Kasetsart J. (Nat. Sci.) 18 : 192—202 (1984)

Electron Microscopic Structure of White Blood Cells of Swamp Buffaloes *(Bubalus bubalis)* I. Lymphocytes and Monocytes

Somchai Pongjunyakul[1] Worawidh Wajjwalku[2] and Teerasak Prapong[1]

ABSTRACT

Lymphocytes and monocytes from the buffy coat of 10 adult swamp buffaloes *(Bubalus bubalis)* were studied. General feature of the lymphocytes was round or oval and the cell outline was slightly irregular. The nuclei were round or oval and had indentations. The cytoplasm contained abundant ribosomes. Two to ten round, oval or elongated mitochondria were seen. The small Golgi complex was situated near the nuclear indentation and was closely associated with the centrioles. There was a small quantity of endoplasmic reticulum while the number of azurophilic granules was quite variable.

The monocytes were round or oval and were the largest among the cell types appearing on the electron microscope. The nuclei were lobule, round, oval or irregular round, in which consisted of fine chromatin granules, distributed compactly or loosely. A few to several dense round, oval or rod-shape granules were observed in the cytoplasm. Several round, oval or elongated mitochondria with clear cristae were seen. The centrioles and ill or well-developed Golgi complex were present near the nuclear indentation. A great number of smooth-surfaced endoplasmic reticulum were distributed on over the cytoplasm while a few rough-surfaced endoplasmic reticulum were also seen.

INTRODUCTION

Lymphocytes and monocytes are regarded as the agranulocytes of white blood cells with similar morphologic features. The general microscopic and ultrastructural features of the lymphocytes and monocytes are well recognized and the hematologic investigator has analyzed these cells with a wide variety of cytologic techniques. Electron microscopic studies of lymphocyte have been directed primarily toward the characterization of the typical lymphocyte. (Brittinger, G. et al, 1968; Brooks, R.E. and Siegel, B.V., 1966; Heiniger, H.J. et al, 1967; Low, F.N., 1960; Movat, H.Z. and Fernando, N.V.P., 1964; Murray, R.G. et al, 1965; Weber, A.F. and Joel, D., (1966) Subsequently the ultrastructures of lymphocytes have been studied in pig (Nafstad Per, H.J. and Nafstad, I. 1968), dog (Sonoda, M. and Kobayashi K., 1970) and man (Ackerman G.A., 1970).

Monocyte is the largest of all the leukocytes. There is considerable difficulty in identifying some monocytes, because there are transitional forms between the small and large lymphocytes, all of which resemble one another. However, cytochemical identification of monocyte has been studied (Yam, L.T. et al, 1971) and the ultrastructures of monocytes have been described in pig (Nafstad Per, H.J. and Nafstad I., 1968) and dog (Sonoda, M. and Kobayashi, K. 1970)

In the present investigation, lymphocytes and monocytes of swamp buffaloes *(Buba-*

1 Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University.
2 Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University.

lus bubalis) have been studied. The fine structures of lymphocytes and monocytes will be described.

MATERIALS AND METHODS

Observations were made on materials obtained from 10 adult swamp buffaloes *(Bubalus bubalis).* Approximately 20 ml. of blood obtained by aortic puncture was collected in a sterile test tube with 15 mg. ethylenediaminetetraacetic acid (EDTA-2K) and was centrifugated at 4,000 rpm for 15 minutes. The plasma above the buffy coat was carefully withdrawn with a pipette and discarded, and cold 2.5% glutaraldehyde (pH 7.4) in sodium cacodylate buffer was added to the buffy coat. Thirty minutes later, the buffy coat was removed from the test tube, sliced into smaller pieces, and fixed in similar 2.5% glutaraldehyde solution for another one and a half hour. The tissues were washed in sodium cacodylate buffer for thrice and post-fixed in cold 1% osmium tetroxide for one to two hours. After dehydration in graded alcohols, the tissues were embedded in epon-araldite. The ultrathin sections were cut with glass knives on LKB V ultramicrotome. After mounting on copper grids the sections were double stained with uranyl acetate (stempak, J.G. and Ward, R.T., 1964) and lead citrate (Renolds, E.S. 1963) and examined under electron microscope (Hitachi Hu-12 A) at magnification varying from 7,000 – 60,000.

RESULTS

Lymphocytes

General feature of the lymphocytes was round or oval and the outline of the cells was slightly irregular with many sharp and blunt pseudopodic projections.

The nuclei were generally round or oval and frequently had blunt indentations or deep incisions, therefore, they looked irregular in form and they occupied the greater part of the cells. The nucleoplasm was mainly differentiated into a dark, densely stained chromatin beneath the nuclear membrane and pale central area. The nucleolus appearance was not so marked. In appropriate sections a nucleolus was located in the center of the nucleus.

The cytoplasm of the cell was limited by a fine membrane. This showed narrow band around the nucleus. The cytoplasm of lymphocyte was moderately dense and contained abundant ribosomes. Ribosomes were randomly distributed and their numbers vary within individual cells, fews were attached to the outer nuclear enveloped; many were aggregated and formed so-called polysomes. Two to ten mitochondria were usually seen in the cytoplasm of the lymphocytes in the thin sections. The shape of the mitochondria was round, oval and elongate and clear cristae were seen. The sizes of the round, oval and elongated ones were 0.57 (0.38 – 0.71) μ, 0.52 (0.4 – 0.67) by 0.71 (0.4 – 0.86) μ and 0.34 (0.33 – 0.35) by 1.38 (0.95 – 1.89) μ respectively. A few mitochondria, however, showed some disorientation of their cristae and some exhibited poorly defined internal structure. The Golgi complex was situated near the nuclear indentation and was closely associated with the centrioles. The Golgi was small and consisted of but few stacks of cisternae and vesicles.

There was a small quantity of endoplasmic reticulum which was mainly rough-surfaced endoplasmic reticulum, while smooth-surfaced endoplasmic reticulum was present, too. The number of azurophilic granules present in lymphocytes was quite variable. In some thin sections of lymphocytes no azurophilic granules may be seen while in others up to ten may be present. Ultrastructures of azurophilic granules were round to ovoid in contour, were electron dense, and were of variable size. Their size was 0.34 (0.31 – 0.39) μ in the present observations. Most azurophilic granules had homo-

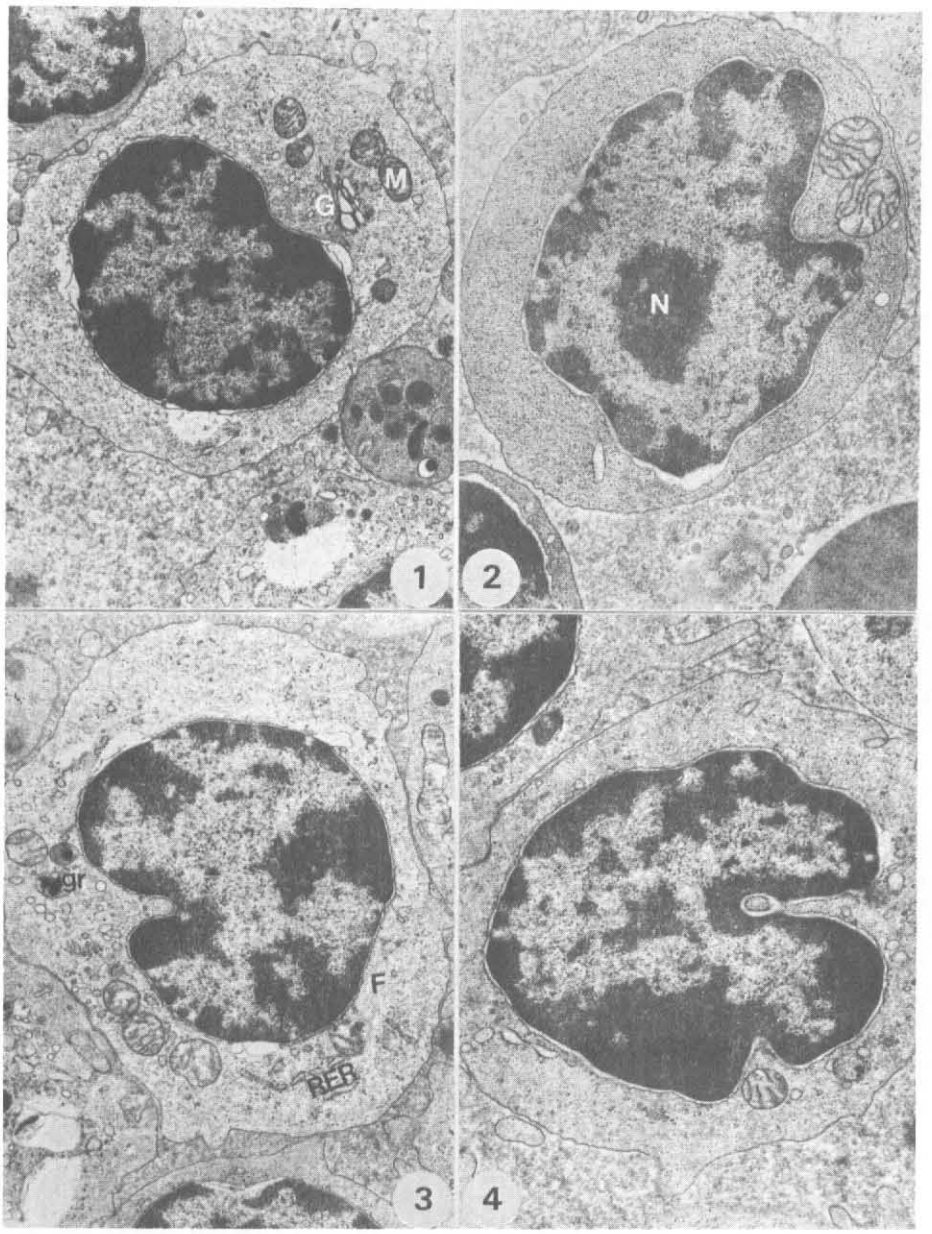

Plate I

General features of 4 lymphocytes are shown.

Fig. 1. The cell has oval nucleus and scanty cytoplasm. A few mitochondria (M) and the Golgi complex (G) are gathered in the part of cytoplasm. 9,300 x

Fig. 2 & 3 The nuclei have blunt indentations. A nucleolus (N) is clearly seen in the nucleus in figure 2. A few rough-surfaced endoplasmic reticulum. (RER) and groups of fibrils (F) are seen in the part of cytoplasm in figure 3. The dense granules (gr) are present. Fig. 2; 15,300x Fig. 3; 13,900 x

Fig. 4. The nucleus of the cell has a deep incision. 15,000 x

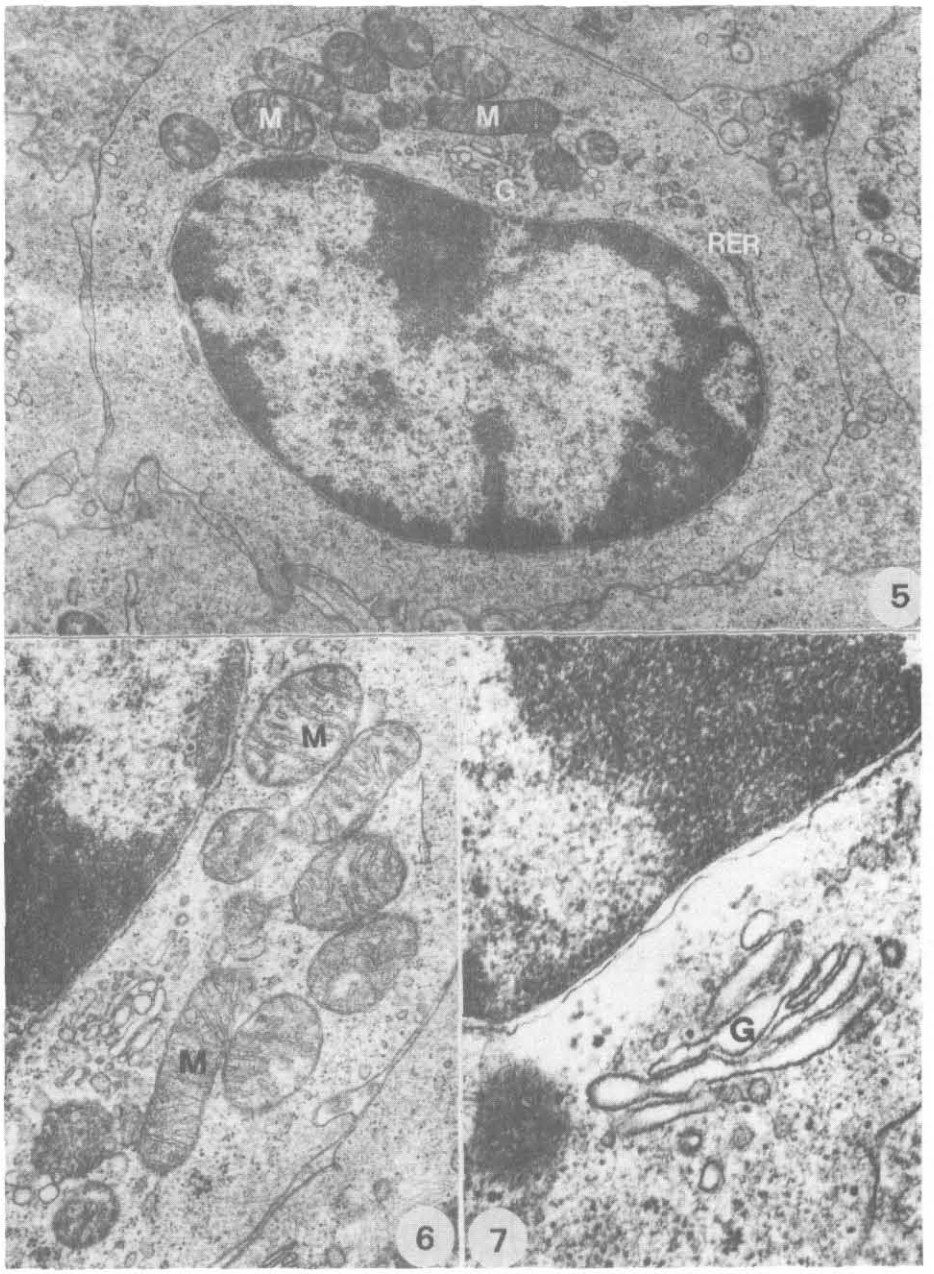

Plate II

Structures of lymphocytes.

Fig. 5. General feature of lymphocyte is shown. The nucleus is oval in form. Ten mitochondria (M), poorly-developed Golgi complex (G) and rough-surfaced endoplasmic reticulum (RER) are seen 15,750 x

Fig. 6. Different shapes of mitochondria (M), which have clear cristae, are shown. 26,800 x

Fig. 7. An ill-developed Golgi complex (G) consisting of small vesicles is seen in a part of the cytoplasm. 56,000 x

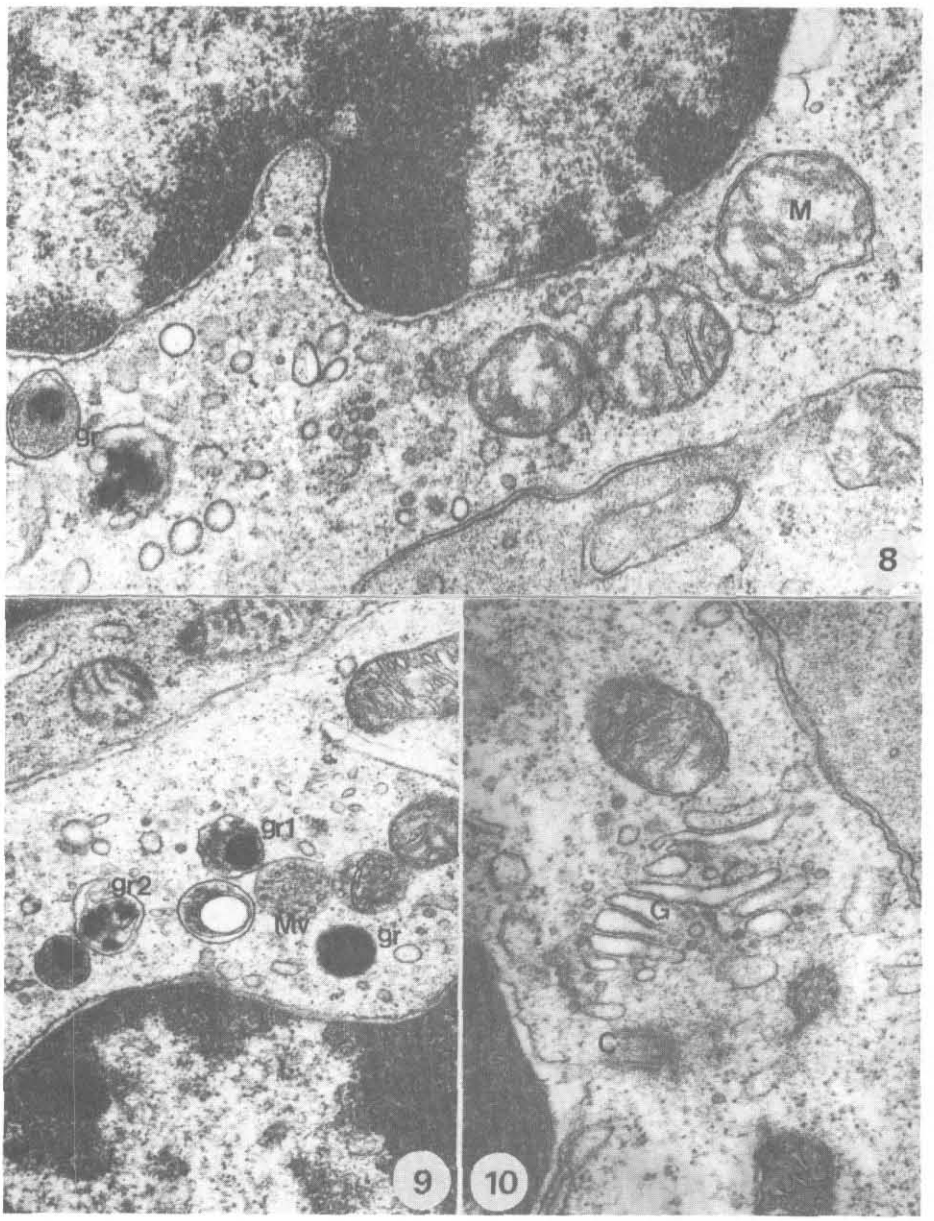

Plate III

Structures of lymphocytes.

Fig. 8. Three mitochondria (M) are present collectively in the cytoplasm near the nuclear indentation. Two dense granules (gr) are seen. 36,000 x

Fig. 9. The dense granules (gr) and the multivesicular bodies (MV) are shown. One azurophilic granule (gr) has homogeneous internal structure while one (grl) has an irregular density. Another (gr 2) has a membranous substance like a unit membrane in the matrix of granule. 30,300 x

Fig. 10. A centriole (C), which is closely associated with the Golgi complex (G), is seen. 40,000 x

geneous internal structure while others may had an irregular density, or may had dense central cores. Furthermore, in the matrix of granules, sometimes, a few membranous substances like a unit membrane were seen. A few multivesicular bodies were present in some lymphocytes. The density of the matrix material in the multivesicular bodies and also the individual internal vesicle may be quite variable. Small fibrils were seen occasionally in the cytoplasm. These fibrils may occured singly or in groups.

Monocytes

The general morphology of the monocyte was round or oval. The outline of the cell was irregular with many large and small cytoplasmic projections. The size of this cell type was the generally largest among the cell types appearing on the electron microscope.

The forms of the nuclei of the monocytes were lobule, round oval or irregular round. Almost of the nuclei had one lobe with deep indentation but some showed two lobes depending on the cut planes. The nuclei were bounded by clear nuclear membrane. The karyoplasm consisted of fine chromatin granules distributed compactly or loosely. The central part of the karyoplasm was light and the peripheral part was usually dark because of the aggregation of chromatin granules.

The cytoplasm of monocyte was filled with very fine particles and vesicles and they were gray in appearance. A few to several dense granules with variable sizes were observed. Almost all of them were round but some were oval or rod-shape which were bounded by clear membrane. In general, they were distributed in groups on the wide areas of cytoplasm near the concave part of the nuclei but some were anywhere in the cytoplasm. The size of the round one was 0.25 (0.14 – 0.33) μ in average diameter. Several mitochondria with clear cristae were seen in the cytoplasm. They were round, oval or elongate but most of them were round in form. The sizes of round and elongated ones were 0.39 (0.27 – 0.52) μ and 0.41 (0.38 – 0.48) by 1.12 (0.9 – 1.33) μ respectively. Near the nuclear indentation, there were ill to well-developed Golgi complexes consisting of cisternae and visicles. Near these areas, one or two centrioles were sometimes observed. A great number of smooth-surfaced endoplasmic reticulum were distributed on all over the cytoplasm. A few rough-surfaced endoplasmic reticulum with the short canalicular form were seen, too. A lot of ribosomes distributed evenly in the cytoplasm were observed. The multivesicular bodies were present in some monocytes. Near the nuclear membrane, small fibrils were observed in cytoplasm.

DISCUSSION

The ultrastructure of lymphocyte in swamp buffalo blood was similar to those described in human[1], (Ackerman, G.A., 1970), dog (Sonoda, M. and Kobayashi, K., 1970) and pig (Nafstad Per, H.J. and Nafstad, I., 1968). On the basis of these observations, scanty microorganelles, little developed endoplasmic reticulum, a reduced Golgi complex, few dense granules in the cytoplasm and slight condensation of chromatin granules in the nucleus seem to be the characteristics of the fine structures of the cells. However, in our observations, the number of ribosomes were vary within individual cells. The presence of scattered ribosomal clusters or polysomes indicated that the lymphocytes were engaged to some degree in protein synthesis, either for maintenance of cellular integrity or for other unknown functional activities. The Golgi complex of lymphocyte was small and ill-developed. Anyhow, in our results, the Golgi complex developed more than those described in man (Ackerman, G.A., 1970) and dog (Sonoda, M. and Kobayashi,

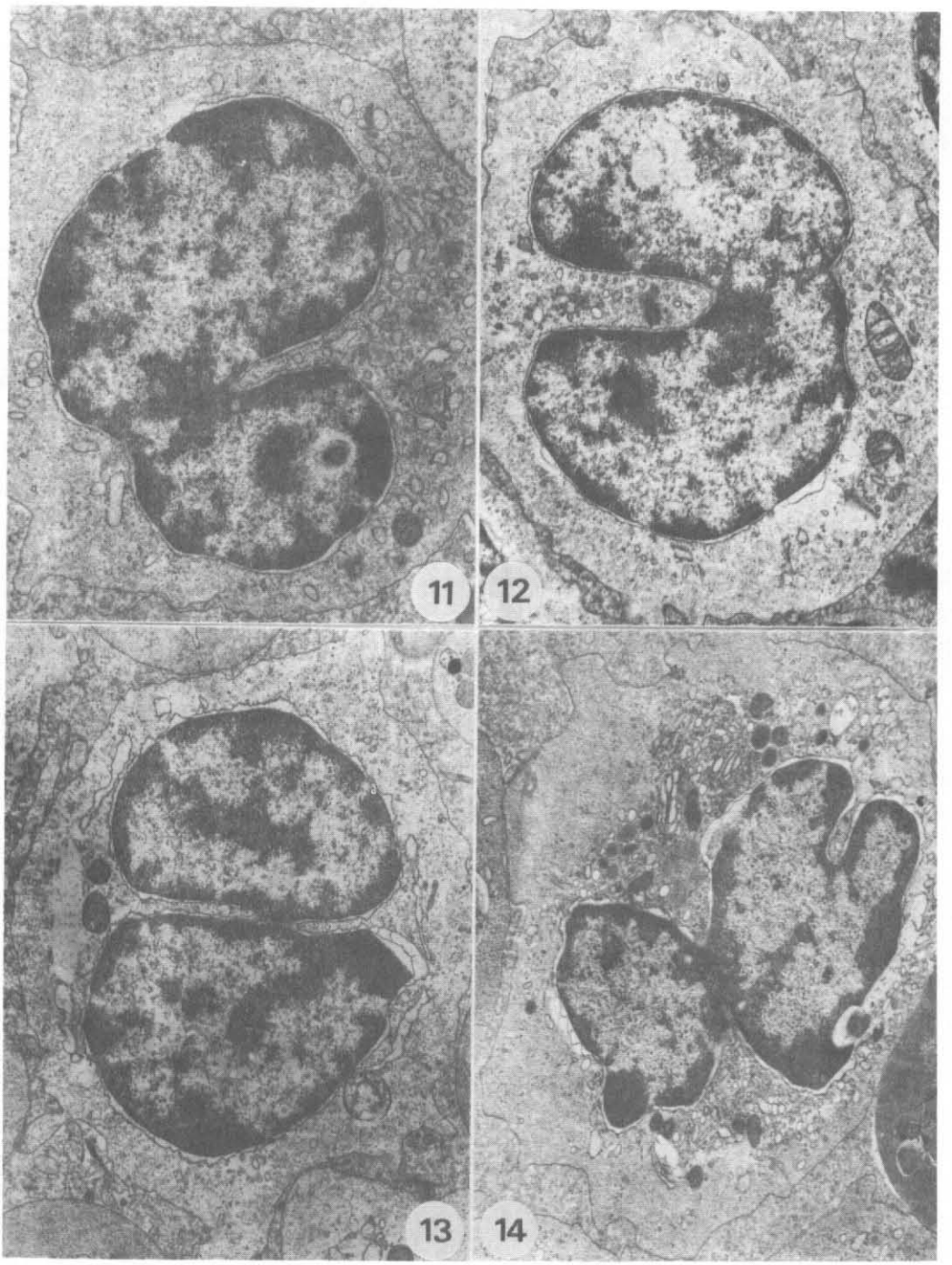

Plate IV

General features of 4 monocytes are shown.

Fig. 11—14 **The outlines of the cells are irregular and the lobulated nuclei are present. In cytoplasm, there are abundant ribosomes and a few mitochondria in figures 11, 12 and 13. Many dense granules and smooth-surfaced endoplasmic reticulum are present in figure 14. Fig. 11; 12,200 x, Fig. 12, 13; 10,600 x, Fig. 14; 11,500 x**

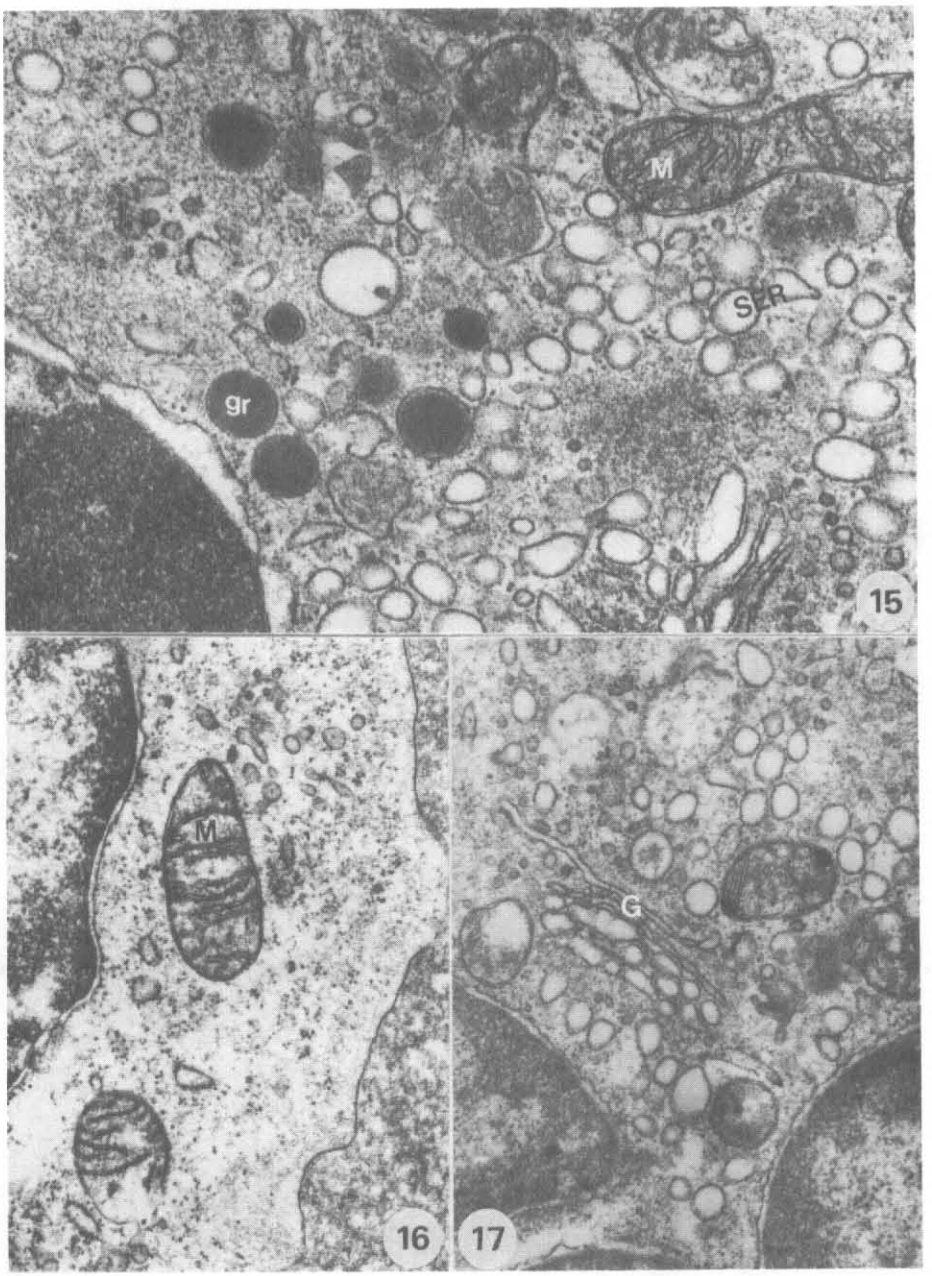

Plate V

Structures of monocytes

Fig. 15. Several dense granules (gr) are present in the part of cytoplasm. A great number of smooth-surfaced endoplasmic reticulum (SER) and a few mitochondria (M) are seen. 42,000 x

Fig. 16. The oval shaped mitochondria (M) with the clear cristae are present. 30,600 x

Fig. 17. A well-developed Golgi complex (G) is seen in the cytoplasm. 32,500 x

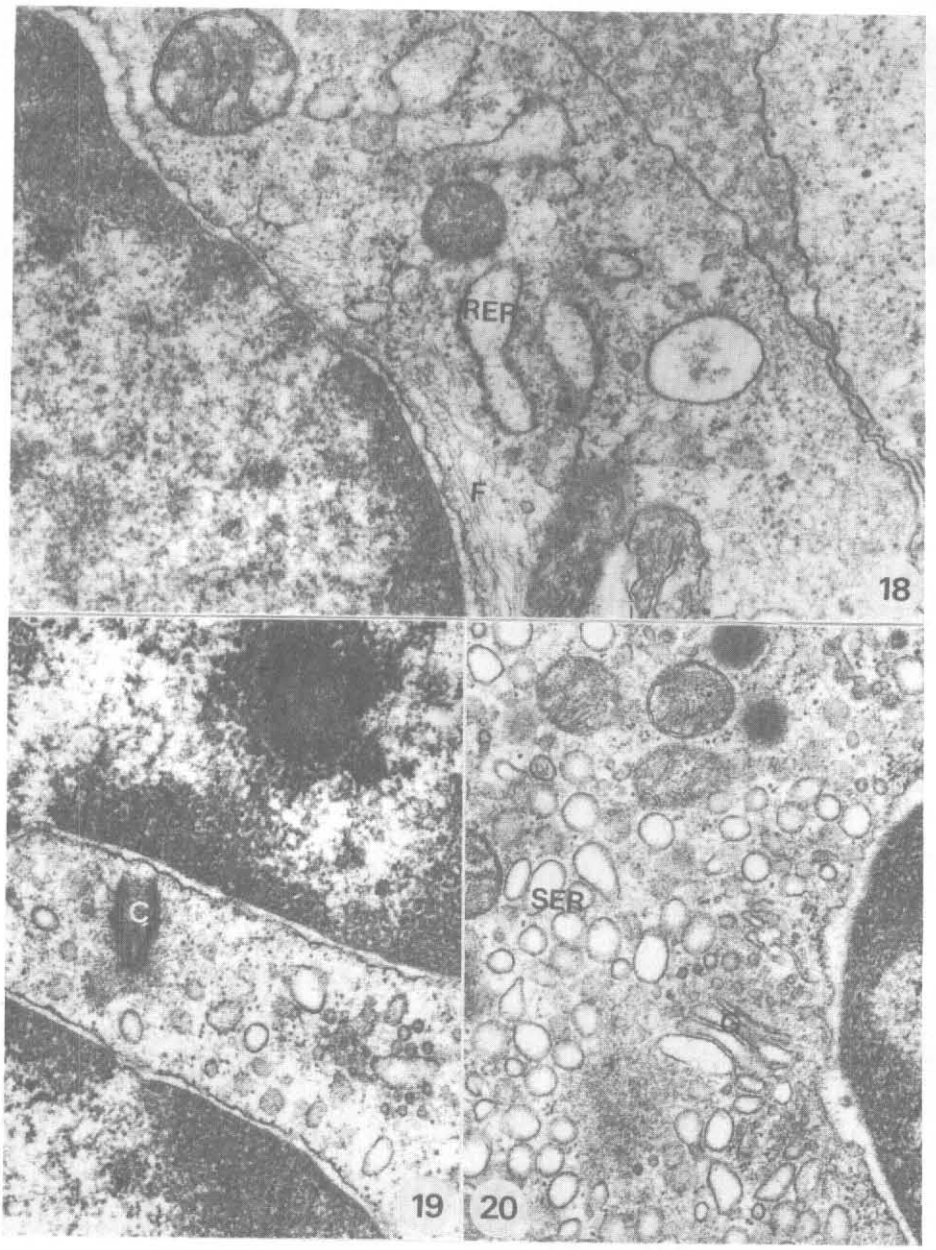

Plate VI

Structure of monocytes

Fig. 18. A few rough-surfaced endoplasmic reticulum (RER) with the short canalicular form are seen. Small fibrils (F) are observed in cytoplasm near the nuclear membrane. 43,100 x

Fig. 19. A centriole (C) is present in the cytoplasm near the nuclear indentation. 38,400 x

Fig. 20. Ill-developed Golgi complex (G) and a great number of smooth-surfaced endoplasmic reticulum (SER) are seen. 32,600 x

K. 1970). The size and complexity of the Golgi in lymphocytes was apparently related to the degree of cellular metabolism and protein synthesis. The sizes of mitochondria in these observations were nearly the same as those described in dog (Sonoda, M. and Kobayashi, K., 1970) and some disorientation of their cristae may be due to the mitochondrial activity.

Our studies of the monocytes revealed a round, oval, irregular round and lobulated nuclei, well-developed smooth-surfaced endoplasmic reticulum and a lot of mitochondria, which closely parallelled to the description of the monocyte in human (Low, F.N. and Freeman, J.A. 1959) and dog (Sonoda. M. and Kobayashi, K., 1970). The mitochondria of monocytes were smaller than those of the lymphocytes. This result was similar to those reported in human (Low, F.N. and Freeman, J.A., 1959 and dog (Sonoda M. and Kobayashi K. 1970). In the present observations, it was shown that in general the monocytes did not have any dense granules in their cytoplasm and only few monocytes had quite exceptionally dense granules with varying large sizes in cytoplasm, in which were similar to those reported in human (Low, F.N. and Freeman, J.A., 1959). In contrast, dense granules were always present in the cytoplasm of the canine monocyte (Sonoda, M. and Kobayashi, K., 1970) and it was demonstrated that bovine monocyte contained numerous fine azurophilic granules in light microscope (Schalm, O.W. etal, 1975).

Acknowledgements

This investigation was supported by grants from Kasetsart University Research and Development Institute. The references were kindly supported by Dr. Claes Rhebinder, Assistant Director of FAO/SIDA International Postgraduate Programme in Veterinary Pathology. The authors wish to thank Professor Gosta Winqvist for his advice in collecting blood sample technique.

LITERATURE CITED

Ackerman, G.A. 1970. Structural studies of the lymphocyte and lymphocyte development, Regulation of hemopoiesis, Vol. II. A.S., Gordon (ed.). Appleton-Century-Crofts, New York. pp. 1,297–1,337.

Brittinger, G., R. Hirschhorn, S.D. Douglas and G. Weissmann. 1968. Studies on lysosomes : XI. characterization of a hydrolase rich fraction from human lymphocytes. J. Cell Biol. 37 : 394–411.

Brooks, R.E. and B.V. Siegel. 1966. Normal human lymph node cells : an electron microscopic study. Blood. 27 : 268–705.

Heiniger, H.J., H. Riedwyl, Giger, H. et al 1967. Ultrastructural differences between thymic and lymph node small lymphocytes of mice : Nucleolar size and cytoplasmic volume. Blood. 30 : 288–300.

Low, F.N. and J.A. Freeman. 1959. Electron microscopic atlas of normal and leukemic human blood. New York, Toronto, London, McGraw-Hill Book Company Inc.

Low, F.N. 1960. Electron microscopy of the lymphocyte, pp. 54–66. In J.W. Rebuck (ed.). The lymphocyte and lymphatic tissue. New York, Hoeber.

Movat, H.Z. and N.V.P. Fernando. 1964. The fine structure of lymphoid tissue. Exp. Molec. Path. 3 : 546–568.

Murray, R.G., A. Murray and A. Pizzo. 1965. The fine structure of the thymocytes of young rats. Anat. Rec. 151 : 17–39.

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

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol.

17 : 208 – 212.

Schalm, O.W., N.C. Jain and E.J. Carroll. 1975. Veterinary Hematology. 3rd ed., Philadelphia, Lea & Febiger.

Sonoda, M. and K. Kobayashi. 1970. Lymphocytes of canine peripheral blood in electron microscopy. Jap. J. Vet. Res. 18 : 71 – 74.

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

Stempak, J.G. and R.T. Ward. 1964. Improved staining method for electron microscopy. J. Cell Biol. 22 : 697 – 701.

Weber, A.F. and D. Joel. 1966. Tubular and ultrastructural studies of agranulocytes of the thoracic duct of calves. Blood. 27 : 266 – 279.

Yam. L.T., C.Y. Li, and W.H. Crosby. 1971. Cytochemical identification of monocytes and granulocytes. Amer. J. Clin. Path. 55 : 283 – 290.

รายนามผู้ทรงคุณวุฒิ

วิทยาสารเกษตรศาสตร์ สาขา Natural Sciences ปีที่ 18 พ.ศ. 2527 ได้รับการตรวจแก้ไขต้นฉบับ จากผู้ทรงคุณวุฒิดังรายนามต่อไปนี้

ดร. ดวงใจ ชูปัญญา
คุณปรีชา อารีกุล
ดร. สุรเชษฐ จามรมาน
ดร. ธานีรัตน์ ผานติวัตร
ดร. นงลักษณ์ ศรินทุ
ผศ. วิจิตร วังใน
ผศ. จิรายุพิน จันทรประสงค์
ศ. ปวิณ ปุณศรี
ผศ. ดร. จวงจันทร์ ดวงพัตรา
ผศ. พรรณจิตต์ นิลกำแหง
ผศ. ดร. วรรณดา สุจริต
ดร. สุรพล อุปดิสสกุล
ผศ. ดร. สายชล เกตุษา
ดร. ประจิตร วงศ์รัตน์
รศ. ดร. สุรนันต์ สุภัทรพันธุ์
ผศ. ดร. ไพบูลย์ ประพฤติธรรม
ผศ. ดร. ศุภมาศ พนิชศักดิ์พัฒนา
ดร. ชำนาญ ฉัตรแก้ว
ดร. เนื่องพนิช สินชัยศรี
ดร. พัธกุล จันทนมัฏฐะ
ดร. ชลอ ลิ้มสุวรรณ
ผศ. ดร. กิติ ศรีสุภาพ
ผศ. บุญธรรม จงเจริญ
ดร. วิวัฒน์ แดงสุภา

ห้างหุ้นส่วนจำกัด
โรงพิมพ์ ชวนพิมพ์
469 ถนนพระสุเมรุ แขวงบวรนิเวศน์ เขตพระนคร กรุงเทพฯ
นายชวน ศรสงคราม ผู้พิมพ์ผู้โฆษณา พ.ศ. 2527 โทร. 2822114, 2810541

Business Office :

Kasetsart University Research and Development Institute (KURDI)
Kasetsart University, Bangkhen, Bangkok 10900

Exchange Publications should be addressed to
The Librarian,
Main Library,
Kasetsart University,
Bangkok 10900, Thailand

THE KASETSART JOURNAL

Natural Sciences

Volume 18, No. 3 1984

Serial number : ISSN 0075—5192

The Official Journal of Kasetsart University
Published by the Kasetsart University, Bangkok 10900, Thailand.