The Ultrastructure of Common Palm Civet Blood Cells and Platelets

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Abstract

The ultrastructure of blood cells and platelets were studied by collecting blood samples from the cephalic vein of anaesthetized common palm civets of both sexes. The average diameters of red blood cells, platelets, neutrophils, eosinophils, basophils, lymphocytes and monocytes were 4.0, 2.6, 6.5, 7.2, 7.0, 6.4 and 8.5 μm respectively.

The neutrophils had two types of granules, the primary and the secondary granules. The primary granules were larger, round and more electron dense. They were called lysosomes in other cell types and had average diameter about 0.18 μm (n=35). The secondary or specific granules were smaller and varied in density and shape. The average diameter of round granules was about 0.13 μm (n=100) and the average of the rod length was about 0.42 μm (n=35). The ratio of the primary and the secondary granules was about 2:3.

The specific granules of eosinophils were large round-shaped (0.9 μm, n=50) and high electron dense while those of basophils were large spindle-shaped (0.65 μm x 1.45 μm, n=25) with thick membrane. The ultrastructures of other organelles were similar to that observed in human and other mammals.

1. Introduction

The common palm civet (*Paradoxurus hermaphroditus*) is a carnivore of the Viverridae family. Other members of this family are many other civet species and mongoose[1]. The distribution of common palm civet is from Kashmir through India to Ceylon, southern China to Indochina, every part of Thailand, Malaysia, Sumatra, Java, Borneo, Philippines, and other smaller Indonesian islands. The animal has spots on the flanks and the hair on the side of the neck forms a whorl. The general body color is grayish with the muzzule, ears, lower legs and distal half of the tail black. There is a white mask across the forehead, a small white patch under each eye, one white spot on each side of the nostrils and a narrow dark line between the eyes. On the back, black spots merge to form three indistinct lines running longitudinally from both shoulders to the root of the tail; on the flanks, the black spots lie in rows separately. This species was originally forest living, now it has adapted to live near areas of human habitation, coming out at night to catch rats, mice and some fruit [1].

The research work on the morphology of blood cells in wild animals at the light microscopic level has already been done on mountain lion, leopard and cheetah but not at the electron microscopic level. There are many kinds of wild animals in Thailand and the Department of Zoology, Kasetsart University can get these wild animals for studies in gross anatomy so it was a good opportunity to use these specimens in this research field. The results could be used as references in future work to compare with other Thai native wild animals.

2. Materials and Methods

2.1 Blood Collection

Adult common palm civets of both sexes with an average weight of about 4 kg. were captured from the Khoakewe Open Zoological Park, Ministry of Forestry, Chonburi province in January 1995 and were sent to the Department of Zoology within one week.

Blood samples were collected from the cephalic vein of anaethetized common palm civets and transfered to 10 ml. vials, mixed gently with the anticoagulant (EDTA: ethylenediamine disodium tetraacetate) (May&Baker Ltd.,England) by the ratio of 1 ml. of blood to 1 mg. of EDTA and immediately processed for the transmission electron microscopic studies.

2.2 Blood Cells Preparation for Transmission Electron Microscopic Studies

A mixture of blood and EDTA was transfered into a small capillary tube (Vitrex, Denmark). The bottom of the tube was plugged with plasticene and centrifuged (Kokusan, Japan) at 2,500 cycles/min. for 10 minutes. The blood was separated into 2 layers with platelets and white blood cells in buffy coat on the top of the red blood cell layer. The capillary tube, a few millimeters above the buffy coat was cut using a diamond knife. Cell layers were pushed out of the capillary tube using a small wooden stick inserted beneath the bottom. Both layers were fixed in 2.5% glutaraldehyde (EMS, U.S. A) overnight. Washed 3 times in 0.2 M phosphate buffer pH 7.2 for 10 minutes each time and postfixed in 2% omium tetroxide (EMS, U.S.A.) for 2 hours. Then washed 3 times in distilled water for 10 minutes each time and processed by Conventional technique.

The TEM was JEM 100SX and JEM 200CX (Jeol, Japan) of Scientific and Technological Research Equipment Center, Chulalongkorn University, were operated at 65 and 80 kV respectively. The photographs were taken at the amplification x3,700-x30,000.

3. Results

3.1 Red Blood Cells (RBC)

Red blood cells of common palm civet were round with biconcave shape without nuclei and organelles. The cytoplasm was homogenous similar to the RBC of other mammals[4] (Fig. 1,5,12). The average disc diameters (Mean $\pm$ S.D.) were 4.0 ± 0.84 μm (n=40).

3.2 Blood Platelets

Platelets of common palm civet were biconvex disklike cell fragments. They could be round or oval and non-nucleated with some pseudopodia. The average disc diameters (Mean $\pm$ S.D.) were 2.6 ± 0.61 μm (n=25). Platelets contained a system of channels called the open canalicular system. Organelles found were mitochondria, electron dense granules and mostly found microtubules located around the edge of the platelets.

There were two types of granules distributed mainly in the central part of the platelets[9]. (Fig. 2,12) The alpha granules were either round or oval and lower electron dense with average diameter (Mean $\pm$ S.D.) of 0.45 ± 0.19 μm (n=50). The membrane-bound dense granules were smaller and very high electron dense with average diameter (Mean $\pm$ S.D.) of 0.29 ± 0.09 μm (n=20).

3.3. White Blood Cells (Leukocytes)

3.3.1. Granulocytes

3.3.1.1 Neutrophils (Fig. 3, 4). The diameter of neutrophils of common palm civet varied in size from 5.0-8.0 μm (Mean $\pm$ S.D. = 6.5 ± 1.59 μm) (n=40). They could be round or irregular shapes. The nuclei had 1-4 lobes and the nuclear membrane was a double layer membrane. Each lobe contained two parts of chromatin. The heterochromatin had high electron density located at the periphery of the lobe and the euchromatin had lower electron density located at the central of the lobe. The cytoplasm contained two types of granules of different sizes and electron density. The more abundant and lower electron dense granules were specific granules or secondary granules. They were round with average diameter (Mean $\pm$ S.D.) of 0.13 ± 0.09 μm (n=100) or in rod shaped form with average length (Mean $\pm$ S.D.) of 0.42 ± 0.12 μm (n=35). The other type was primary granules or azurophilic granules. They were round and more electron dense than specific granules and had larger average diameter (Mean $\pm$ S.D.) of 0.18 ± 0.10 μm (n=35). These granules were primary lysosomes in other types

of white blood cells. The ratio of the primary and the secondary granule was about 2:3. The other organelles that could be examined were mitochondria, free ribosomes and Golgi complex (Fig. 3).

3.3.1.2 Eosinophils (Fig. 5, 6). The eosinophils of common palm civet varied in size from 6.6-7.8 μm (Mean ± S.D. = 7.2 ± 0.74 μm in diameter (n=15)). They could be round or oval shapes with 1-2 lobes of nuclei, with double layers of nuclear membrane. Each lobe contained two parts of chromatin similar to those in neutrophils. Cytoplasm contained very large round, high electron dense specific granules with average diameter (Mean ± S.D.) of 0.9 ± 0.31 μm (n=50). The small amount of vacuoles and other organelles such as rough endoplasmic reticulum and mitochondria could be seen in some eosinophils.

3.3.1.3 Basophils (Fig. 7, 8). The basophils of common palm civet varied in size from 6.5-7.4 μm (Mean ± S.D. = 7.0 ± 0.65 μm in diameter (n=5)). They were round shape with 1-2 lobes of nuclei, with double layers of nuclear membrane. Each lobe cantained two parts of chromatin similar to those in neutrophils and eosinophils. Cytoplasm contained very large spindle-shaped specific granules with average diameter (Mean ± S.D.) of 0.65 ± 0.24 μm in width and of 1.45 ± 0.47 μm in lenght (n=25). These granules were medium electron dense type that were surrounded by thick but less electron dense unit membrane. Many mitochondria were located on the opposite side of the specific granules and some vacuoles were also found.

3.3.2. Agranulocytes

3.3.2.1 Lymphocytes (Fig. 9, 10, 12). The lymphocytes were round and varied in size from 4.6-7.2 μm (Mean ± S.D. = 6.4 ± 0.97 μm in diameter (n=40)). There were many cytoplasmic projections from the cell membrane called the microvilli. The nuclei were large round or oval and surrounded by double layers of nuclear membrane. The nucleus contained two parts of chromatin similar to those of other leukocytes. Cytoplasm also contained mitochondria, azurophilic granules or lysosomes, free ribosomes, some vacuoles, and in some cells there were rough endoplasmic reticulum.

3.3.2.2 Monocytes (Fig. 11, 12). The monocytes of common palm civet varied in size from 8.1-9.2 μm (Mean ± S.D. = 8.5 ± 0.83 μm in diameter (n=10)) with microvilli similar to those of lymphocytes. They could be round or oval shapes with a large oval, horseshoe-shaped or kidney-shaped nucleus and were eccentrically placed. Nucleus was surrounded by double layers of nuclear membrane and contained two parts of chromatin similar to those found in other leukocytes and some contained 1-2 nucleoli. Cytoplasm also contained mitochondria, azurophilic granules or lysosomes, free ribosomes, phagosomes, and in some cells there were rough endoplasmic reticulum at the edge.

4. Discussion and Conclusion

These studies showed that ultrastructures of common palm civet blood cells and platelets were similar to those of human and other mammals. Cell sizes and some organelles that were found in each blood cell types were slightly different. The most obvious difference was the morphology of the cytoplasmic granules in some granulocytes. The neutrophils had two types of granules; the larger were round with more electron dense primary granules, and the smaller secondary or specific granules varied in density and shape, similar to those found in human neutrophils[2,3]. They could not be separated into three types as in the rabbits[4,5]. The specific granules of eosinophils were large round-shaped with high electron dense but did not have very high electron band called the crystalloid characteristic. Whereas the specific granules in human and other mammals are spindle or round shaped and have a crystalloid characteristic. The crystalloid morphology varies among species such as needle-like in rabbits[3,4], doughnut or cylindrical shape in cats[3], long band in human and mouse[3] and can not be found in donkeys [6]. The specific granules in basophils were large spindle-shaped with thick membrane whereas in human and other mammals were round-shaped granules. Human basophils also had the myelinlike granule[7].

The ultrastructures of eosinophilic and basophilic specific granules in common palm civet were similar to those studied in the light microscopic examination[8]. They reported that the specific granules of common palm civet eosinophils and bosophils in Wright's Giemsa stain were large, round, pink stained granules; and large spindle-shaped, grayish-blue stained granules respectively. Ultrastructures of other observed organelles were not different to those in human and other mammals excepted their sizes and numbers. According to the literatures review, we could not find research work on the morphology of blood cells in wild animals at the electron microscopic level. Studies, only at the light microscopic level, were done on mountain lion, leopard, cheetah, tiger, bear, elephant giraffe, raccoon, mink, deer and hyena [9].

The results of the blood cell and platelet ultrastructures could be used as references in the future works to compare with other Thai native wild animals.

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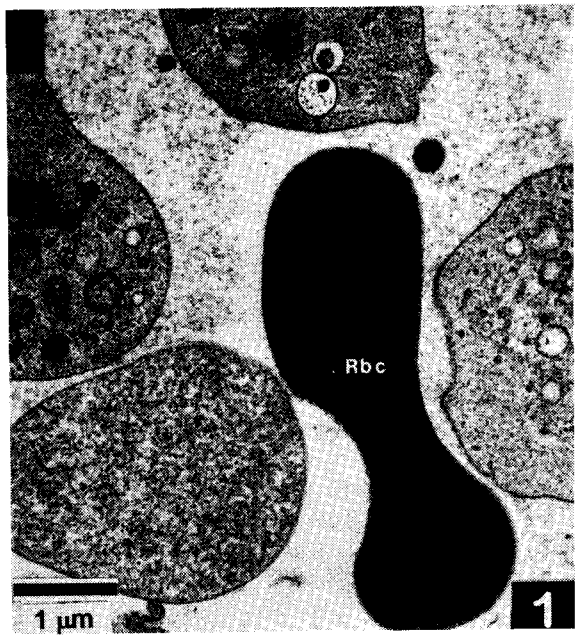

Fig. 1 TEM micrograph of a red blood cell among blood platelets.(x 15,000)

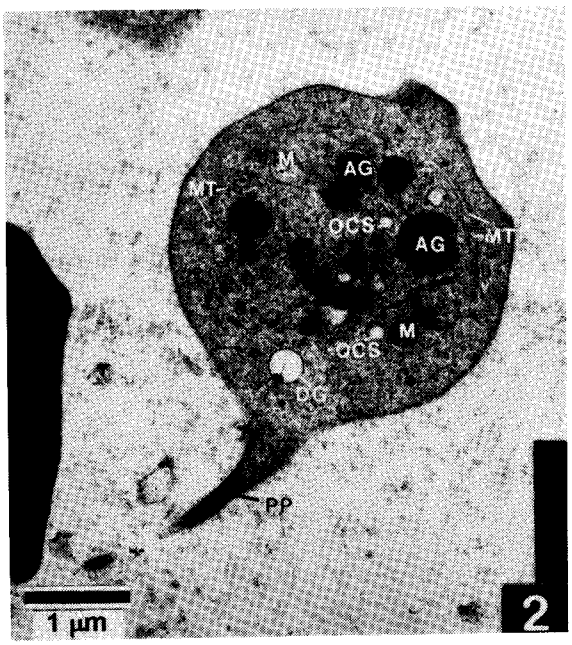

Fig. 2 TEM micrograph of a blood platelet. (x 15,000)

AG= *alpha granule*
DG= *very dense granule*
M = *mitochondria*
MT= *microtubule*
OCS= *open canalicular system*
PP= *pseudopodic projection*

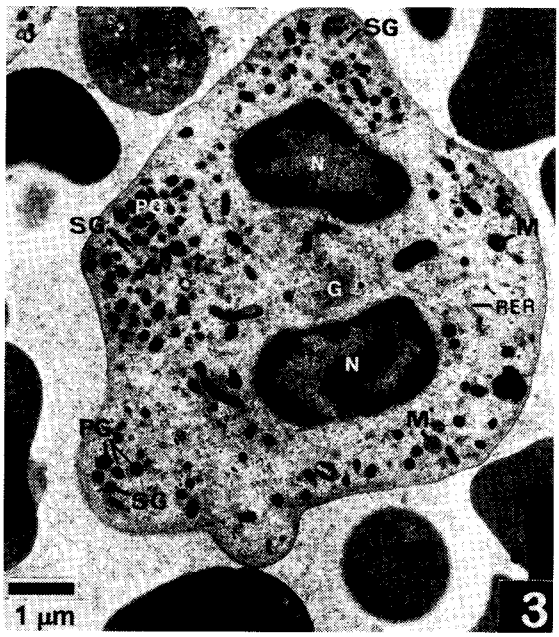

Fig. 3 TEM micrograph of a neutrophil. (x 9,000)

G = *Golgi complex*
M = *mitochondria*
N = *nucleus*
PG= *primary granule*
RER= *rough endoplasmic reticulum*
SG= *specific granule*

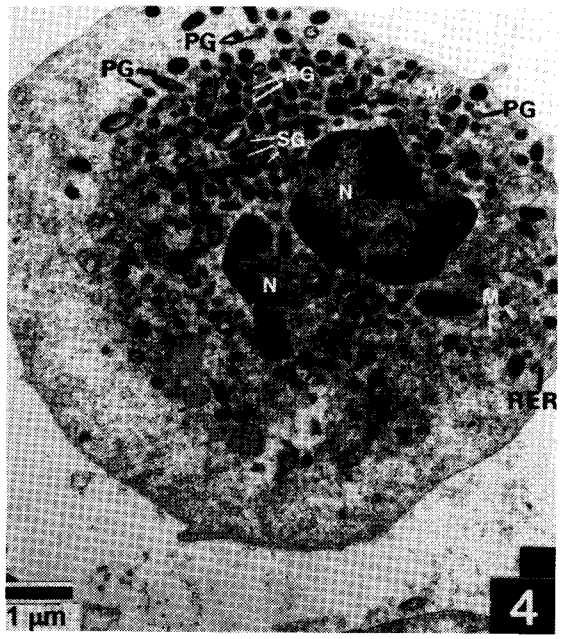

Fig. 4 TEM micrograph of a neutrophil.(x 10,950)

M = *mitochondria*
N = *nucleus*
PG= *primary granule*
RER= *rough endoplasmic reticulum*
SG= *specific granule*

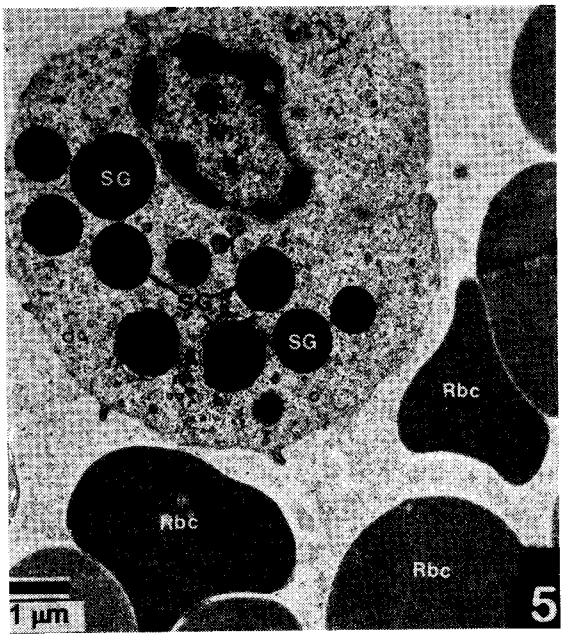

Fig. 5 TEM micrograph of an eosinophil among red blood cells. (x 9,000)
M = *mitochondria*
N = *nucleus*
SG= *specific granule*
Rbc= *red blood cell*
V = *vacuole*

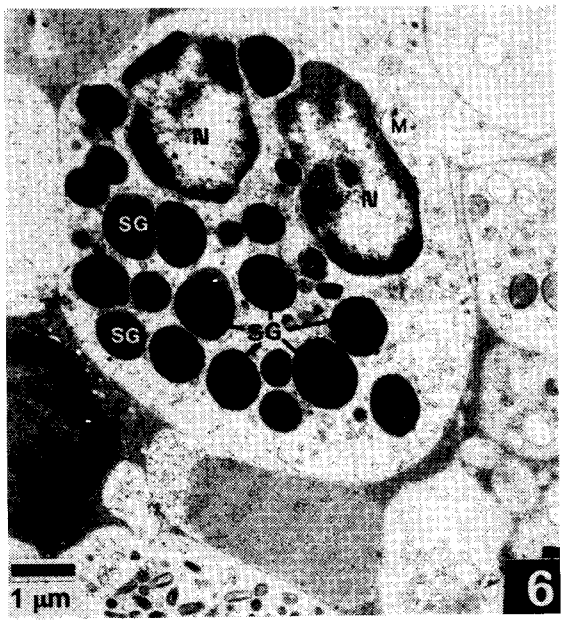

Fig. 6 TEM micrograph of an eosinophil.(x 9,000)
M = *mitochondria*
N = *nucleus*
SG= *specific granule*

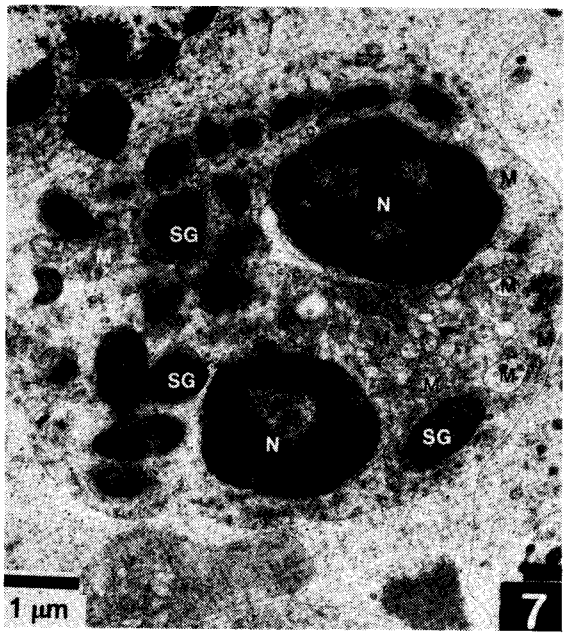

Fig. 7 TEM micrograph of a basophil. (x 10,950)
M = *mitochondria*
N = *nucleus*
SG= *specific granule*
V = *vacuole*

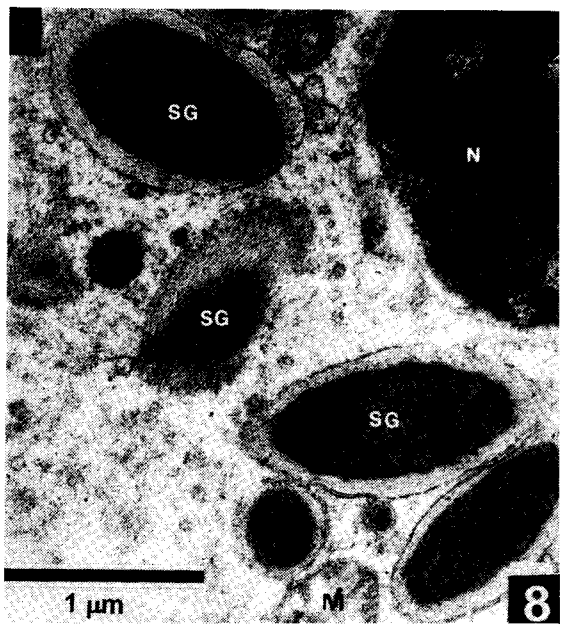

Fig. 8 TEM micrograph of a basophil. (x 30,000)
M = *mitochondria*
N = *nucleus*
SG= *specific granule*

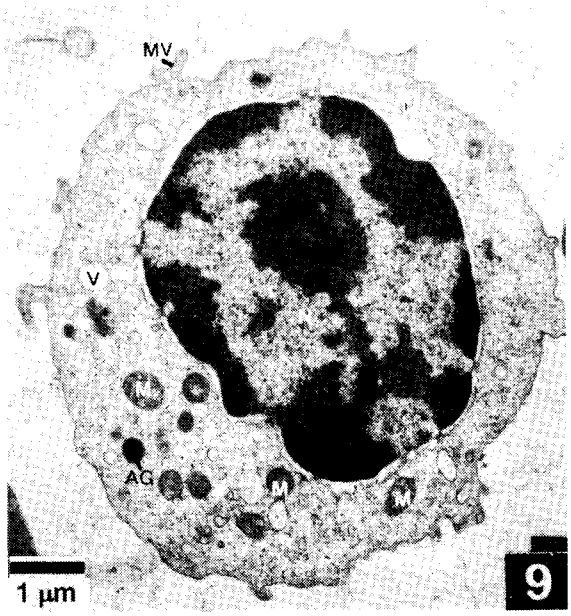

Fig. 9 TEM micrograph of a lymphocyte.(x 10,950)

M = *mitochondria*
N = *nucleus*
AG= *azurophilic granule*
MV= *microvilli*
V = *vacuole*

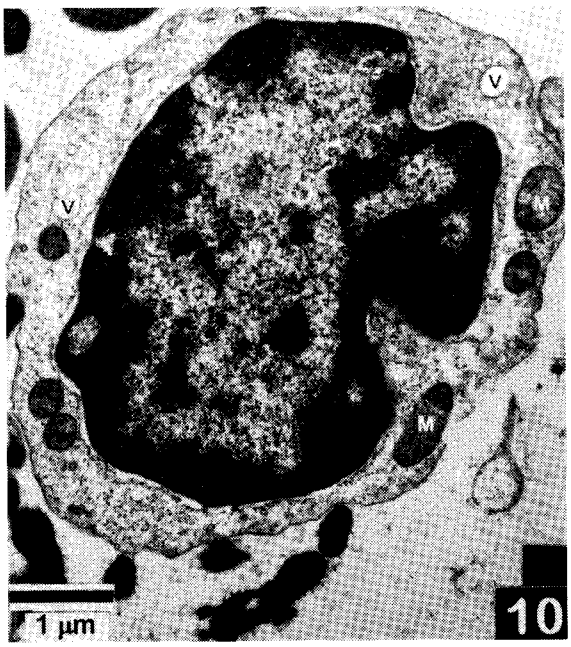

Fig. 10 TEM micrograph of a lymphocyte. (x 10,950)

M = *mitochondria*
N = *nucleus*
V = *vacuole*

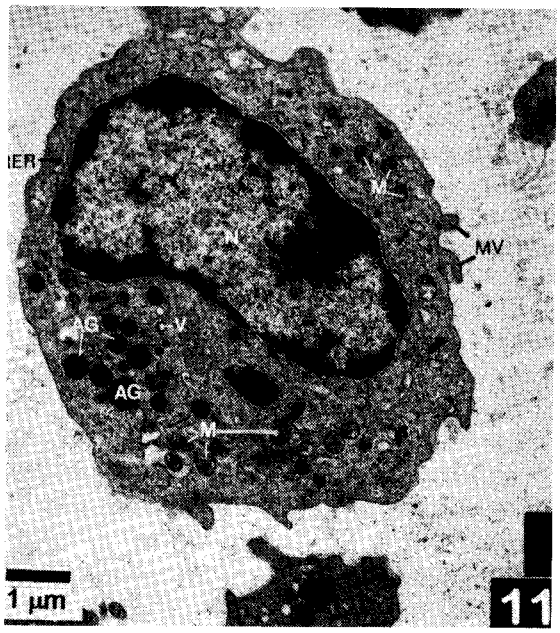

Fig.11 TEM micrograph of a monocyte. (x 9,000)

M = *mitochondria*
N = *nucleus*
AG= *azurophilic granule*
MV= *microvilli*
RER= *rough endoplasmic reticulum*
V = *vacuole*

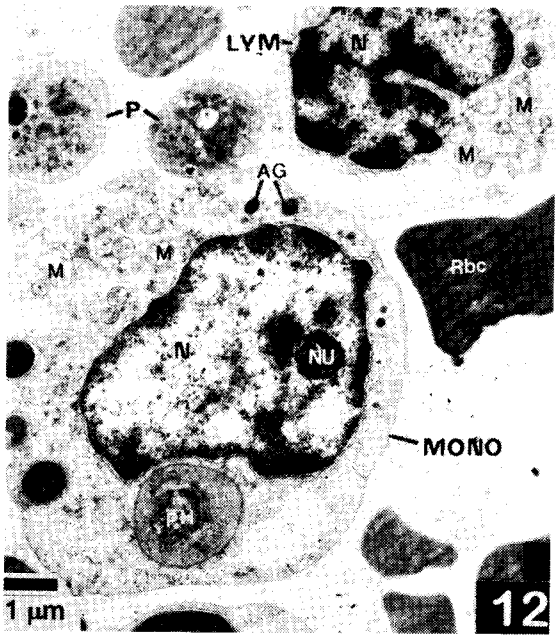

Fig.12 Comparision in sizes of monocyte (MONO) and lymphocyte (LYM). Some platelets and red blood cells are shown. (x 7,500)

AG= *azurophilic granule*
N = *nucleus*
NU= *nucleolus*
M = *mitochondria*
P = *platelet*
PH= *phagosome*
Rbc= *red blood cell*