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## NEUTROPHILS OF CANINE PERIPHERAL BLOOD IN ELECTRON MICROSCOPY

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The neutrophils of the canine peripheral blood were observed through electron microscopy.

The results thus obtained were summarized as follows.

- 1) The fine structures of the canine neutrophils were fundamentally identical with those of humans and other animals reported already.
- 2) Slight or moderate maculous appearances on the nuclear lobes were observed in the nuclei.
- 3) The specific granules of the cells were classified into three types, such as compact homogeneous ones with high electron density, moderately dense ones with granular or lattice-like structures, and low dense or almost vacant ones.

### INTRODUCTION

There are a lot of publications on the morphological observations of canine blood in light microscopy.

However, there are only two descriptions<sup>7,16)</sup> of electron microscopic observations of canine blood cells in the literatures. This is why the authors undertook the subject of this study.

In this study, the fine structures of the neutrophils of peripheral blood obtained from clinically normal dogs were described.

### MATERIALS AND METHODS

#### The dogs

Five clinically healthy dogs were provided for this experiment. They were all mongrel dogs, male or female, 2~3 years of age. The hematological findings of the peripheral blood of the dogs were listed in the table.

#### The blood

Approximately 20 ml of blood obtained by venipuncture of the saphena vein by the use of a sterile syringe wetted with 10% ethylenediaminetetraacetic acid (EDTA-2Na) solution was dripped into test tubes.

The tubes were placed diagonally at 37°C in the chamber of an incubator for 30 minutes. Thereafter, the upper parts of the plasma were transferred to small test tubes and centrifuged

*Hematological findings of dogs used in the experiment*

DOG NO.	AGE	SEX	ERYTH.	LEUK.	DIFFERENTIAL COUNT					
					Neut.	Ly.	Mon.	Eos.	Bas.	Atypical Ly.
1	3	Male	6.46	8.8	64.0	28.0	2.0	4.5	+	1.5
2	3	Male	6.69	12.0	58.5	35.0	0.5	6.0	+	+
3	2	Male	5.94	9.4	67.0	21.5	2.0	9.0	+	0.5
4	2	Male	5.67	9.8	62.0	24.5	4.5	8.0	+	1.0
5	4	Female	5.81	10.6	78.0	14.5	3.0	4.0	+	0.5

Note: + Visible in the thick smear preparations of buffy coats

for 5 minutes at 2,000 rpm. The supernatants were taken away as perfectly as possible. The sediments thus obtained consisted of leukocytes, thrombocytes and a small number of erythrocytes.

#### Fixation, dehydration and embedding

The techniques used for the fixation, dehydration and embedding of the sediments were exactly the same as those already reported by the authors<sup>17)</sup>.

#### Sections and observations

The ultrathin sections were cut with glass knives on a JUM-5A ultramicrotome. After mounting on copper grids, the sections were double stained with uranyl acetate and lead citrate, and examined under an electron microscope, JEM 7 type, at magnifications varying from 3,000 ~ 60,000.

### OBSERVATIONS

In all of the visual fields under the microscope, the neutrophils always exceeded any kinds of leukocytes in number of appearance, and the neutrophils were examined at random.

#### 1 Nucleus

The nuclei of the neutrophils were generally separated into two to several nuclear lobes in the cytoplasm. The numbers of lobes in the cells were variable in accordance with the cut directions of the cells.

The neutrophils with only one nuclear lobe were rare and ones with no nuclear lobes—only cytoplasm—were not uncommon. However, generally speaking, the cells with two or three nuclear lobes were most frequently observed.

The nuclear lobes varied in form and size according to the differences in the cut directions. The outlines of the nuclear lobes were usually smooth, but a few of them had sharp indentations.

In form, almost all of the nuclear lobes were round, ovoid, and slightly irregular but some had a short rod-like shape in the cut planes.

In some sections, it was seen that the lobes were connected to each other by a thin

nuclear strand.

The nuclear lobes were located everywhere in the cytoplasm of each cell but in general, they were placed slightly eccentrically in the cells. The cytoplasm surrounding the nucleus was of varying width.

The nuclear lobes were lined with nuclear membranes, which consisted of a double-layered structure separated by clear space. In several parts of the nuclear membrane, there were observed some perforations. These nuclear pores were clear especially in parts of the light areas of the nuclear lobes.

The nuclear lobes of the cells showed a maculous appearance with two parts more or less dense depending upon the amount of chromatin condensation. The maculous parts with greater density were usually found attached to the nuclear membranes and they formed so-called chromatin nodes which looked dark in the micrographs.

On the other hand, in the less dense parts of the nuclear lobes, the fine granular particles dispersed comparatively homogeneously. In these parts, there were some small clusters of granular particles, too.

## 2 Cytoplasm

The cytoplasm was delimited with a visible thin membrane. The contours were indented by many small or large cytoplasmic projections.

The cytoplasm was filled with numerous fine dust-like particles and these formed the back-ground of the cytoplasm.

Mixing with these fine particles, point-like particles called glycogen granules with high electron density were scattered in large numbers throughout the cytoplasm.

There were a number of specific granules randomly distributed in the cytoplasm except at the areas of the pseudopodic projections. These granules had a distinct unit membrane. The form of the granules was variable, such as round, ovoid, rice grain- or rod-like, the round ones always exceeded by far any of the other forms in number.

On the basis of the internal structures and densities, the granules were classified into three types. The granules of the first type had compact homogeneous substances with high electron density, therefore, they were noticed as the most dark granules in the cytoplasm. In size, the round ones were about  $0.1\sim 0.24\ \mu$  in diameter and rod- or rice grain-like ones were  $0.34\sim 0.84\ \mu$  long.

Those of the second type were characterized by the granular or lattice-like interior and they were visible to moderate electron density. They were almost identical with those of the former type in size.

The third type were the ones with least density. The contents of the granules looked slightly gray or almost vacant in the micrographs, however, they were certainly bounded by unit membranes. They were round or slightly irregular in form and almost all of these granules were smaller than the former two types in size.

Among the specific granules in the cytoplasm, a small number of mitochondria were distinguished by the presence of cristae. Their size was about  $0.16\sim 0.28\ \mu$  in diameter.

Occasionally, an ill-developed Golgi complex consisting of some vacuoles and lamellar structures was found in the cut planes of some neutrophils. It was usually found in the

central portion of the cytoplasm.

Near the areas of the Golgi complex, one or two centrioles were rarely observed.

In some parts, especially near the peripheral areas of the cytoplasm, round or irregular vacuoles and vesicles of various size were found and in some of them, several small vesicles were observed.

#### CONSIDERATIONS

The fine structures of the neutrophils of the canine peripheral blood were fundamentally similar to those of humans<sup>3,4,8,10,13,14,21,22</sup> and other animals<sup>9,12,15~21,23</sup> described by many investigators.

In the authors' previous paper on the equine neutrophils<sup>17</sup>, the very distinct maculous appearance on the nuclear lobes was reported.

In the present observations on the canine neutrophils, the maculous figures were observed on the nuclear lobes, too. However, they were slighter than those of the horses.

It has been reported that there are two to four types in the specific granules of the neutrophils of humans<sup>1,3,5,6,11,22</sup> and other animals<sup>2,16,19~21,23</sup>. In canine neutrophils, SHIVELY et al. observed three types of granules such as azurophilic, specific and large pale ones in the cytoplasm.

In the authors' observations, the specific granules of the canine neutrophils were divided into three types on the basis of their fine structures and densities, too. However, there were so many intermediate ones among them that the typing of these granules except the typical ones should not be considered strictly.

Recently, some investigators<sup>5,6,9,11</sup> have pointed out that there are some structures inside the specific granules. In our observations, the granular or lattice-like structures were observed in some of the specific granules. At the present time, the authors can not give additional details about structures to those described above, because our pictures are not clear enough for that purpose. It is thought that the appearance of the internal structures seems to be influenced by the difference of fixatives and the conditions in dehydration and embedding<sup>6,11,16,22</sup>. Further experiments will be needed for further clarification of the fine structures of the canine neutrophilic granules.

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## EXPLANATIONS OF PLATES

PLATE I  $\times 10,000$

General figures of 4 typical neutrophils are shown in figs. 1 ~ 4.

The nuclei of these cells have two to four nuclear lobes. Two distinct densities are evident on the nuclear lobes of the cells. 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. There is an occasional extension of the lighter density toward the nuclear membrane.

The specific granules are randomly distributed in the cytoplasm except the pseudopodic portions near cell membranes. Ill-developed Golgi complexes are seen in the central areas of the three cells in figs. 1 ~ 3.

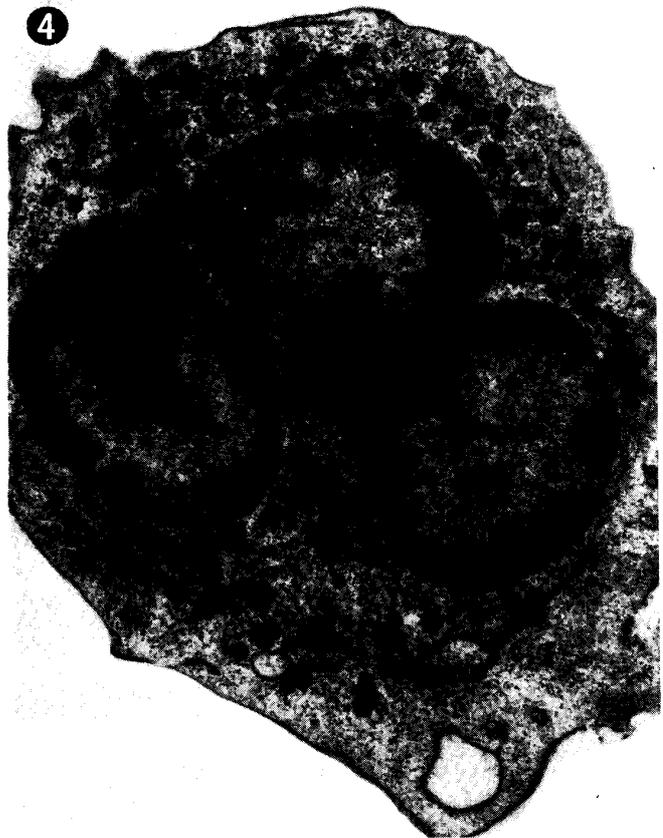
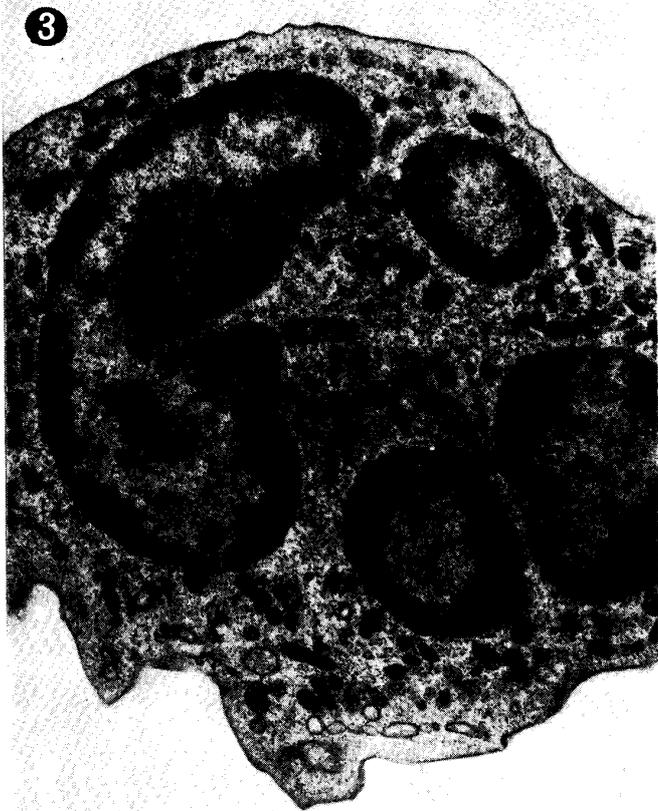
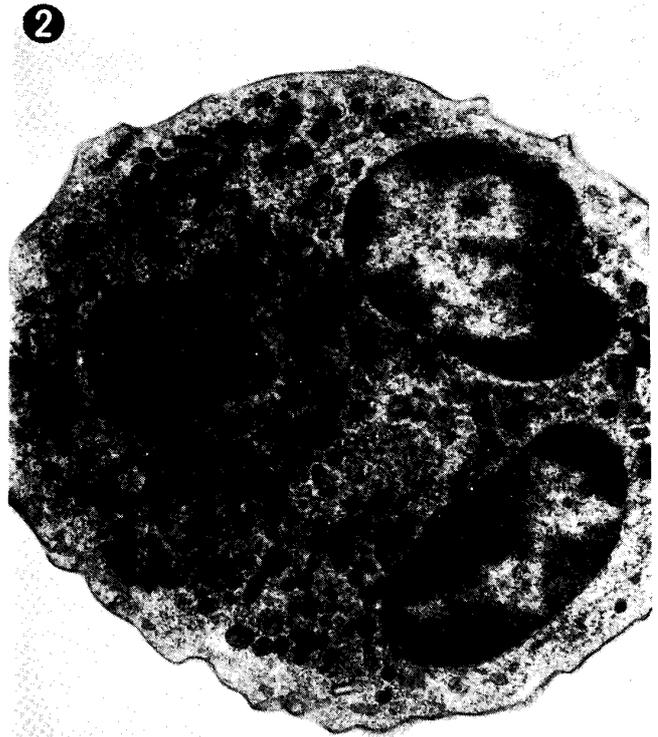
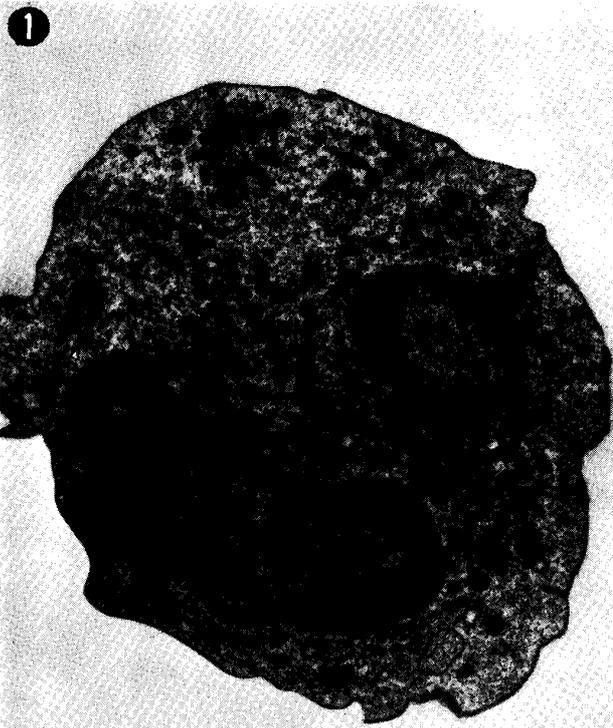


PLATE II × 25,000

An enlarged figure of a typical neutrophil is shown.

On the nuclear lobes, maculous figures are not so distinct in this cell. The nuclear pores are clear at the part indicated by an arrow. In the cytoplasm, a moderately developed Golgi complex (G) and a distinct centriole (C) are present in the central area. Mixed glycogen particles and specific granules are distributed on all over the cytoplasm. Several mitochondria (M) are distinguished by the presence of cristae. A number of vacuoles (Va) and vesicles with or without contents (Ve) are observed in the cytoplasm.

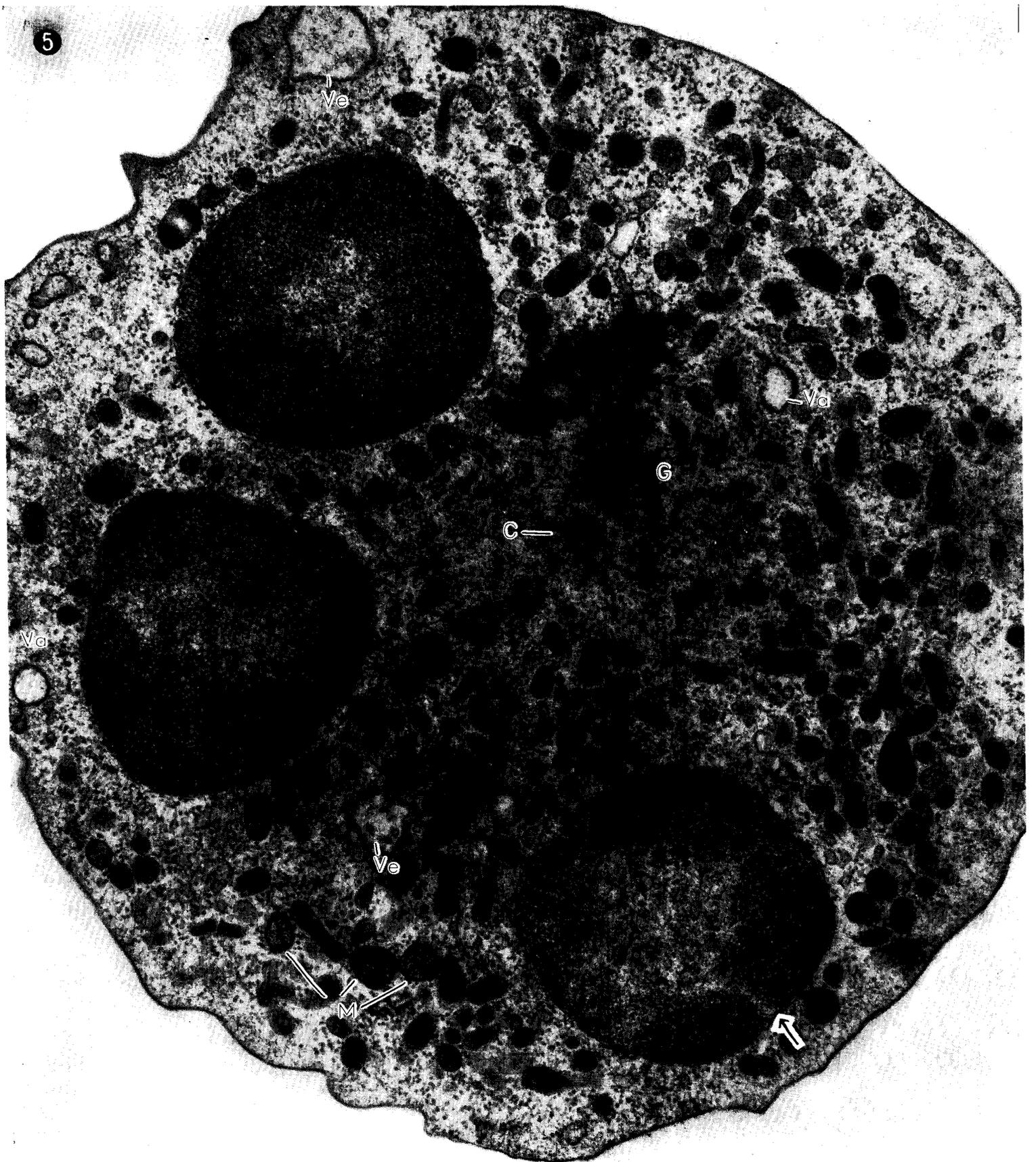


PLATE III × 50,000

Two parts of the cytoplasms are shown in figs. 6 & 7.

Several number of rice grain- and rod-like granules are observed conspicuously in fig. 6. Three types of specific granules are distributed in both figures. They are marked I, II and III, respectively.

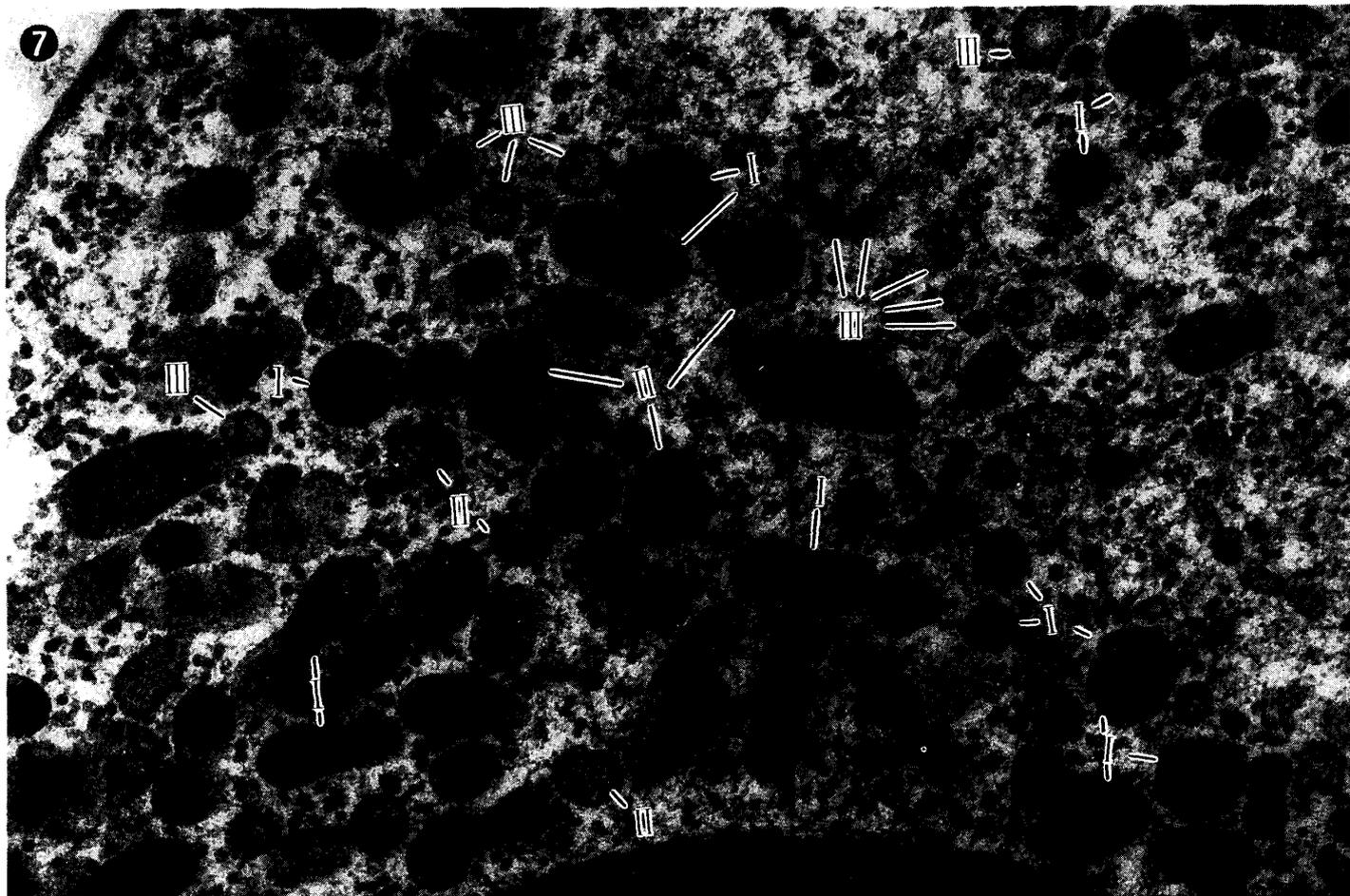
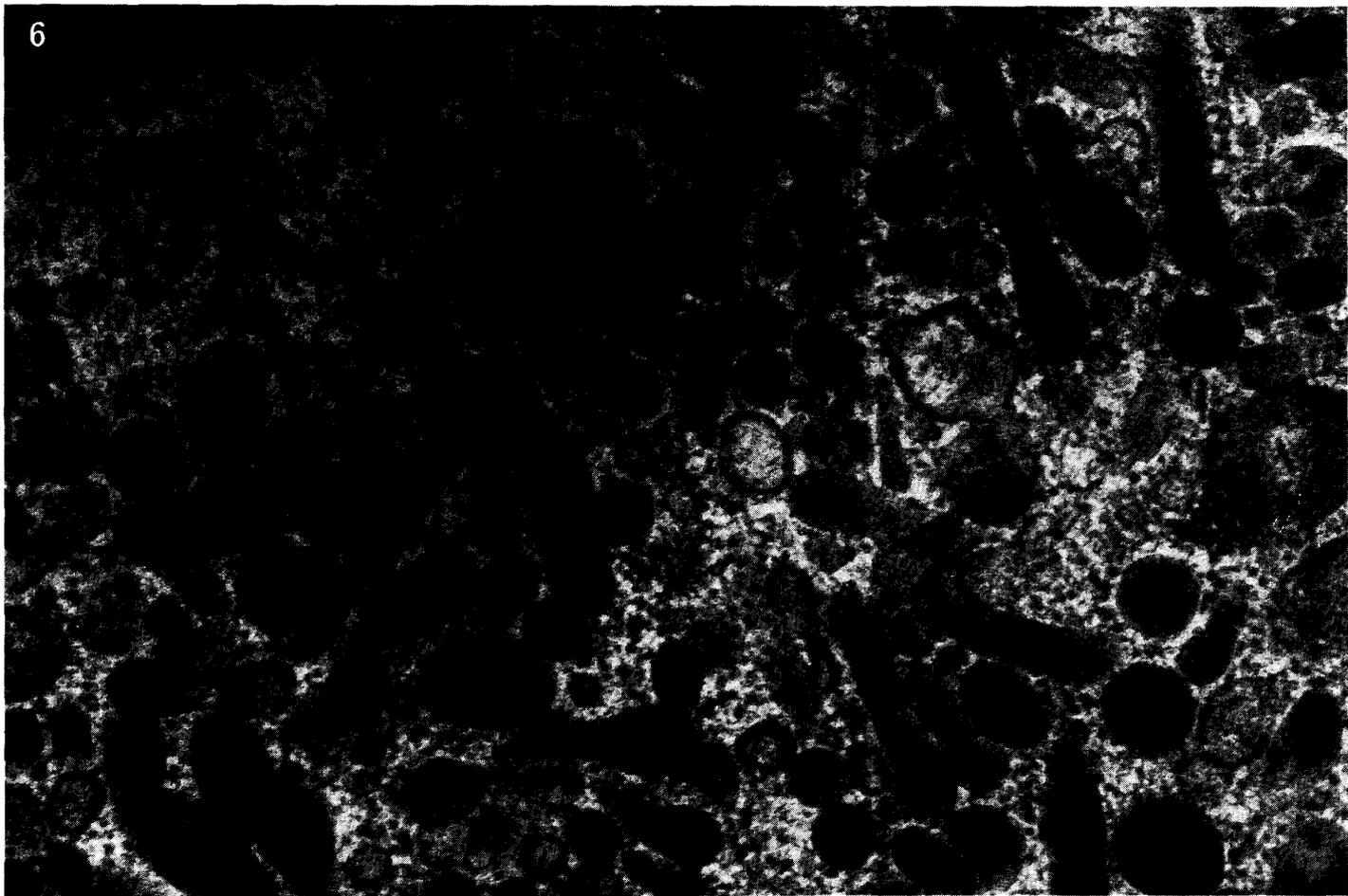


PLATE IV  $\times 50,000$

Four parts of the cytoplasm of neutrophils are shown in figs. 8 ~ 11.

In figs. 8 & 9, two granules with dense cores are seen (one arrow). In the ones of the second type, granular structures are observed and in one of them, a lattice-like structure is evident (two arrows).

In fig. 10, three types of granules are seen.

In fig. 11, an extra-large granule can be seen (arrow). The size of the granule is  $0.5 \mu$  in diameter.

