. Invest. 61: 677-690.

Groves, C. 2005. Macaques. In: Mammal Species of the World. 3rd ed. Wilson, D.E. and Reeder, D.M. (ed.). Johns Hopkins University Press. 161-165.

Hovig, T., Jeremic, M. and Stavem, P. 1968[a]. A new type of inclusion bodies in lymphocytes. Scand. J. Haemat. 5: 81-96.

Hovig, T., Jorensen, L., Packham, M.A. and Mustard, J.F. 1968[b]. Platelet adherence to fibrin and collagen. J. Lab. Clin. Med. 71: 29-40.

Jain, N.C. 1986. The eosinophils. In: Schalmís Veterinary Hematology. Philadelphia: Lea & Febiger: 731-755.

Jain, N.C. 1993. Essentials of Veterinary Hematology. Philadelphia: Lea & Febiger. 417pp.

Johnson, E.H., Muirhead, D.E., al-Busaidy, R. and Musa, B.E. 1999. The ultrastructural morphology of the camel eosinophil. Vet. J. 157: 79-84.

McEwen, B.J. 1992. Eosinophils: a review. Vet. Res. Commun. 16: 11-14.

Mitsunaga, F., Nakamura, S., Hayashi, T. and Eberle, R. 2007. Changes in the titer of anti-B virus antibody in captive macaques *(Macaca fuscata, M. mulatta, M. fascicularis)*. Comp. Med. 57: 120-124.

Nafstad, H.J. and Nafstad, I. 1968. An electron microscopic study of normal blood and bone marrow in pigs. Vet. Pathol. 5: 451-470.

Nichols, B.A., Bainton, D.F. and Farquhar, M.G. 1971. Differentiation of monocytes. Origin, nature and fate of their azurophilic granules. J. Cell Biol. 50: 498-515.

Osaka, R. 1959. An electron microscopic observation on the specific granules of eosinophil leukocytes of vertebrates. Acta Haemat. Jap. 22: 134-139.

Pothiwong, W., Sakulwira, K., Prachammuang, P. and Vetchagarun, S. 2006. Observations on the structures of red blood cells, white blood cells and platelets in some *Felis* spp. and *Panthera* spp. Thai J. Vet. Med. 36: 55-62.

Pothiwong, W. and Vetchagarun, S. 1986. Electron microscopic study from blood cells and platelets of donkeys. Kasetsart Anim. Hosp. J. 2: 199-208.

Pothiwong, W., Vetchagarun, S., Sajjarengpong, K. and Adirekthaworn, A. 2002. An electron microscopic study from blood cells and platelets of swamp buffaloes *(Bubalus bubalis)*. Thai J. Vet. Med. 32: 43-52.

Robinson, T.M., Sidhu, M.K., Pavlakis, G.N., Felber, B.K., Silvera, P., Lewis, M.G., Eldridge, J., Weiner, D.M. and Boyer, J.D. 2007. Macaques co-immunized with SIV *gag/pol*-HIV env and IL-12 plasmid have increased cellular responses. J. Med. Primatol. 36: 276-284.

Sakulwira, K., Pothiwong, W., Prachammuang, P. and Vetchagarun, S. 2004. The structure of red blood cells, white blood cells and thrombocytes in freshwater crocodiles *(Crocodylus siamensis)*. Thai J. Vet. Med. 34: 83-90.

Salakij, C., Salakij, J., Apibal, S., Narkkong, N., Chanhome, L. and Rochanapat, N. 2002. Hematology, morphology, cytochemical staining, and ultra-structural characteristics of blood cells in king cobra *(Ophiophagus hannah)*. Vet. Clin. Path. 31: 116-126.

Shively, J.N., Feldt, C. and Davis, D. 1969. Fine structure of formed elements in canine blood. Am. J. Vet. Res. 30: 893-905.

Skalak, R. and Branemark, P.I. 1969. Deformation of red blood cells in capillaries. Science 164: 717-719.

Sokol, R.J., Hudson, G., Wales, J. and James, N.T. 1988. Morphometry of human blood leukocyte ultrastructure: its potential value in haematology. Haematologia (Budap) 21: 129-139.

Sonoda, M. and Kobayashi, K. 1966. Electron microscopic observations on the blood of the horse. I. Neutrophils in the peripheral blood of the clinically healthy horse. Jpn. J. Vet. Res. 14: 71-78.

Sonoda, M. and Kobayashi, K. 1970. Monocytes of canine peripheral blood in electron microscopy. Jpn. J. Vet. Res. 18: 67-69.

Stefani, S.S. and Tonaki, H. 1970. Fibrillar bundles in the nucleus of blood lymphocytes from leukemic and non-leukemic patients. Blood 35: 243-249.

Tanaka, Y. and Goodman, J.R. 1972. Electronmicroscopy of human blood cells. New York: Harper & Row. 432 pp.

Taylor, D.W., Perri, S.F., Sorensen, C.A. and Palumbo, N.E. 1980. A comparative study of rosette formation in 12 species of nonhuman primates. J. Med. Primatol. 9: 76-82.

Tranzer, J.P., Pletscher, A. and Da Prada, M. 1966. The increase of 5-hydroxytryptamine in submicroscopic organelles of blood platelets. Helv. Physiol. Pharmacol. Acta. 68: 108-110.

Ward, J.W., Wright, J.F. and Wharran, G.H. 1972. Ultrastucture of granulocytes in the peripheral blood of the cat. J. Ultrastruct. Res. 39: 389-396.

Watanabe, I., Donahue, S. and Hoggatt, N. 1967. Method for electron microscopic studies of circulating human leukocytes and observation of their fine structure. J. Ultratruct. Res. 20: 336-382.

Yamada, Y. 1970. The leukocytes of ovine peripheral blood in electron microscopy. Jpn. J. Vet. Res. 18: 99-106.

Zucker-Franklin, D. 1967. Electron microscopic study of human basophils. Blood 29: 878-890.

หน้า 26
โฆษณา ขาวดำ
บ. เวท เรคคอมเมนท์ จำกัด