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Title	ELECTRON MICROSCOPIC OBSERVATIONS ON THE BLOOD OF THE HORSE I : NEUTROPHILS IN THE PERIPHERAL BLOOD OF THE CLINICALLY HEALTHY HORSES
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Citation	Japanese Journal of Veterinary Research, 14(1-2), 71-78
Issue Date	1966-06
DOI	<a href="https://doi.org/10.14943/jjvr.14.1-2.71">https://doi.org/10.14943/jjvr.14.1-2.71</a>
Doc URL	<a href="https://hdl.handle.net/2115/1841">https://hdl.handle.net/2115/1841</a>
Type	departmental bulletin paper
File Information	KJ00002369256.pdf



**ELECTRON MICROSCOPIC OBSERVATIONS ON  
THE BLOOD OF THE HORSE I\*  
NEUTROPHILS IN THE PERIPHERAL BLOOD OF  
THE CLINICALLY HEALTHY HORSES**

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(Received for publication, February 4, 1966)

INTRODUCTION

Recently, in the field of the human hematology, electron microscopic methods have been becoming more and more important as one of the most useful methods for differentiating the blood cells in the states of health and various diseases, and several research publications have been presented.

On the other hand, in the field of veterinary hematology, some reports on the blood cells of several domestic animals using electron microscopy have been presented, but in these publications<sup>1,3,5,7,9,13,17,19)</sup>, only a few of the blood cells were described and there is no systematic observation using electron microscope on the blood cells of the peripheral and bone marrow of each of the domestic animals.

Since several years ago, the authors have attempted to study the blood cells of several domestic animals using the electron microscope.

In this paper, the fine structures of the neutrophils of the peripheral blood, obtained from clinically healthy horses, were described.

MATERIALS AND METHODS

Approximately 20 ml of blood obtained by venipuncture of the jugular vein of clinically healthy horses was drawn into test tubes with heparin soda. The tubes were placed diagonally at 37.0°C in the chamber of an incubator for 10 minutes. Thereafter, the upper parts of the plasma containing a large portion of leucocytes and thrombocytes and a few red cells were transferred to the test tubes and centrifuged for 5 minutes at 1,000 rpm. The supernatants were taken away as perfectly as possible. About 3 ml of phosphate-buffered 1% osmic acid solutions of pH 7.4 were poured into the tubes and shaken gently for mixing with the sediments. For fixation, the tubes were put in an ice box at 0°C for one hour. The osmic acid solutions containing blood cells were transfused into small polyethylene conical micro-

\* This work was presented at the 55th Meeting of the Japanese Society of Veterinary Science at Tokyo, April 6, 1963

tubes, and were again centrifuged for 5 minutes at 10,000 rpm. The strongly packed sediments were cut, with the polyethylene conical microtubes, to about 0.5 mm<sup>3</sup> in size. They were dehydrated in a graded series of ethanol or acetone and were embedded in Epon 812.

The thin sections were cut with glass knives on a JUM-5A ultra-microtome. After mounting on copper grids, the sections were stained with uranyl acetate and examined with JEM CHD-4.

## OBSERVATIONS

Of the cells in the visual field of the cut planes, the neutrophils always exceeded in number any other kinds of white blood cells. This cell type is readily identified from other kinds of white blood cells by the numerous specific granules, the typical shape of the nucleus and the clearly differentiated cytoplasm in characteristic pattern.

### 1 Nucleus

The nuclei of the neutrophils often show several separated nuclear lobes in the cytoplasm because the connecting strands of the nuclei are usually not included in the planes of the section. In the authors' observations, the number of nuclear lobes appearing in the cut plane of each cell are variable in accordance with the cut directions of the cells, but generally speaking, the cells with one to three nuclear lobes are most frequent in number. However, cells with over four nuclear lobes are also commonly observed. Cells with no nuclear lobes—only cytoplasm—are not uncommon.

The nuclear lobes vary in form and size determined by the difference in the cut directions. The outlines of the nuclear lobes are usually smooth. Occasionally, one of the thin strands connecting nuclear lobes is so visible in the section of some of the cells that for a short or somewhat long distance. These nuclear lobes appear to have tails. Some of the nuclear strands separate perfectly from the nuclear lobes and sometimes form rings of nuclear strands in the cytoplasm.

At the sites enclosed partly with these nuclear strands or in the rings of the nuclear strands, the same substances that are usually found in the cytoplasm of this cell type are present.

The locations of the nuclear lobes are everywhere in the cytoplasm of each cell but are generally centrally placed. The cytoplasm surrounding the nucleus is of varying width.

The nuclear lobes are lined with nuclear membranes. These membranes are constituted from a double-layered structure which is separated by clear space. There are perforations in the membranes (nuclear pores). In some of our micrographs, the double nature of the nuclear membrane is evident, especially at the site of the light areas of the nuclear lobes, and the nuclear strands. The nuclear pores are clear at the part of the light areas of the nuclear lobes.

The back-ground materials of the nuclear lobes present a very fine, relatively diffuse, light grayish density, which are almost similar to that of the cytoplasm.

The nuclear lobes of the cells show maculous appearance with two parts more or less dense depending upon the amount of chromatin condensation, but there are no limiting materials in the boundary between them, nor is there a definite shape to either of the areas. The

maculous parts with more density are usually found attached to the nuclear membrane and they form so-called chromatin node (nuclear dark part). They look dark in the micrographs because they are filled with closely aggregated granular fine-particles of very high density.

On the other hand, in the parts with less dense nuclear lobes (nuclear light part), the granular fine-particles disperse comparatively homogeneously, but in some parts of them, the particles tend to gather in small clumps and form small scattered chromatin clusters. These parts with less dense nuclear lobes usually attach to the nuclear membrane at the smaller frontages than those of the parts of the chromatin nodes.

In the cut planes of the nuclear lobes of some neutrophils, the clear point-like particles about 140~300 Å in size with very high electron density, and larger in size than the granular fine-particle described above are found sparsely on the both of the light and dark parts of nuclear lobes, but these particles are not found at all on any parts of the cytoplasm.

On parts of the nuclear strands, chromatin materials with high density are observed like a somewhat thick string between the clear nuclear membranes.

In the authors' observations, no nuclear lobe with a nucleolus is able to find in the cut planes of any sections of the neutrophils.

## 2 Cytoplasm

The cytoplasm surrounding the nucleus is delineated sharply by a very fine external membrane. In the cells which are free from other cells in the cut planes, the shapes are round or ovoid in general. Most of the cells are irregular in contour because of the many small or large pseudopodic projections present extending from the peripheral cytoplasm. The fine, dust-like, grayish opacity making up the cytoplasmic background is analogous to those present in nuclear plasm. However, their electron density is slightly less than that of the nuclear plasm. On the cut planes of the cells, there are innumerable fine point-like granules which are less dense and smaller in size than that of the nuclear lobes. They are distributed closely and homogeneously in all of the cytoplasm. In the cytoplasm, there are usually found specific granules, mitochondria, vacuoles and some other organelles.

The observations of the specific granules of the neutrophils indicate that the granules are randomly distributed in the cut planes of the cells except for the pseudopodic projections and a narrow peri-nuclear band in the cytoplasm. These granules have a distinct limiting membrane and a rather homogeneous interior.

The form and size of the granules are variable depending upon the planes in which the granules are sectioned. In general, the form of these granules are round, ovoid, rice grain- or rod-like, the round ones always exceed by far any of the other forms in number. The round or ovoid granules are about 0.05~0.2 μ in diameter and have comparatively variable electron density in each of the granules. Sometimes, in the cut planes of the cells, the rod-like or rice grain-like granules are found in large number. Their size is also variable but usually they are about 0.5~0.6 μ long by 0.05~0.2 μ wide. Frequently, they are not uniform homogeneous density in each of the granules therefore they appear to have a slightly mottled patterns.

In the specific granules, there is a small number of mitochondria in transverse or longitudinal section. They are round, ovoid or elongate in form and identical to the specific

granules, and they are generally about the same size as the specific granules except at times, some larger ones are present. Usually, the mitochondria are discriminated from the other characteristics by the presence of the double layered limiting membrane and cristae; however, in the neutrophils of the peripheral blood of the horse, the fine structures of these mitochondria are not always so prominent as those of other blood cell types such as lymphocytes and monocytes, therefore, there is some difficulty in differentiation between specific granules and mitochondria, especially in the case of the round forms of the mitochondria.

Occasionally, clusters of vacuoles and ill-developed lamellar structures are found in the cut planes of some neutrophils. They represent Golgi apparatus which has been shown by some investigators to be present in the human neutrophils. Usually, the apparatus is found in the central portion of the cytoplasm enclosed by nuclear lobes, near which, one or two centrioles are sometimes observed. The frequency of the appearance of the Golgi apparatus and the centriole in the cut planes of the cells are comparatively rare.

The cytoplasm of this cell type contains round and ovoid vacuoles or vesicles of various small sizes in considerable number, scattered on the entire cytoplasm. Certain ones are filled with a less dense substance or with a clear center. They are usually smaller than the specific granules in size, but in some cut planes of this cell type, a few randomly distributed slightly larger ones with several small vesicles are observed; these seem to be multi-vascular bodies.

In the cytoplasm of some cut planes, round or large ovoid bodies with smooth or slightly irregular contour, measuring about  $0.5\sim 1.0\mu$  are observed. They have a clearly defined limiting membrane. In our observations, they are divided into three types. The first is the round body type with or without slightly irregular contour, and containing a few fine fibrin-like or granular materials, sometimes small vesicles are observed. The second type is exactly round in form and filled with homogeneous or slightly aggregated materials of a slightly higher density. The last one of the bodies is the round body demarcated from the cytoplasm but containing the same materials as the cytoplasm. All three types of bodies were seen very rarely in our observations.

#### CONSIDERATION

On the basis of the results of our observations, it may be said that the fine-structures of the neutrophils of the equine peripheral blood are fundamentally identical with those of human neutrophils as presented by many workers<sup>2,6,8,10,14,19</sup>. However, in some fine-structures of this cell type, there are some differences between them. First of all, the maculous appearances with dark and light areas on the nuclear lobes of the equine neutrophils are clearer than those of the human neutrophils. SCHALM stated that in Giemsa-stained smears, the nuclear chromatin of the equine neutrophils is very heavily plaqued and causes the nuclear membrane to be jagged and tends to give the impression of multi-lobulation. The specific staining character of the nuclei of the equine neutrophils is probably due to the variation of aggregation of the fine nuclear particles. The surroundings of the nuclear membranes are always smooth, and the jagged structure of the nuclear

lobes is not observed at all in the authors' micrographs, so it will be said that the jagged structure observed in Giemsa-stained smears may perhaps be due to an artifact at the time of smear-making or fixation.

From the micrographs findings, it may be suggested that the connecting substance between lobes is a considerably wide membrane-like substance about  $60\text{ m}\mu$  in thickness and not one like a string. No regions have been observed with two double nuclear membranes in direct contact without a layer of nuclear material separating the two membranes.

In our observations, the particles with high electron density about  $140\sim 300\text{ \AA}$  are observed in the nuclear lobes of some cells, but their actual entity is not possible to decide by only our findings.

The shape of the specific granules separated by the special method, TAKAHASHI reported that they were spherical or oval shaped granules in the cytoplasm of the neutrophils of the horse. On the other hand, several workers<sup>2,6,8,10,19)</sup> reported that those of the human neutrophils were round, rice grain or small club in shape and were generally  $0.1\sim 0.5\mu$  in size. In our observations, in the large number of the cut planes of the cells, the round or oval granules in shape are abundantly seen. These granules are  $0.05\sim 0.2\mu$  in diameter, which is smaller than those granules of the human neutrophils. In some parts of the cut planes of the cells, there are a lot of granules of the rod-like shape. From this finding, it may be thought that the real shape of the specific granules are rod-like in the great number of them. Their size are  $0.5\sim 0.6\mu$  long by  $0.05\sim 0.2\mu$  wide, viz., they are generally larger than those granules of the human neutrophils. The round profiles of the granules show variability in the electron density, this may be due to the variety of the constituents of the granules themselves, and not due to differences of the granules like WATANABE<sup>19,20)</sup> described on the human specific granules, because the difference in electron density in the rod-like granules has been certified.

It has been recognized by WATANABE<sup>20)</sup> that the mitochondria of the human neutrophils are usually under  $0.15\mu$  in size, viz., they are slightly smaller than the specific granules and their number in the cut planes of the cells is few.

In the cut planes of the equine neutrophils, few mitochondria are similarly identifiable but they are similar or slightly larger than those of the specific granules in size.

In our observations of the mitochondria of the equine neutrophils, the double layered nature of the limiting membrane and cristae which are the characteristics for the organelle are, as a rule, not so evident as those of the lymphocytes and monocytes, so in differentiation from the mitochondria, there are some difficulties. In this respect, GOODMAN et al. also pointed out the same findings in the human

neutrophils.

RINEHART described a degeneration of the mitochondria to the specific granules in the human neutrophils and postulated this mechanism as a source of the granules formation. On the other hand, WATANABE<sup>19,20</sup> suggested that the specific granules are formed in the Golgi apparatus. On the basis of the results of our observations, we have not seen any evidence of specific granules which are derivation. However, it is a noteworthy finding that the mitochondria and specific granules of the equine neutrophils are nearly the same size and both of them are very identical in morphology.

The authors also identified the Golgi apparatus which is not always observed in the cut planes of the cells. However, the authors suspect that this organelle always remains in the cytoplasm, but its appearance in the cut planes is variable in accordance with the cut directions of the cells, because this apparatus usually occupies a position near the center of the cells.

The Golgi apparatus of the equine neutrophils is so ill-developed that we have not seen vacuoles, lamellae or granules, which are characteristics of the Golgi apparatus in the normal human neutrophils.

It has been said<sup>10,19</sup> that the human neutrophils are characteristically poor in the endoplasmic reticulum which appears as small circular profiles having a dark membrane and a light, apparently empty center.

In the equine neutrophils, a few number of round or ovoid vacuoles in various size disseminated throughout the cytoplasm are encountered. These vacuoles in the cytoplasm of the human neutrophils, BESSIS & THIERY divided into 5 hypothetic substances, viz., they: (1) derive from the endoplasmic reticulum; (2) result from pinocytosis; (3) are part of the Golgi corpuscles; (4) represent a particular stage in the development of the neutrophilic granules; or (5) represent the localization of glycogen dissolved by fixation or embedding technique.

The authors are in doubt as to whether they belong to any of the substances described above, but the majority of the vacuoles observed in the cytoplasm of the equine neutrophils must be the endoplasmic reticulum.

Furthermore, the 3 kinds of spherical or round large bodies observed in the cytoplasm of the equine neutrophils are supposed to phagosome which is due to phagocytosis and lysosome or cytolysome which are due to the cell degeneration<sup>11,18</sup>.

#### SUMMARY

The fine structures of sectioned neutrophils of the peripheral blood obtained from the clinically healthy horses were examined with an electron microscope and the findings were discussed comparing with those of the human neutrophils

as reported by many workers.

The results thus obtained are summarized as follows.

1) The nuclear lobes of the cells showed clear maculous appearance with two parts of more or less density according to the amount of the chromatin condensation.

2) The connecting substance between nuclear lobes seemed to be a considerable wide membranous one about  $60\text{m}\mu$  in thickness and not the one like a string.

3) The shapes of the specific granules were thought to be rod-like in the great number of them and they were  $0.5\sim 0.6\mu$  long by  $0.05\sim 0.2\mu$  in size.

4) In the cytoplasm, few mitochondria similar to or slightly larger than the specific granules in size were identified.

5) The ill-developed Golgi apparatus and the centrioles were observed rarely in the cut planes of the cells.

6) A few round or ovoid vacuoles of various size disseminated throughout the cytoplasm seemed to be the endoplasmic reticulum

7) Three kinds of large spherical or round bodies which were presumed to phagosome, lysosome or cytolysosome were observed in the cytoplasm of some neutrophils.

#### ACKNOWLEDGMENTS

The authors wish to express their sincere gratitude to Mr. Y. MIFUNE, the Electron Microscope Laboratory, Faculty of Veterinary Medicine, Hokkaido University, for his technical assistance.

#### REFERENCES

- 1) BARGMAN, W. & KNOOP, A. (1956): *Z. Zellforsch. mikrosk. Anat.*, **44**, 282
- 2) BESSIS, M. & THIERY, J. (1961): *Int. Rev. Cytol.*, **12**, 199
- 3) BOCCIARELLI, D., TENTORI, L. & VIVALDI, G. (1959): *Rc. Ist. sup. Sanità*, **22**, 1059
- 4) BRAUNSTEINER, H. & PAKESCH, F. (1957): *Acta haemat.*, **17**, 136
- 5) BRAUNSTEINER, H. & PAKESCH, F. (1962): *Ibid.*, **28**, 163
- 6) GOODMAN, J. R., REILLY, E. B. & MOORE, R. E. (1957): *Blood*, **12**, 428
- 7) ITO, Y. (1964): *Kachiku-eisei shikenjyo nenpo*, **4**, 386 (in Japanese)
- 8) KAUTZ, J. & DEMARSH, Q. B. (1954): *Blood*, **9**, 24
- 9) KNOCKE, K. W. (1964): *Zentbl. VetMed. B*, **11**, 1
- 10) LOW, F. N. & FREEMAN, J. A. (1958): Electron microscopic atlas of normal and leukemic human blood, 1 ed., New York, Toronto, London: McGraw-Hill Book Company, Inc.
- 11) NOVIKOFF, A. B. & ESSNER, E. (1962): *J. Cell Biol.*, **15**, 140
- 12) OGIHARA, T. (1963): *Med. J. Osaka Univ.*, **15**, 183 (in Japanese)

- 13) ŌSEKO, R. (1959): *Acta haemat jap.*, **22**, 134 (in Japanese with English summary)
- 14) RINEHART, J. F. (1955): *Am. J. clin. Path.*, **25**, 605
- 15) SCANGA, F. (1964): *Atlas of electron microscopy*, 1 ed., Amsterdam, London, New York: Elsevier Publishing Company
- 16) SCHALM, O. W. (1961): *Veterinary hematology*, 1 ed. 122, Philadelphia: Lea & Febiger
- 17) TAKAHASHI, H. (1961): *Nagoya J. med. Sci.*, **24**, 1
- 18) TANAKA, H. (1963): (translated title) "Electron microscopic analysis on the processes of uptake of substances by various cells" *Compendium of haematology I*, Ed. AMANO, S. & HIBINO, S., 1 ed. 249, Tokyo: Maruzen (in Japanese)
- 19) WATANABE, Y. (1956): *Acta haemat. jap.*, **19**, 327 (in Japanese with English summary)
- 20) WATANABE, Y. (1964): (translated title) "The fine structure of leucocyte" *Compendium of haematology I*, Ed. AMANO, S. & HIBINO, S., 1 ed. 327, Tokyo: Maruzen (in Japanese)

#### EXPLANATIONS OF PLATES

##### PLATE I

###### Fig. 1

In the plane of this section, a typical polymorphous neutrophil is visible showing three nuclear lobes. The contour of this cell is slightly irregular because of the pseudopodic projections. Two distinct densities, one light (NL) and other dark (ND), are evident in the nuclear lobes. The darker of the two is located peripherally, constituting a thick band subjacent to the nuclear membrane. The central area of the nuclear lobe is occupied chiefly by the lighter density, but occasionally the darker nucleoplasm invaginates it. There is also an occasional extension of the lighter density toward the nuclear membrane. In general these densities correspond to the chromatin pattern characteristic of these nuclei in light microscopy. This cell possesses a large number of small, round, medium dense, specific granules (SG), although rice grain- or rod-shaped ones are sometimes observed. Among them, several mitochondria (M) are sparse but they are not so clear in the characteristics. A considerable number of vacuoles (V) in variable size are seen scatteringly in the entire of the cytoplasm. The cluster of small vesicle-like substance observed in the central area of the cut plane of the cell is perhaps ill-developed Golgi apparatus (G).     × 35,000

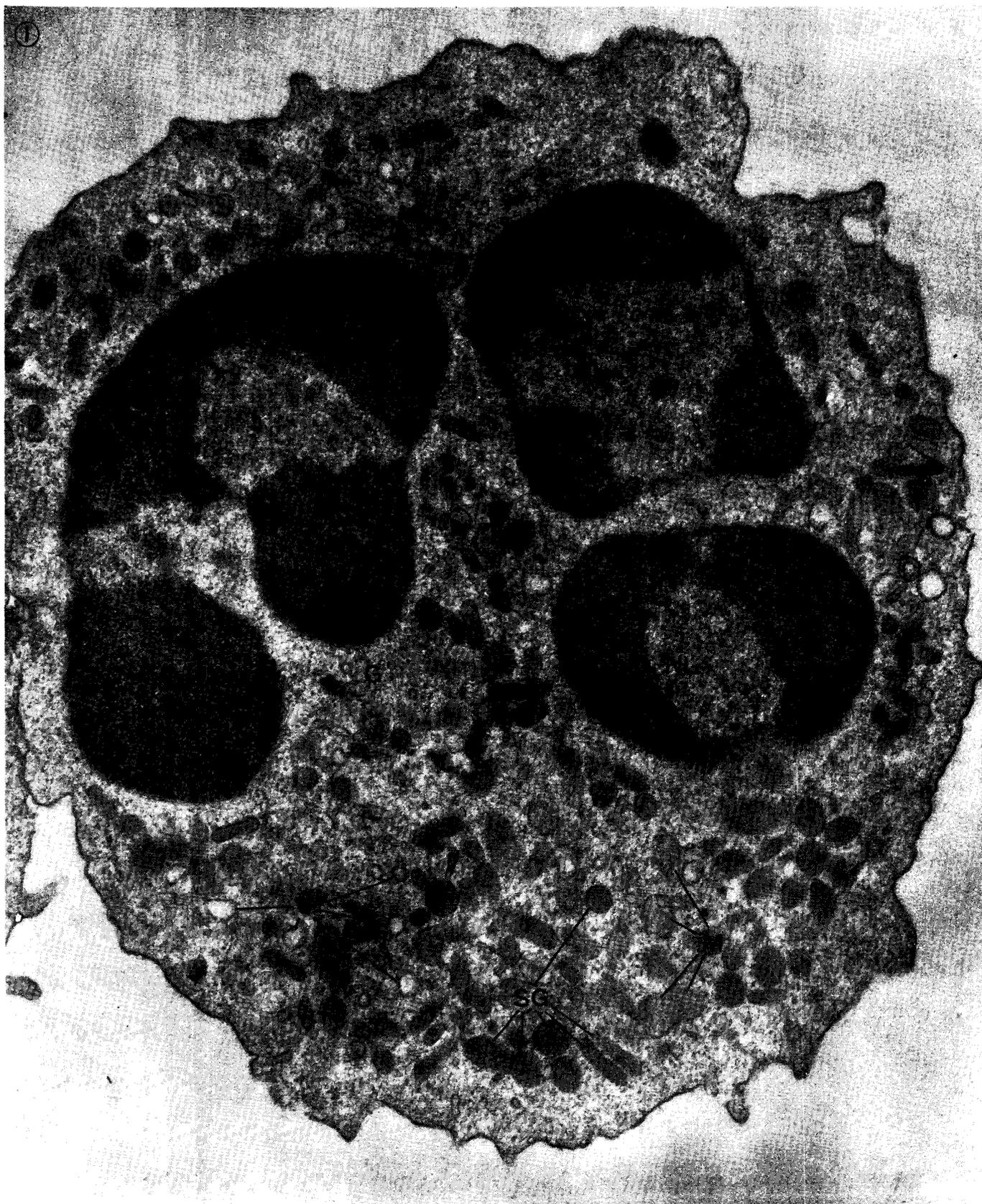


PLATE II

Fig. 2

In the area between nuclear lobes, the Golgi apparatus which is constituted from vacuoles, vesicles, ill-developed lamelle structures and two centrioles (C) are observed. There are some areas of cytoplasm free of specific granules, especially, around the lower nuclear lobe and in the peripheral cytoplasm. A multi-vascular body (MV) is present.  $\times 35,000$

Fig. 3

Two multi-vascular bodies are evident.  $\times 35,000$

Fig. 4

In both of the light and dark parts of the nuclear lobes, clear, point-like particles with high density are observed sparsely.

The double layered nature of the nuclear membrane is evident at the light part of the nuclear lobe (arrow). Golgi apparatus is present.  $\times 35,000$

Fig. 5

The neutrophilic granules are variable in form and size. The cluster of small vesicles which is supposed to represent ill-developed Golgi apparatus is observed.  $\times 35,000$

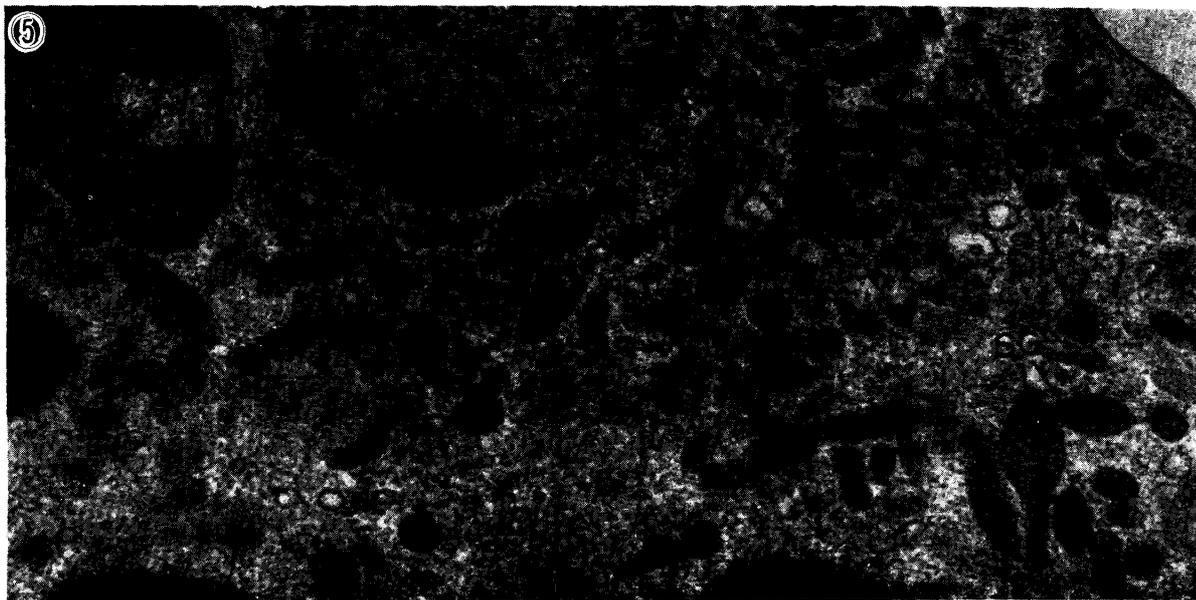
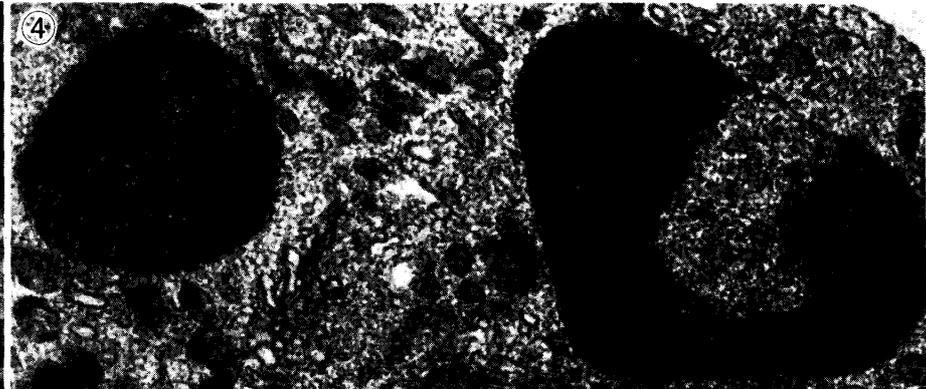
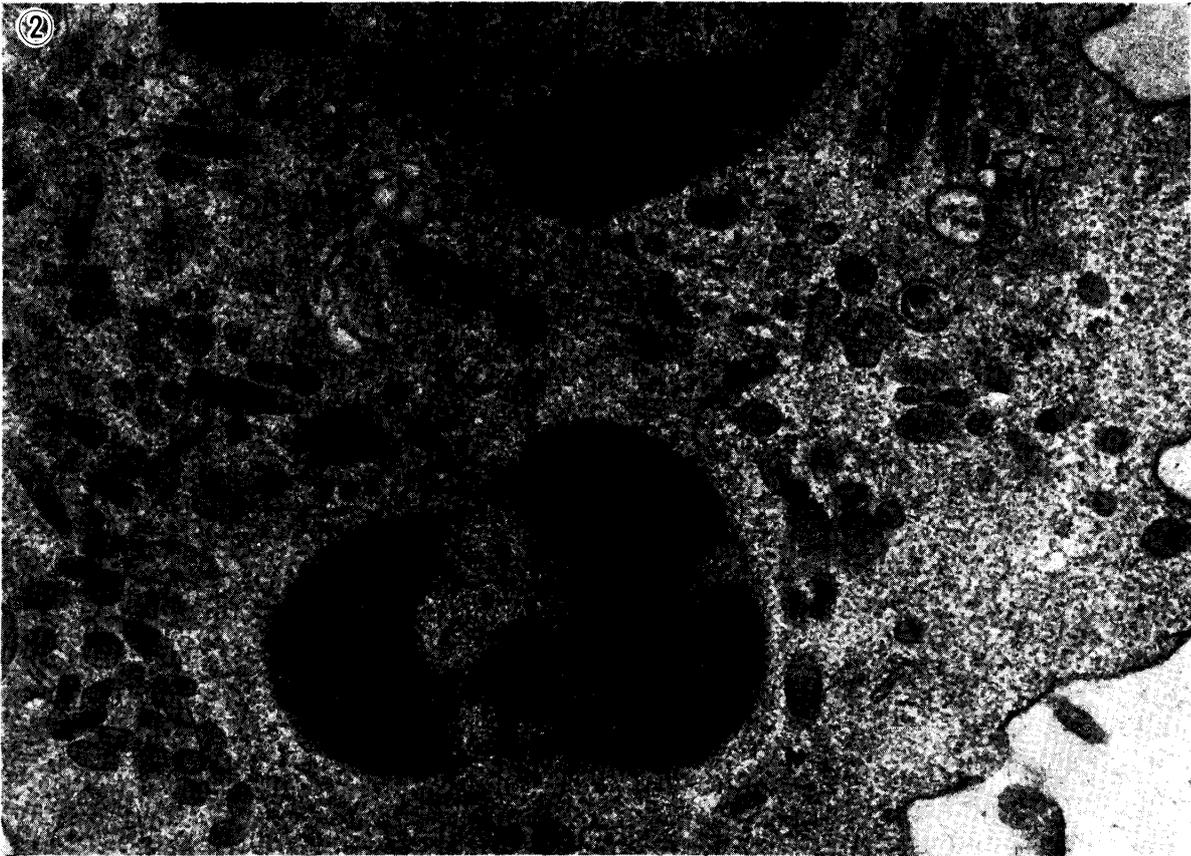


PLATE III

Fig. 6

A multi-vascular body-like body which is different from those depicted in figures 2 and 3 is observed (arrow). Furthermore, in this plane, it is clear that one of the specific granules is rod-like in form and particularly large in size.  $\times 35,000$

Fig. 7

The chromatin aggregates along the inner nuclear membrane and constitutes an irregular thick band with high electron density subjacent to the nuclear membrane. In the central part of the nuclear lobe, a clump of chromatin separated from the dark nuclear part is observed clearly.  $\times 35,000$

Fig. 8

This is a portion of the strands connecting the lobes of the nucleus. The double layered nature of the nuclear membrane can be seen and it is apparent that the dense nuclear materials are continuous far the full length of the strand.  $\times 35,000$

Fig. 9

This ring is a profile of the nuclear strand in the cut plane. The structure of the strand is the same as that shown in figure 8. The substance observed inside the ring is the same as that usually observed in the cytoplasm of this cell type. A considerable number of rod-like, specific granules are observed in this cell, and they appear to have a somewhat spotted appearance.  $\times 35,000$

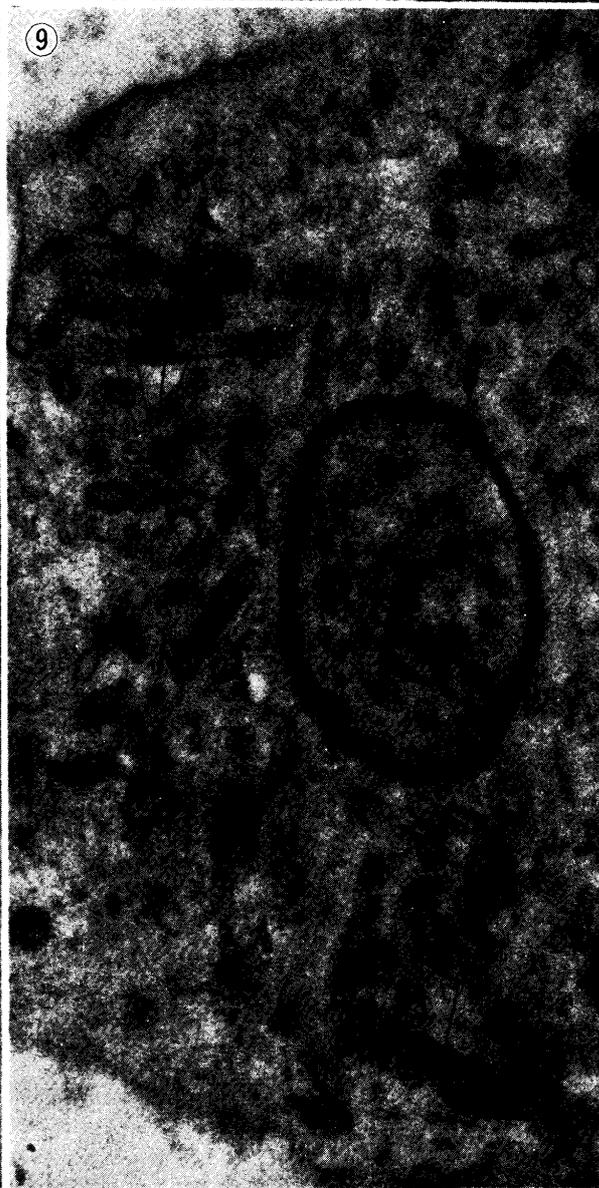
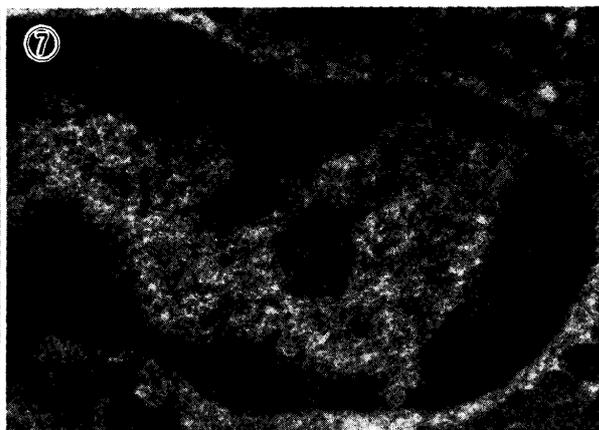
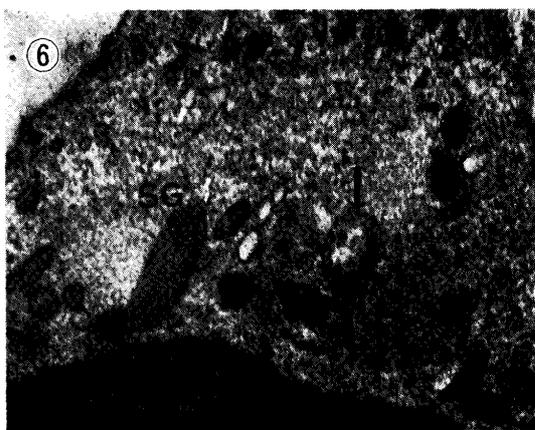


PLATE IV

Fig. 10

In this figure, two kinds of bodies are observed, one of them seems to be a cytolysome (CY) and the other seems to be a phagosome (PH).  
× 35,000

Fig. 11

The body observed may be a phagosome. × 30,000

Fig. 12

The body observed may be a lysosome. × 25,000

Fig. 13

The arrows point to what seems to be the initial change in cytolysome formation. × 25,000

Fig. 14

The cytoplasmic substance is demarcated and forms a round body. This may be a cytolysome. This cell may be degenerated, because the specific granules are not clear and the scalloped nature of the cell surface is evident. × 25,000

