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NEUTROPHILS OF OVINE PERIPHERAL BLOOD IN ELECTRON MICROSCOPY

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Fine structures of the neutrophils obtained by hemolytic method from the peripheral blood of 5 clinically normal sheep were examined by an electron microscope. The results thus obtained were summarized as follows.

1) The nuclei of the cells showed one to several nuclear lobes, and the nuclei with 2 to 4 nuclear lobes were observed most commonly.

2) The nuclear lobes of the cells showed a clear maculous appearance with two distinct densities, one light and the other dark, according to the amount of the chromatin condensation.

3) The specific granules were classified into two types on the basis of their electron density, the one with high density and the other with less density.

4) Most of the specific granules had the homogeneous interior without any internal structures. However, some of them had internal structures such as a middle plate-like substance, round defects with or without vacancy, sacs with small vesicles and clear spaces between the membrane and matrix of the granules.

5) A small number of mitochondria measuring 0.3 by 0.7 μ on the average, Golgi complex, a number of smooth-surfaced and a few rough-surfaced endoplasmic reticulum, and a few large round or oval bodies supposed to be lysosome were observed in the cytoplasm.

INTRODUCTION

In the field of veterinary hematology, there are many reports on the electron microscopic observations of the blood cells of domestic animals such as horses^{9,10,16,21,23,28,30,31,36,44}), cattle^{18,19,35,37~39,45,46}), pigs^{25,26}), dogs^{27,29,33,34}), cats^{4~6,23,32,42~44}), minks^{21,36}) and chickens^{12,23,44}). However, so far as the authors know, no observations of the blood cells of the sheep through an electron microscope have been reported.

In this paper, the ultrastructures of neutrophils in the peripheral blood of clinically normal sheep will be described.

MATERIALS AND METHODS

Five clinically normal female sheep were used for the experiments. They were all

Corriedale and from 2 to 8 years of age. The leukocytes were obtained by ODAJIMA's methods modifying the hemolytic methods of BEHRENS & ESCH, viz., 5 ml of the peripheral blood anticoagulated with EDTA-2Na obtained from the jugular vein was hemolysed by the addition of 7.5 ml of water and restored to its isotonic state exactly after 20 seconds by the addition of 1.5 ml of 5.4% NaCl solution. The hemolysates were transferred to conical test tubes and centrifuged for 5 minutes at 1,000 rpm. The supernatants were discarded as perfectly as possible. The sediments at the bottom of the test tubes were mixtures consisting of leukocytes and thrombocytes. The sediments were fixed by phosphate-buffered 1% osmic acid solution (MILLONIG's method) in the test tubes for 50 minutes. Then, they were dehydrated by a graded series of acetone and embedded in Epon 812. Polymerization was carried out at 60°C for 48 hours. The ultra-thin sections were cut with glass knives on a Porter-Blum MT-1 model ultramicrotome. After mounting on nickel grids, the sections were double stained with uranyl acetate and lead citrate.

The sections were examined and photographed in a JEM 7 type electron microscope, at magnifications varying from 5,000~60,000.

OBSERVATIONS

The cell in this type was readily identified from the leukocytes in other types by its special nuclear shape and the presence of many specific granules.

The general shape of the cells was round or oval. Usually, their outlines were smooth, but some of them were irregular because they had many small or large cytoplasmic projections.

1 Nucleus

The nuclei of the neutrophils showed several separated nuclear lobes in the cytoplasm. In the present observations, the numbers of nuclear lobes appearing in the cut plane of each cell were variable in accordance with the cut directions of the cells, but in general, they were 2~4. However, cells with over 5 nuclear lobes were also sometimes observed.

The shape and size of the nuclear lobes were variable in accordance with the different cut directions. Round, oval or short strand ones were most frequently observed in the cytoplasm. The outlines of the nuclear lobes were usually smooth.

Occasionally, the nuclear lobes were connected with thin nuclear strands for a short distance. The nuclear lobes were enclosed by a distinct nuclear membrane composed of a double-layered structure which was separated by clear spaces.

The lobes were filled with diffusely dispersed fine granular materials. The nuclei of the neutrophils showed two areas with distinct densities, one light and the other dark, depending upon the amount of chromatin condensation. The darker area usually attached to the nuclear membrane and was located peripherally. The lighter area occupied chiefly the central areas of the nuclear lobes, but it occasionally extended toward the nuclear membrane.

In these studies, up to the present time, the nuclei with a nucleolus have not been observed in the cut planes of any sections of the neutrophils.

2 Cytoplasm

The cytoplasm was delineated by a thin cell membrane. In the cytoplasm, there were a lot of specific granules, a small number of mitochondria, Golgi complex and some other micro-organelles.

The specific granules The specific granules of the neutrophils were randomly distributed in the cytoplasm except for the pseudopodic projections and a narrow perinuclear area. These specific granules were opaque and had a distinct unit membrane. The shape and size of the granules were variable depending upon the cut planes in which the granules were sectioned. In general, they were round, oval, rice grain- or short rod-like in shape.

The electron densities of these granules were considerably variable. However, on the basis of their density, they were divided into two types. The 1st type was dense and filled compactly with homogeneous materials at a high density, and the 2nd type was less dense with fine granular materials relatively coarsely distributed. The shape of the 1st type granules was, in general, rod-, spindle-like or round. The size of the granules varied considerably and they were measured as 0.18 (0.1~0.4) by 0.54 (0.3~0.7) μ on the average. The 2nd type granules were usually round or oval in shape. The diameter of these granules was 0.4 (0.3~0.6) μ on the average.

Interiors of the most specific granules were homogeneous and without any internal structures. However, in some granules, particular internal structures were observed. They were middle plate-like structures, round defects with or without vacancy, sacs with small vesicles and clear spaces between the membranes and inner substances.

Mitochondria There were a small number of mitochondria scattered among the specific granules in the cytoplasm. They were round, oval or rod-like in shape in the cut planes. Their average size was 0.3 (0.2~0.4) by 0.7 (0.4~1.0) μ . The cristae mitochondriales were not always clear so that it was sometimes difficult to differentiate the mitochondria from the specific granules, particularly when the mitochondria were round in shape.

Golgi complex Occasionally, Golgi complex composed of clusters of small vesicles and ill-developed lamellar structures was found in the cytoplasm.

In general, the Golgi complex was observed in the central portion enclosed by the nuclear lobes in the cytoplasm. One or two centrioles were rarely seen near the Golgi complex.

Endoplasmic Reticulum A considerable number of smooth-surfaced endoplasmic reticulum were seen scatteringly throughout the cytoplasm. They were smaller than the specific granules in size, and were filled with a more or less dense transparent substance or had clear contents. They were round or oval in shape. A very few roughsurfaced endoplasmic reticulum were rarely observed in the cytoplasm. They were short, thin canalicular in shape.

Others Besides the organelles described above, there were free ribosomes and polysomes, which were scattered at random throughout the cytoplasm. The multivesicular bodies were frequently observed in the cytoplasm. They were round or oval in shape, and in general, they were larger than the smooth-surfaced endoplasmic reticulum and smaller

than the specific granules in size. The multivesicular bodies were characterized by containing several smaller vesicles and granules in their ground substances. In the peripheral areas of the cytoplasm, several numbers of phagocytic vacuoles were seen, too.

Furthermore, in the cytoplasm, large round or oval bodies with smooth or slightly irregular contours, measuring about $0.7\sim 1.7\ \mu$ were observed. They had a clearly defined limiting membrane. They contained small or large vesicles and granules of various densities in their finely granular ground substances. Some of them had fine structures closely similar to those of thrombocytes.

DISCUSSION

On the basis of the authors' observations, it might be said that the ultrastructures of the neutrophils of ovine peripheral blood were fundamentally similar to those of the human^{1,8,20,40,42~44}) and other animal^{3,9,16,18,24,26,27,30,36,41,47}) neutrophils reported already. However, in the fine structures of the specific granules of ovine neutrophils, some interesting findings were obtained, that is; the specific granules were classified into 2 types on the basis of electron density.

It has been reported that there were one to four types of granules in the human and other animal neutrophils. Namely, in animals, one type was in horses^{9,16,30}) and pigs²⁵), two types were in cats^{42,43}), rabbits^{2,3}), guinea-pigs^{41~43}), rats²⁴), mice¹⁴), gorillas¹³) and chimpanzees¹³) and three types were in orangutans¹³), dogs²⁷), rabbits^{47,48}) and minks³⁶), respectively.

In the human neutrophils, the specific granules were so variable by each report that there were one⁸), two^{15,20,44}), three^{1,40,42,43}) or four¹¹) types in the specific granules.

The authors' typing of the granules was similar to those in guinea-pigs^{41~43}), cats^{42,43}), mice¹⁴), rats²⁴), gorillas¹³), chimpanzees¹³), rabbits^{2,3}) and the human^{15,20,44}), though there were some differences in the scale of classification.

Recently, the internal ultrastructures of the granules have interested some workers. WATANABE et al. observed that the granules of the human neutrophils contained crystals arranged parallel to the longitudinal axis and displaying a lattice arrangement on the cross section. DAEMS reported that in the human neutrophils, there were granules with 3 kinds of internal structures, such as; the granules containing a crystal inclusion consisting of a number of rigid rods arranged perfectly parallel with spaces of about $100\ \text{\AA}$, the granules with a somewhat floccular matrix containing a number of strictly unparallel filaments measuring about $60\ \text{\AA}$ in diameter and the granules containing rather coarse or fine floccular materials. KAMIYAMA observed the crystals consisting of a number of rods arranged parallel with spaces of about 50 to $60\ \text{\AA}$ in the granules of the human neutrophils. Furthermore, in the neutrophils of some animals^{13,27,48}), the human^{20,44}) and chickens¹²), the granules with clear spaces between the membrane and the

matrix of the granules, and the granules with middle plate-like structures have been reported.

In the present observations of the ovine neutrophils, the specific granules with various internal structures were observed by the authors, viz., the granules with middle plate-like substance, the granules containing round defects with or without vacancy, the granules with sacs containing many small vesicles and the granules with clear spaces between the membrane and matrix of the granules. The granules with a middle plate-like structure and with clear spaces between the membrane and matrix observed in the ovine neutrophils were identical with those of chickens¹²⁾ and orangutans¹³⁾, of dogs²⁷⁾ and the human^{20,24)}, respectively. However, the granules with other inner structures* observed by the present authors have not been reported by any other workers.

The large round or oval bodies observed in the cytoplasm of the ovine neutrophils described above were thought to be so-called lysosomes or phagosomes. Some of them seemed to be phagocytosed thrombocytes, because they had fine structures closely similar to those of thrombocytes. Furthermore, some were thought to be cytolysome due to cell degeneration.

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EXPLANATION OF PLATES

PLATE I

- Fig. 1 The contour of this cell is nearly smooth, but has a few small pseudopodic projections. The nucleus is separated into 2 lobes. The maculous appearance is not so clear in the lobes. There are a large number of specific granules (SG) with variable densities in the cytoplasm. Among the granules, a few mitochondria (M) are seen. A considerable number of vacuoles (V) in variable size are scattered throughout the cytoplasm. $\times 10,000$
- Fig. 2 The nucleus is separated into four lobes. A large number of specific granules are in the cytoplasm. The size and shape of the granules are considerably variable. Two granules with the middle plate-like structures are seen (arrows). $\times 10,000$
- Fig. 3 Many specific granules are seen in the cytoplasm. They are variable in shape. A granule with round defects containing a filamentous substance is seen (arrow). The differences of electron densities among the granules are clear. Mitochondria (M) are present, too. $\times 20,000$
- Fig. 4 There are many specific granules of variable size and shape in the cytoplasm. The differences of electron densities among the granules are clear, viz., they are classified into two types, the one with high density and the other with less density. $\times 20,000$

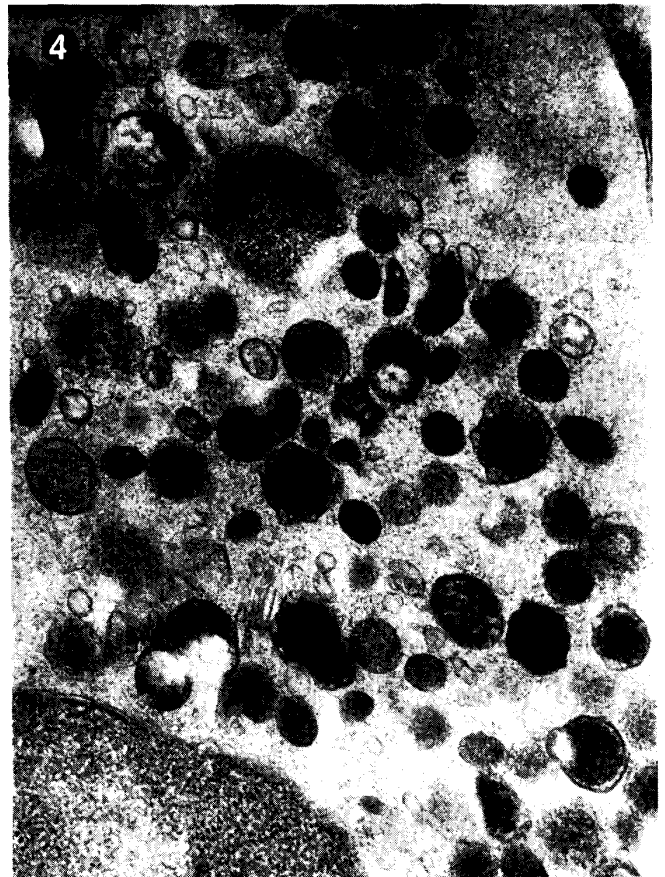
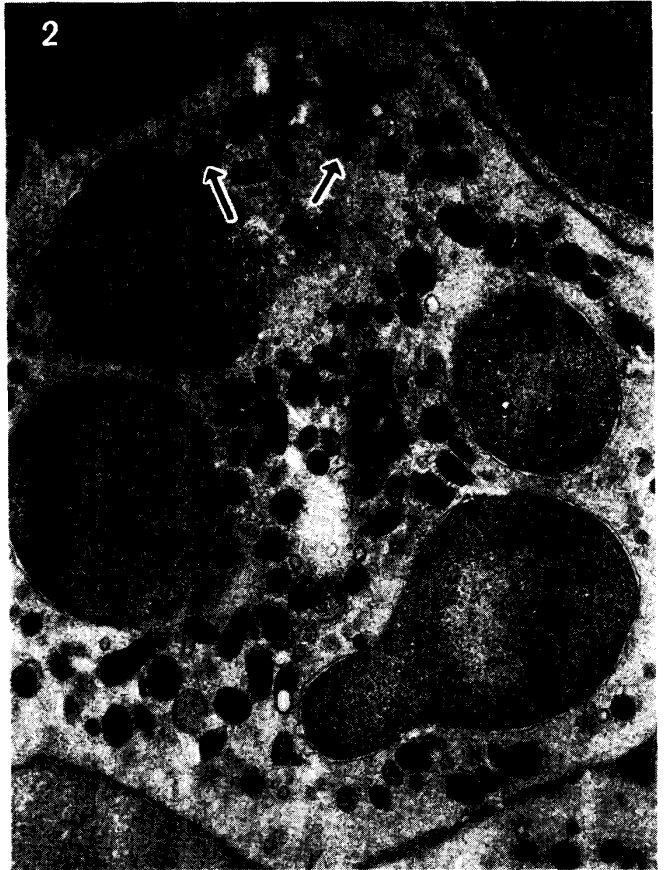
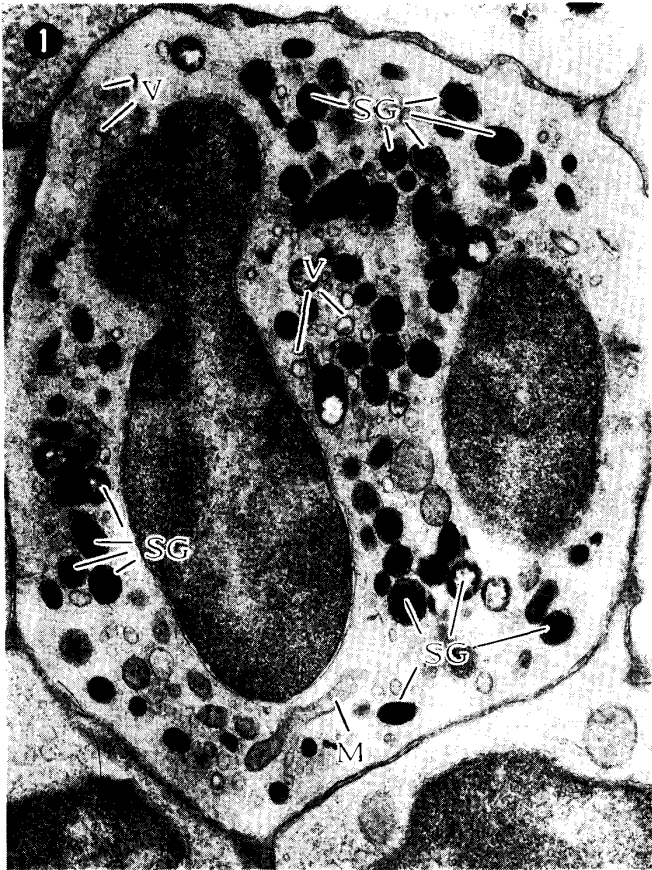


PLATE II

- FIG. 5 A spindle-shaped granule has a middle plate-like substance being parallel to the longitudinal axis of the granule. $\times 45,000$
- FIG. 6 Two kinds of granules with different electron densities are seen. The short rod-like granules have a homogeneous high density. An oval granule has less density and a fine granular structure. These granules are enclosed by a distinct double-layered unit membrane. $\times 90,000$
- Fig. 7 A granule with homogeneous interior is shown. $\times 90,000$
- Fig. 8 An oval granule with a defect is shown. $\times 90,000$
- Fig. 9 A large oval body observed in the cytoplasm is shown. It has a distinct membrane. It seems to be a phagocytosed thrombocyte, because it has internal structures closely similar to those of thrombocytes. $\times 45,000$
- Fig. 10 The large round body observed in the cytoplasm is shown. It contains several granular structures in its ground substances. It seems to be a so-called phagosome or cytolysosome. $\times 45,000$

