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EOSINOPHILS OF OVINE PERIPHERAL BLOOD IN ELECTRON MICROSCOPY

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The fine structures of the eosinophils of the peripheral blood obtained from 5 clinically normal sheep were examined under an electron microscope. The results thus obtained were summarized as follows: 1) The cells with nuclei of two to four lobes were most commonly observed on the cut planes. 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) On the basis of the internal structures of the specific granules, they were classified into six types and the basic type of the granules seemed to be those containing middle plates. 4) A small number of mitochondria measuring 0.3 by 0.7 μ on the average, Golgi complex, a number of smooth- and a few rough-surfaced endoplasmic reticulum, and some other micro-organelles were observed in the cytoplasm.

INTRODUCTION

There have been a lot of publications on the fine structures of the eosinophils in the peripheral blood of the human[1,2,5,10,11,16,18,20,35,37,38] and other animals[6~8,14,17,21,23,29~31,34], and it is well known that the internal structures of their specific granules are very different in accordance with the difference of the animal species. However, up to the present time, there are no reports on ovine eosinophils in electron microscopy.

In this paper, the fine structures of the eosinophils in the peripheral blood obtained from clinically normal sheep will be described.

MATERIALS AND METHODS

Blocks for electron microscopy and the methods of observation were just the same as those of the previous paper[41].

OBSERVATIONS

The general shapes of the eosinophils were round or oval. The contours of the cells were usually smooth; however, some of them were irregular because of having many small or large pseudopodic projections.

1 Nucleus

The nuclei of the eosinophils showed one to several separated nuclear lobes on the cut planes. In the present observations, however, the nuclei with 2 to 4 lobes were common in number, but the cells with more than 5 nuclear lobes were rarely observed.

In the nuclei of the eosinophils, there were two parts of distinct densities, one light and the other dark, depending upon the amount of the chromatin condensation. The dark part of the two was located peripherally in the nuclear lobes and constituted a thick band subjacent to the nuclear membrane. On the other hand, the light part occupied chiefly the central area of the nuclear lobes, however, it occasionally extended toward the nuclear membrane.

2 Cytoplasm

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

The specific granules The specific granules of the eosinophils were distributed randomly in the cytoplasm except for parts of the pseudopodic projections, the Golgi and narrow peri-nuclear areas. These granules had extraordinary high electron density and were opaque. They were surrounded by a distinct unit membrane. On the cut planes, they varied considerably in size and shape depending upon the cut directions in which the granules were sectioned.

Almost all of the granules had variable internal structures; however, some of them were homogeneous without any internal structures. On the basis of the internal structures, they were divided into 6 types of granules as follows.

1) The 1st type

These were the homogeneously compact high or moderate dense granules without any internal structures. In general, the granules of this type were round in shape, and their size was on the average 0.60 (0.4~0.8) μ in diameter.

2) The 2nd type

These were the granules containing so-called middle plates in their ground substances. The middle plates were more dense than that of the ground substances of the granules, and they consisted of substances with stratified structures or without any structures. Each granule usually contained one to several middle plates. In the longitudinal sections of the granules, the middle plates appeared like bandages running parallel to the long axis of the granule profiles. The middle plates were situated usually in the central portions of the granules, but sometimes they were seen eccentrically in the side areas of the granules. The granules of this type were usually oval or spindle-like and a few were round in form. In size, they were 1.1 (0.8~1.5) μ long by 0.3 (0.2~0.6) μ wide on the average.

3) The 3rd type

These were the granules containing substances with myelin-like structures, viz., they had lamellar structures arranged concentrically, so that they looked just like hair balls. In some of the granules of this type, the substances consisted of 2~3 myelin-like structures, and a dense substance in the central area or periphery were observed. The granules of

this type were usually round. They were 0.7 (0.4~1.0) μ in diameter on the average.

4) The 4th type

These were the granules with fine reticulated structures in some parts of the ground substances. The parts of the ground substances without reticulated structures were homogeneous. By observing enlarged pictures, it was clear that the reticulated structures were composed of fine granular materials arranged checkedly. The granules of this type were round or oval in shape and they were much the same as those of the 2nd and the 3rd type granules in size.

5) The 5th type

These were the granules containing dense homogeneous round structures. They were usually round in shape and much the same size as those of the 1st type granules.

6) The 6th type

These were the granules containing simultaneously some substances of the internal structures described in the 2nd to the 5th type granules. Namely, for example, the granules containing the reticulated structure or myelin-like structure together with the middle plate were observed, frequently. Of course, in others, the ones with various complicated combinations of the internal structures were observed. The granules of this type were round, oval or slightly irregularly round in shape, and approximately similar in size to those of the 2nd and the 3rd type granules.

Among these specific granules, the granules with round or oval vacant defects were observed rarely.

In the appearance rates of the specific granules of each type in the cytoplasm of the cells, the granules of the 2nd type were observed most predominantly and the granules of the 3rd and the 6th types were observed next most predominantly. The granules of other types were only observed rarely.

Mitochondria A few mitochondria distributed randomly among the specific granules throughout the cytoplasm. They were round or oval in shape. Their size was 0.3 (0.2~0.4) μ by 0.7 (0.4~1.0) μ on the average.

Golgi complex Golgi complex consisted of lamellar membranes and vesicles were frequently observed in the central areas of the cells.

Endoplasmic reticulum A large number of smooth-surfaced endoplasmic reticulum were seen randomly throughout the cytoplasm. They were smaller than the specific granules in size and were filled with more or less dense substances or had clear contents. A few rough-surfaced endoplasmic reticulum were observed in the cytoplasm. They were short or long thin canalicular in shape.

Others A number of free ribosomes and polysomes were present in the cytoplasm. The multivesicular bodies enclosed by the unit membrane and containing a few small granular or vesicular substances were rarely observed. The bodies with the structures similar to those of thrombocytes were present in the cytoplasms of a few cells. They were supposed to be phagocyted thrombocytes.

CONSIDERATIONS

It has been well known that the fine structures of the specific granules of the eosinophils are very variable according to the difference of animal species. On the basis of the observations reported already, the fine structures of their specific granules will be fundamentally grouped into the following four types, viz., they are the type of granules containing middle plates as seen in those of the human[1,2,5,9~11,16,18,20,21,35,37~39,42], guinea pigs[12,13,20,21,36~38], rabbits[21,40,43], mice[20], rats[20,22~24], orangutans[14] and chimpanzees[14], the ones with the middle trunks[21] which are composed of a very characteristic lamellar structure as seen in those of cats[2~4,21,37~39], the ones with two or three thick stratified concentric structures as seen in those of cattle[34], dogs[31,32] and minks[19], and homogeneous dense granules without any internal structures as seen in those of horses[6,7,15,21,30,39], pigs[27], gorillas[14] and chickens[8,39].

In the present observations of the ovine eosinophils, the specific granules of the cells have showed various internal structures. Namely, the homogeneous granules without any internal structures, the ones with middle plates, the ones with myelin-like structures, the ones with small fine reticulated structures, the ones with homogeneous dense round substances, and the granules containing simultaneously some of the internal structures described above were observed.

It was very interesting to discover that in the individual eosinophils, the internal structures of the granules were very variable from one granule to another.

Furthermore, considering the cubic structure of the granules, the 1st, the 2nd and the 5th type granules are supposed to be granules with similar internal structures. The morphological differences among them on the cut planes are thought to be derived from the different directions of cutting.

On the basis of the apperance rate of the granules of each type, the basic type of the specific granules of the ovine eosinophils seems to be the granules with the middle plates.

Recently, attention has been paid to the fine structures of the substances contained in the granules. SHELDON & ZETTERQUIST and others[20,35] reported that the middle plates or crystals of the specific granules had a repeat period of about 30 to 40 Å in the human, rats, mice and guinea pigs. MILLER et al. suggested that the crystals of the granules had cubic lattices. RUDOLPH observed that the specific granules of the eosinophils in the mast cell tumor of dogs had lamellar structures in the peripheral or central areas of their homogeneous matrix.

In the present observations of the ovine eosinophils, it was seen that the middle plates or crystalloids of the specific granules had a lamellar structure similar to that of the observations described above. In addition, the authors

observed the fine reticulated and lattice-like structures in the granules as shown in the micrographs of the 4th type granules. This is the first report on the presence of such structures in the granules of the eosinophils. Furthermore, the 5th and the 6th type granules classified by the authors have not been reported yet.

The granules with round or oval vacant defects in the ovine eosinophils were rarely observed by the authors. SONODA and KOBAYASHI observed round or oval vacant defects in the granules of the canine eosinophils, and supposed that they were morphological findings related to the function of the eosinophils.

The presence of a unit membrane of the specific granules of the eosinophils has been disputed by many workers[1,8,11,16,20,21,25,26,29,31,32,35,37,39]. In the present observations, it was clear that the specific granules of the ovine eosinophils had a unit membrane consisting of three layers.

In the cytoplasm of the human eosinophils, moderately dense, homogeneous and irregularly shaped bodies with much the same size as the specific granules have been observed by some workers[1,9,18,39,42]. Especially LOW & FREEMAN called them basophilic bodies and WATANABE[39] supposed that these bodies were the lipid bodies. However, in the present study on the ovine eosinophils, any of such bodies in the cytoplasm had not been observed at all.

REFERENCES

1) ANDERSON, D. R. (1966): Ultrastructure of normal and leukemic leukocytes in human peripheral blood, *J. Ultrastruct. Res.*, Suppl. **9**, 1
2) BARGMAN, W. & KNOOP, A. (1956): *Z. Zellforsch. mikrosk. Anat.*, **44**, 282
3) BARGMAN, W. & KNOOP, A. (1956): *Ibid.*, **44**, 692
4) BARGMAN, W. & KNOOP, A. (1958): *Ibid.*, **48**, 130
5) BESSIS, M. & THIERY, J. P. (1961): *Int. Rev. Cytol.*, **12**, 199
6) BOCCIARELLI, D. S., TENTORI, L. & VIVALDI, G. (1969): *Rc. Ist. sup. Sanità*, **22**, 1059
7) BRAUNSTEINER, H. & PAKESCH, F. (1962): *Acta haemat.*, **28**, 163
8) DHINGRA, L. D., PARRISH, W. B. & VENZKE, W. G. (1969): *Am. J. vet. Res.*, **30**, 637
9) FREEMAN, J. A. (1964): Cellular fine structure, New York, Toronto, London: McGraw-Hill Book Company
10) GOODMAN, J. R., REILLY, E. B. & MOORE, R. E. (1957): *Blood*, **12**, 428
11) HIRSCH, J. G. & FEDORKO, M. E. (1968): *J. Cell Biol.*, **38**, 616
12) HUDSON, G. (1966): *Expl. Cell Res.*, **41**, 265
13) HUDSON, G. (1967): *Ibid.*, **46**, 121
14) HUSER, H. J. & WEBB, C. M. (1967): *Experientia*, **23**, 669
15) ITO, Y. (1964): *Nōrinsho Kachiku-eisei-shikenjyō Nenpō*, **4**, 386 (in Japanese)
16) KAUTZ, J. & DEMARSH, Q. B. (1954): *Blood*, **9**, 24
17) KNOCKE, K.-W. (1963): *Folia haemat.* Neue Folge, **7**, 129
18) LOW, F. N. & FREEMAN, J. A. (1958): Electron microscopic atlas of normal and leukemic human blood, New York, Toronto, London: McGraw-Hill Book Company, Inc.
19) LUTNER, M. A., TIERNEY, J. H. & BENDITT, E. P. (1965): *Lab. Invest.*, **14**, 2063
20) MILLER, F., DEHARVEN, E. & PALADE, G. E. (1966): *J. Cell Biol.*, **31**, 349
21) OSAKO, R. (1959): *Acta haemat. jap.*, **22**, 134 (in Japanese with English summary)
22) PEASE, D. C. (1956): *Blood*, **11**, 501
23) RINEHART, J. F. (1955): *Am. J. clin. Path.*, **25**, 605
24) ROSS, R. & KLEBANOFF, S. J. (1966): *J. exp. Med.*, **124**, 653
25) RUDOLPH, R. (1969): *Dt. tierärztl. Wschr.*, **76**, 176
26) SCHULZE, P. (1966): *Arch. exp. VetMed.*, **20**, 767
27) SCHULZE, P. (1967): *Ibid.*, **21**, 1305
28) SHELDON, H. & ZETTERQUIST, H. (1955): *Bull. Johns Hopkins Hosp.*, **96**, 135
29) SHIVELY, J. N., FELDT, C. & DAVIS, D. (1969): *Am. J. vet. Res.*, **30**, 893
30) SONODA, M. (1963): *Proceedings of the 55th* Meeting of the Japanese Society of Veterinary Science, *Jap. J. vet. Sci.*, **25**, 394 (summary in Japanese)
31) SONODA, M. (1969): Proceedings of the 67th Meeting of the Japanese Society of Veterinary Science, *Ibid.*, **31**, Suppl., 98 (summary in Japanese)
32) SONODA, M. & KOBAYASHI, K. (1970): *Jap. J. vet. Res.*, **18**, 43
33) SONODA, M. & MARSHAK, R. R. (1970): *Ibid.*, **18**, 9
34) SONODA, M., MIFUNE, Y. & OHYA, S. (1964): Proceedings of the 57th Meeting

of the Japanese Society of Veterinary Science, *Jap. J. vet. Sci.*, **26**, 440 (summary in Japanese)

35) WATANABE, I., DONAHUE, S. & HOGGATT, N. (1967): *J. Ultrastruct. Res.*, **20**, 366

36) WATANABE, Y. (1954): *J. Electron Microsc., Chiba Cy*, **2**, 34

37) WATANABE, Y. (1956): *Acta haemat. jap.*, **19**, 327 (in Japanese with English summary)

38) WATANABE, Y. (1957): *J. Electron Microsc., Chiba Cy*, **5**, 46

39) WATANABE, Y. (1963): (translated title) "The fine structures of leukocyte", Compendium of haematology, 1, Ed. AMANO, S. & HIBINO, S., 1 ed., 327, Tokyo, Maruzen (in Japanese)

40) WETZEL, B. K., HORN, R. G. & SPICER, S. S. (1967): *Lab. Invest.*, **16**, 249

41) YAMADA, Y. & SONODA, M. (1970): *Jap. J. vet. Res.* **18**, 83

42) ZUCKER-FRANKLIN, D. (1963): *J. Ultrastruct. Res.*, **9**, 325

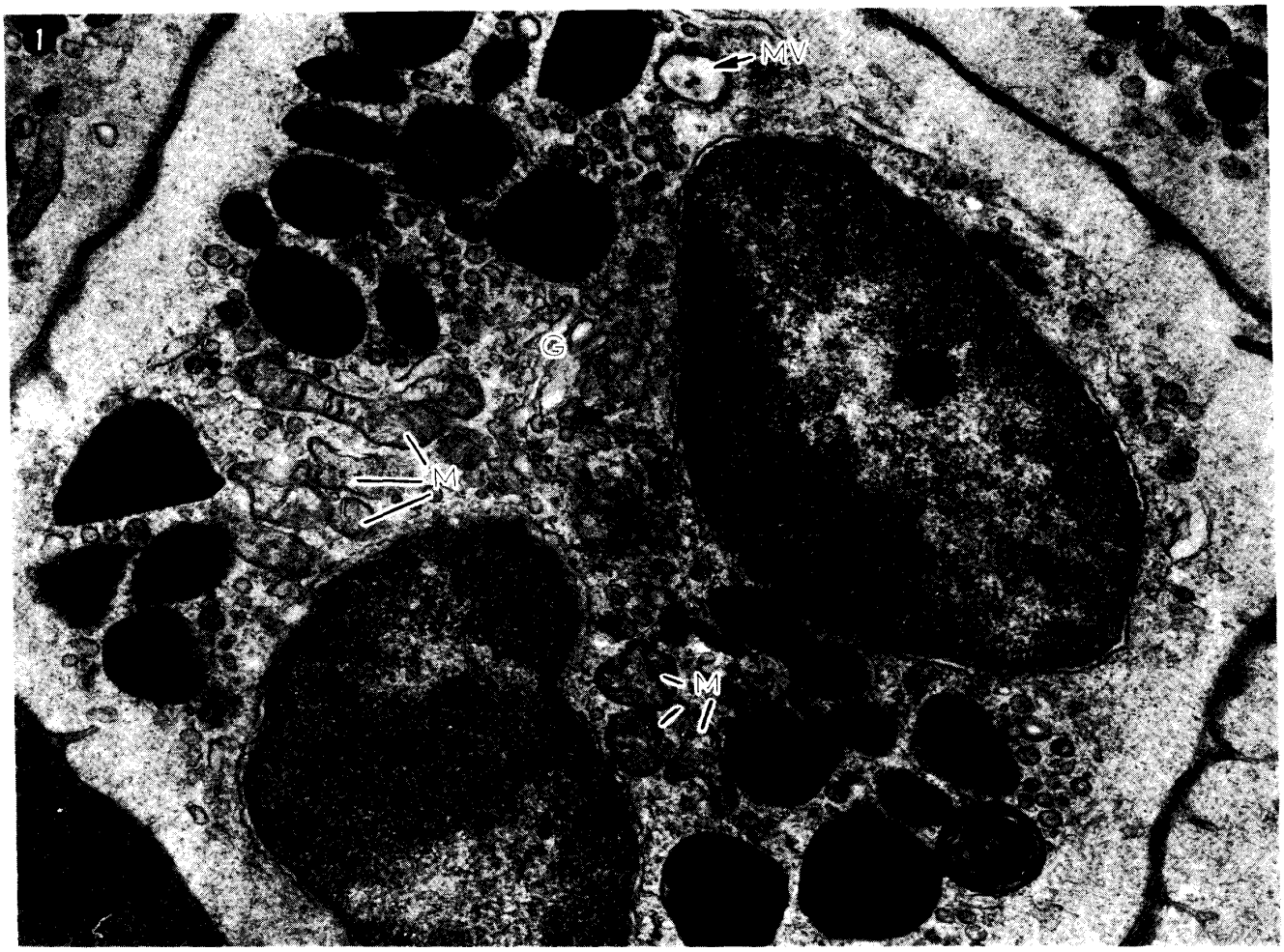
43) ZUCKER-FRANKLIN, D. & HIRSCH, J. G. (1964): *J. exp. Med.*, **120**, 569

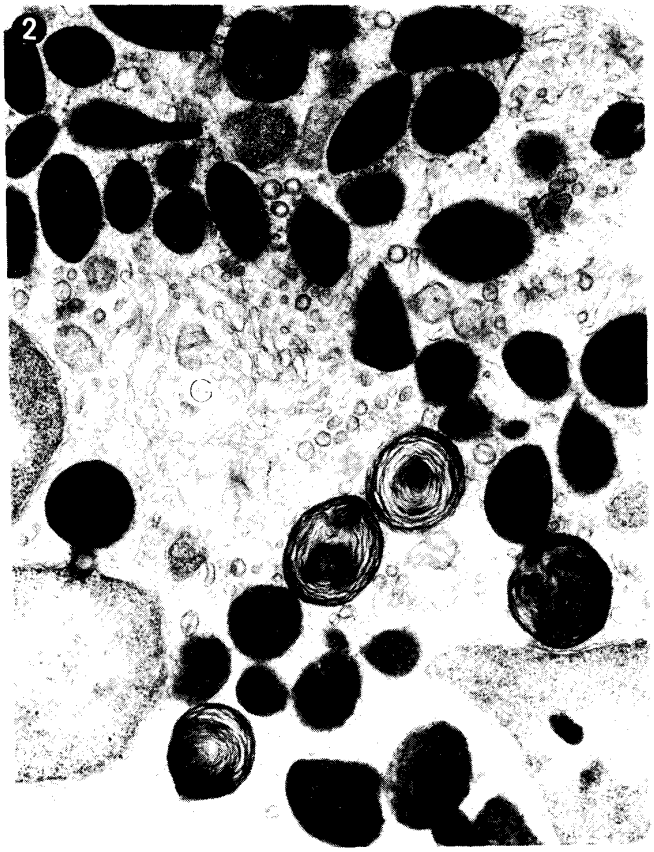
Explanation of plates

Plate I

Fig. 1 The nucleus is separated into 2 lobes. The maculous appearance of the lobes with two distinct densities is clear. There are a number of large specific granules with very high density in the cytoplasm. A few mitochondria (M) are seen. They are variable in shape. Golgi complex (G) is seen in the central area of the cell. A considerable number of small vesicles are scattered throughout the cytoplasm. They are supposed to be smooth-surfaced endoplasmic reticulum. A multivesicular body (MV) is seen.
× 17,500

Figs. 2 & 3 In these figures, many of the specific granules with variable shape and internal structures are shown. Furthermore, Golgi complex (G) and mitochondria (M) are seen.
Fig. 2 × 12,500
Fig. 3 × 20,000


1
MV
G
M
M


2
G


3
G
M
M

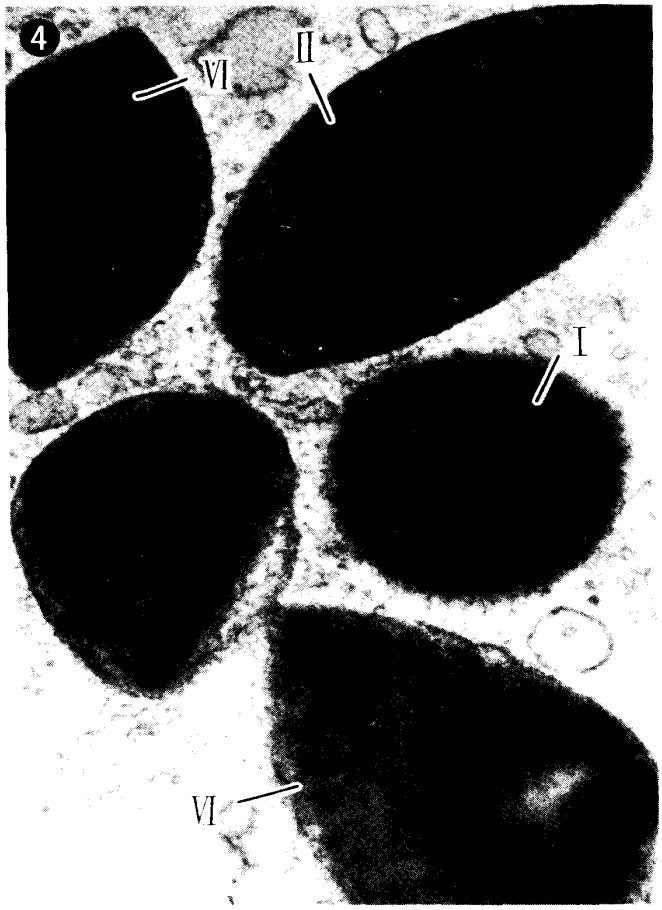
PLATE II

Fig. 4 A small round homogeneous granule (the 1st type, I), a spindle-form granule with wide middle plate (the 2nd type, II), two short spindle-form granules containing simultaneously narrow middle plates and round myelin-like structures (the 6th type, VI) and one other granule are seen.
× 75,000

Fig. 5 A small round granule with fine reticulated structures (the 4th type, IV) and a large almost round granule with a highly dense annular structure (the 3rd type, III) are seen.
× 75,000

Fig. 6 An oval granule with a wide middle plate (the 2nd type) is seen. The middle plate has lamellar structures parallel to each other. The unit membrane consisting of three layers is clear.
× 150,000

Fig. 7 A granule with myelin-like structures (the 3rd type) is shown. Its core consists of three small hair ball-like substances.
× 150,000


4
VI
II
I
VI


5
III
IV


6

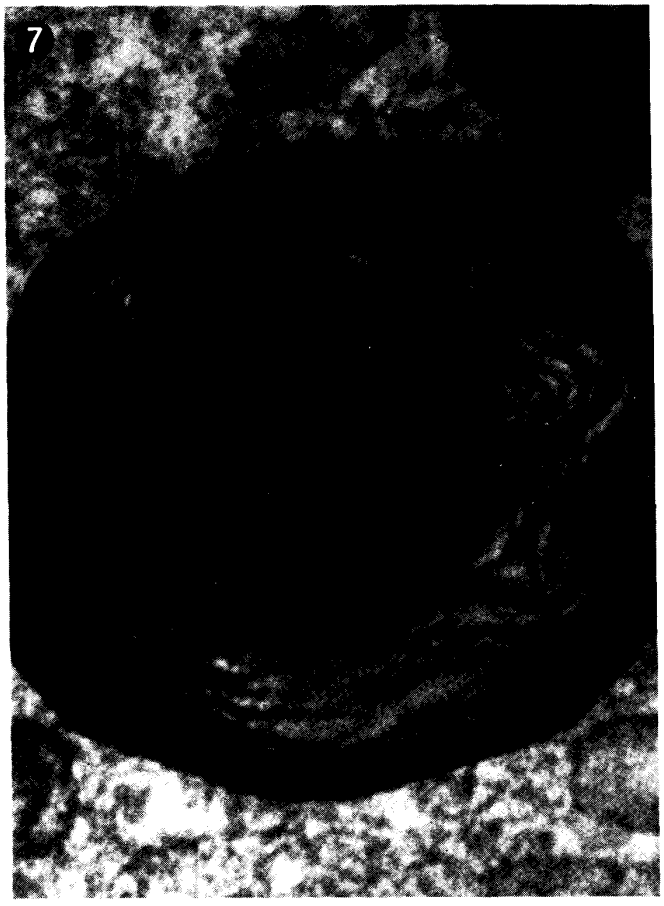

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PLATE III

Fig. 8 This is a round granule with almost homogeneous matrix (the 1st type).
× 90,000

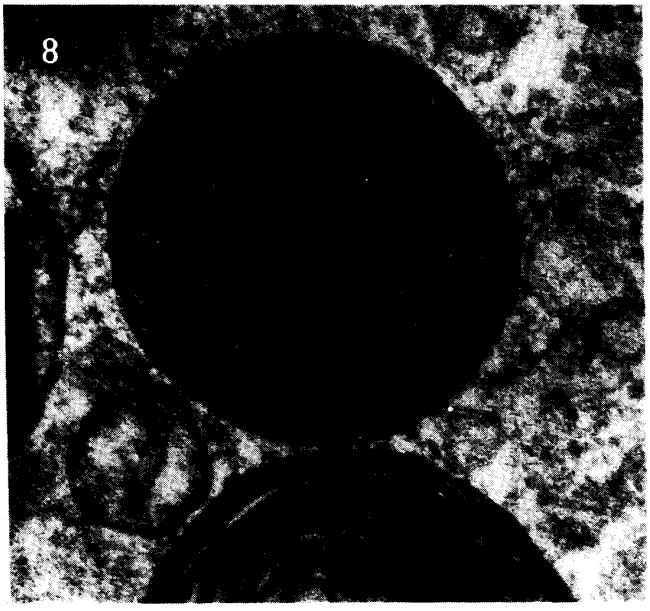
Fig. 9 A round granule with round dense homogeneous structures (the 5th type) is shown.
× 90,000

Fig. 10 An elliptical granule containing a wide middle plate and fine reticulated structures (the 6th type) is shown.
× 90,000

Fig. 11 A round granule containing a bent needle-like middle plate and a concentric myelin-like structure in its matrix (the 6th type) is shown.
× 90,000

Fig. 12 An elliptical granule containing two narrow belt-like middle plates and a myelin-like structure (the 6th type) is shown.
× 90,000

Fig. 13 A slightly irregularly round granule containing myelin-like structure in the peripheral area and a fine reticulated structure in the central area (the 6th type) is shown.
× 90,000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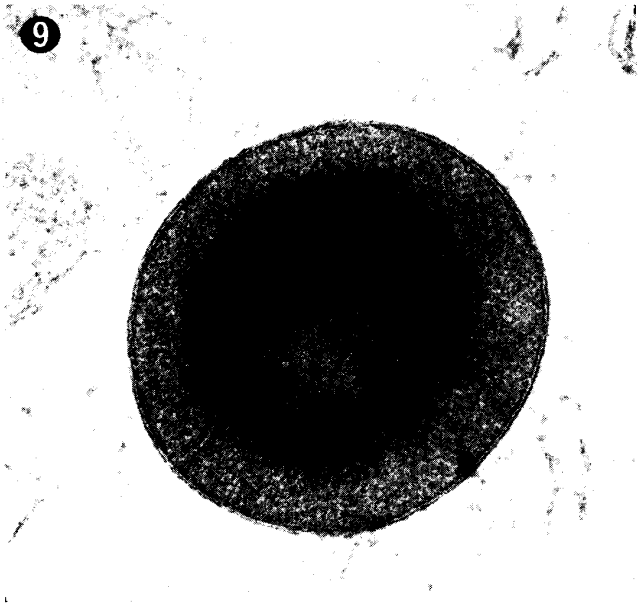
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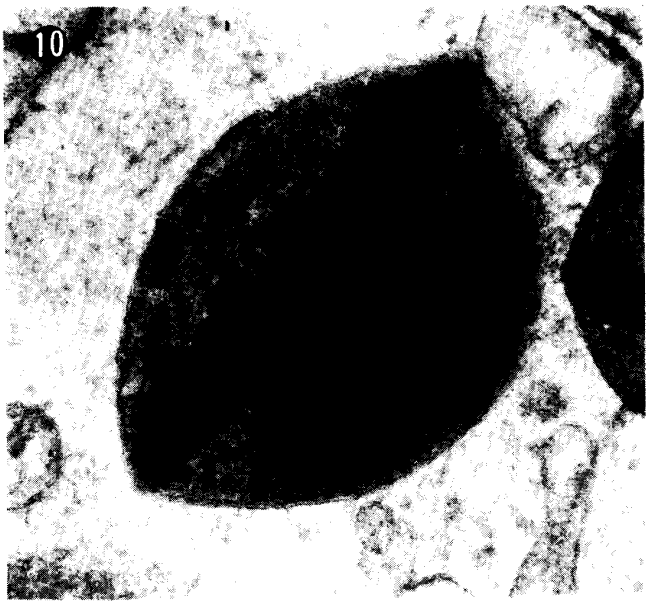
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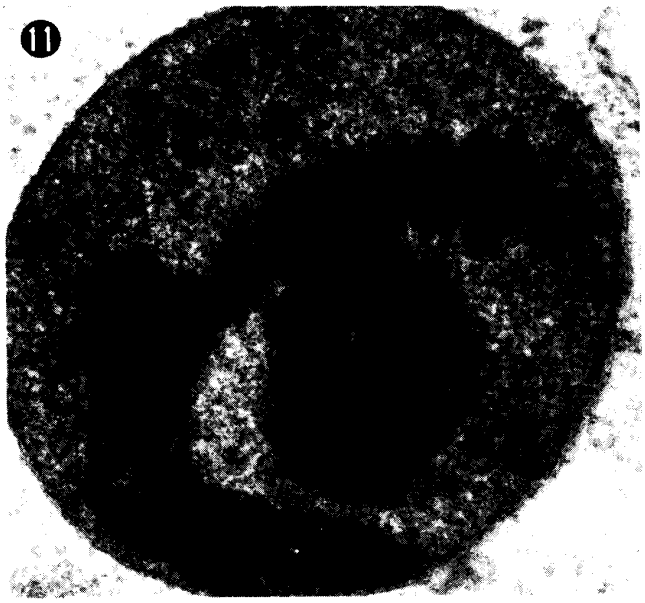
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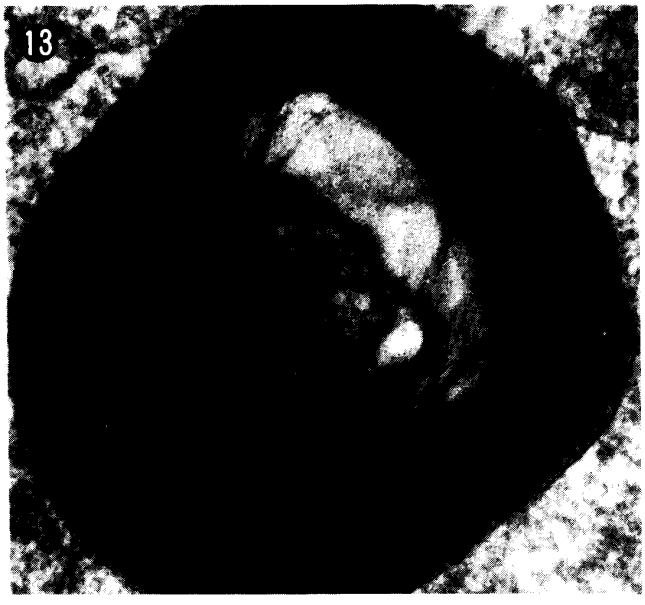
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