

## Fine Structures in the Blood Cells of Japanese Quails (*Coturnix coturnix japonica*)

### I. Heterophils and Eosinophils

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ニホンウズラ (*Coturnix coturnix japonica*) の

血液細胞の微細構造について

I. 好異球と好酸球について

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### Introduction

Many investigators have described the ultrastructure of mammalian blood cells, but there are few studies of the peripheral blood cells of fowls. These reports on fowls were obtained solely from chickens. Recently Japanese quails are attracting considerable attention as experimental animals instead of chickens. However, so far as the authors know, no observations of the blood cells of Japanese quails under an electron microscope have been reported. It was difficult to differentiate between the heterophils and the eosinophils under the light microscope, since both have eosinophilic staining granules in the cytoplasm. Ultrastructural observations on the blood cells of Japanese quails were then undertaken. In this paper, the differences in the fine structures between the heterophils and the eosinophils were determined in the peripheral blood of clinically normal Japanese quails.

### Materials and Methods

Nine adult male Japanese quails which were healthy clinically were used in this study. Peripheral blood (1.5 ml) obtained by cutting the *A. occipitalis* and the *A. vertebralis* was poured into anticoagulant (0.5 ml) which was equally mixed with 1.5% EDTA-2K and 1.5% EDTA-3K solutions. The blood and anticoagulant were mingled gently but thoroughly. This anticoagulated blood was centrifugated at 1,000 rpm. for 5 minutes. Thereafter, the upper parts of the plasma containing a large portion of leukocytes and thrombocytes and a

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few red blood cells were transferred to small polyethylene test tubes and again centrifugated at 2,500 rpm. for 20 minutes.

The supernatant was removed as completely as possible. Some of the resultant buffy coats were fixed in a standard fixative of 1% OsO<sub>4</sub> in Millonig's buffer (pH 7.3) for 90 minutes, and some in a mixture of 1% OsO<sub>4</sub> and 2.5% glutaraldehyde in Millonig's buffer (pH 7.3) or of 1% OsO<sub>4</sub> and 2.5% glutaraldehyde in Caulfield's buffer (pH 7.4) for 90 minutes (YAMASHITA 1971). The remaining buffy coats were placed in a standard fixative of 2.5% glutaraldehyde in Millonig's buffer (pH 7.3) for 20 minutes, followed by postfixation of 1% OsO<sub>4</sub> in Millonig's buffer for 90 minutes, following the method of SABATINI *et al.* (1963). After the primary fixation, the fixative was gently decanted, and samples were washed three times with the same buffer for 20 minutes each.

The fixed tissues were dehydrated in a graded series of ethanol-acetone and embedded in epoxy resins after the method of LUFT (1961). The tissues were sectioned with an ultramicrotome (Porter-Blum MT-I) equipped with glass knives, and placed on grids with and without collodion film. The sections were stained with a saturated solution of uranyl acetate in 50% alcohol followed by lead citrate (REYNOLDS 1963) and observed under a JEM-7 type electron microscope.

## Results

**Heterophils** (Figs. 1-13): The size of the heterophils were about 6-7 $\mu$  in diameter. Heterophils were roundish cells with small pseudopodia (Fig. 1). A very small number of the heterophils had lobopodia (Fig. 3). Usually, their surfaces were smooth, although some of them had a small number of pinocytotic vesicles.

The nuclei of the heterophils 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 from 1 to 3. Cells with 4 nuclear lobes were rarely observed. The shapes of the nuclear lobes were variable according to different cut directions, but round, oval and reniform lobes were most frequent. Occasionally, nuclear lobes had narrow nuclear strands (Figs. 3 & 4). The nuclei of the heterophils showed two areas with distinct density, one clear and the other dark, depending upon the amount of the chromatin condensation. The darker areas usually attached to the nuclear membrane and were located peripherally. The clear area occupied chiefly the central portion of the nuclear lobe, but it occasionally spread toward the nuclear membrane. In this study, up to the present time, nuclei with nucleolus

have not been observed in the heterophils.

The specific granules of the heterophils were randomly scattered throughout the cytoplasm except for in the pseudopodia, lobopodia and Golgi area (Figs. 1, 3, 5, & 6). These specific granules were surrounded by a distinct double limiting membrane (Figs. 10 & 11). The shape and size of the granules were variable depending upon the cut plane in which the granules were sectioned. In general, they were typical fusiform, round, oval or rod-like in shape. The electron densities of these granules were variable. However, they were basically divided into two types on the basis of their electron densities. The dense heterophil granule (type I) was filled compactly with homogeneous materials at high electron density, and the light heterophil granule (type II) was filled with low dense homogeneous materials (Figs. 1-6). The shape of type I granules was, in general, a typically long fusiform structure with pointed ends, although round or oval granules were also observed (Figs. 1 & 6). The size of type I granules varied considerably measuring  $0.6-3.3 \mu$  by  $0.4-0.7 \mu$ . Type II granules were usually round or oval, although occasionally short rod-like or short fusiform in shape (Figs. 5 & 10). The diameter of these granules were  $0.3-1.2 \mu$ . The interiors of most of the specific granules of both types were homogeneous material without any internal structures. Some granules of type I, however, showed particular internal structures, viz., a cleft-like structure (Fig. 7) or small clear spots (Fig. 8). These structures were observed solely in 1%  $\text{OsO}_4$  fixed samples. Further, some granules of type I had a large clear space encompassed by a unit membrane (Fig. 11). In type II granules, filamentous structures were found only in samples which were fixed with 1%  $\text{OsO}_4$  (Fig. 9).

Excepting the granules of both types aforementioned, small cored granules were frequently around the Golgi complex (Figs. 5 & 12), although they were also occasionally randomly distributed in the cytoplasm (Figs. 6 & 11). The shape of these granules was round and their diameters ranged from 100 to 150  $m\mu$ . These cored granules had clear halos around their cores. The electron densities of their cores were variable. Some showed the same density as type I heterophil granules (Figs. 6 & 12) and others were lighter (Figs. 5 & 13).

There was a small number of mitochondria scattered among the specific granules in the cytoplasm. The majority of the mitochondria were small and round, oval or short and rod-like in shape, each containing a dense matrix with pale cristae. A few vesiculated smooth-surfaced endoplasmic reticulum were seen scattered throughout the cytoplasm. Rough-surfaced endoplasmic reticulum, however, were rarely seen in the cytoplasm. Golgi complex composed of clusters of small vesicles and ill-developed lamellar structure were found in the cytoplasm. In general, the Golgi complex was observed in the central portion

enclosed by the nuclear lobes in the cytoplasm. Rarely, one or two centrioles were seen near the Golgi complex (Figs. 1,2,5 & 12). Microtubules were usually found with the centrioles (Figs. 12 & 13). Among the organelles described above, there was a small amount of free ribosomes and polysomes, which were randomly scattered throughout the cytoplasm. Phagosome-like structure was very rarely found.

**Eosinophils (Figs. 14-20):** The general shape of the eosinophils was round or oval. Usually, their outline was smooth, although some of them were irregular because they had small pseudopodic projections and pinocytotic vesicles. The size of the eosinophils were about  $6\mu$  in diameter and were smaller than the heterophils. The nucleus consisted of 2, or in rare case 3, round to spherical lobes (Figs. 14-16). Occasionally, the nuclear lobes had narrow nuclear strands (Fig. 16). The chromatin pattern of the eosinophils shown mostly resembled heterophils. Nucleolus have not been found in the cut planes of any sections of the eosinophils up to the present observations.

The specific granules of the eosinophils were distributed randomly in the cytoplasm except for the parts of pseudopodia and the Golgi complex (Figs. 14-16). These granules were surrounded by a distinct unit membrane (Figs. 17 & 20). The shape and size of the granules were variable depending upon the cut planes. Generally, they were round, oval, quadrilateral or short rod-like in shape. The electron densities of these granules were variable. On the basis of these variable electron densities, they were classified into 2 types of granules as follows. The eosinophil granules of type I were homogeneously compact, vary dense granules without any internal structures (Fig. 15), although small number of the granules had small myelin-like structure on the periphery (Fig. 18). In the materials that were fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ , type I granules were observed as an accumulation of fine granular substances (Fig. 17). In general, the granules of this type were round in shape, and their sizes were ranged from 0.8 to  $1.5\mu$  in diameter. Occasionally, these granules became quadrilateral in form from jostling each other. The outline of this type granule varied with the fixative employed. The granules became transformed into star-like forms in the single fixation with 1%  $\text{OsO}_4$  in Millonig's buffer (Fig. 14), and retained their round form in the double fixation with 2.5% glutaraldehyde and 1%  $\text{OsO}_4$  in the same buffer (Fig. 16). In the mixture fixative with 2.5% glutaraldehyde and 1%  $\text{OsO}_4$  buffered solution, these granules showed traditional form between the star-like form and the round granules (Fig. 15).

Type II eosinophil granules were homogeneously compact and moderately dense granules (Figs. 14-16). Generally, the granules of this type were round,

oval or short rod-like in shape, with round or oval granules observed most predominantly. Short rod-like granules were rarely seen. The size of these granules were 0.6–0.9  $\mu$  in diameter. This type granule had a clear space enclosed by a unit membrane in the granule, these intragranular clear spaces some times being connected with cytoplasm (Fig. 18). Small cored granules were rarely observed in the cut planes of sections of the eosinophils (Figs, 19 & 20). There were instances of these cored granules around the Golgi complex (Fig. 20) and in the peripheral portion of the cytoplasm (Fig. 19). Some granules had 2 dense cores (Fig. 19).

A few mitochondria and smooth-surfaced endoplasmic reticulum were distributed randomly among the specific granules throughout the cytoplasm. Almost all of the smooth-surfaced endoplasmic reticulum were of vesiculated form. The Golgi complex consisted of ill-developed lamellar membranes and vesicles were frequently observed in the central areas of the cells (Figs. 14,15 & 20). Centrioles were very rarely observed near the Golgi complex (Fig. 20). Rough-surfaced endoplasmic reticulums, multivesicular bodies and phagosomes have not been observed in the eosinophils up to the present time. A small number of free ribosomes and polysomes were randomly scattered in the cytoplasm. Microtubules were very rarely found (Fig 20).

### Discussion

The peripheral blood cells of Japanese quails have been well described morphologically and cytochemically at light microscopic level (ATWAL & JAMROZ 1966). The term heterophil was suggested by KYES (1929) for a granulocyte in which the specific granules of homologous cells among the various classes of vertebrates had great diversity in reaction to stain. The heterophil of birds and reptiles is the equivalent of the neutrophil in mammals. Heterophils are the most numerous of the granular leukocytes. The ultrastructure of the heterophil of the quail's peripheral blood is fundamentally similar to that of domestic fowls, as described by various investigators (OSAKO 1959, ENBERGS & KRIESTEN 1968, DHINGRA, PARRISH & VENZKE 1969, KELENYI & NEMETH 1969, MAXWELL & TREJO 1970).

ENBERGS & KRIESTEN (1968) and MAXWELL & TREJO (1970) reported that the heterophil granules were divided into two types in the domestic fowls. Somewhat different results were reported, DHINGRA, PARRISH & VENZKE (1969) who stated that there were granules of three types in the heterophils of chickens. In the present study, the specific granules of heterophils of quails were fundamentally divided into two types on the basis of their electron densities. Type I granules were dense, and were filled compactly with homogeneous materials at

a high electron density. Type I granules of the heterophils had, in general, a typically long fusiform structure with pointed ends, although round granules were also found. It is reasonable that type I heterophil granules were fusiform structure in shape, because various shapes of transitional forms long fusiform to round were observed.

Type II heterophil granules were filled with low density materials. They were generally smaller than type I granules and were usually round or oval, although occasionally short rod-like in shape. In the chicken, some heterophil granules had "elektronenlichtes Zentrum" (ENBERGS & KRIESTEN 1968) and had internal dark stripping (DHINGRA, PARRISH & VENZKE 1969). In the present observations, the interiors of the most heterophil granules of both types consisted of homogeneous substances without any internal structures. However, in some of the type I heterophil granules, particular cleft-like internal structures were observed as well as clear spots. However, these internal structures were observed only in the materials fixed with  $\text{OsO}_4$ . It had been suggested from the results of KAMIYAMA (1970) that the size and electron density of the neutrophil granules varied with the difference in technical procedure of handling specimens, and in the fixative and in materials used in studies. It is possible, therefore, that these internal structures are artifactual structures. Furthermore, some of the type I heterophil granules had a large clear space surrounded by a unit membrane. There was some resemblance between the substance of the large clear space and the cytoplasm. Connections between this space and the cytoplasm have not been found up to the present time. But it is conceivable that such a large clear space connects in somewhere with the cytoplasm.

KAMIYAMA (1970) found a regular arrangement of dense lines in some of the neutrophil granules of leukemic patients. In the type II heterophil granules of the quails, filamentous structures were also observed in some samples, which had been fixed solely with 1%  $\text{OsO}_4$  in Millonig's buffer. KAMIYAMA (1970) found the line structure in the dense and light granules and the same frequency in the samples, which had been fixed with 8 kinds of fixatives. The authors, however, found the filamentous structure in type II heterophil granules and in some samples, which were fixed solely with  $\text{OsO}_4$ . From the above facts, it seems most likely that the filamentous structure of the quail's heterophils differs from the line structure of the human neutrophils which were found by KAMIYAMA (1970). Thus, it may be conceivable that this filamentous structure of type II heterophil granules is an artifactual structure.

Small cored granules were observed frequently around the Golgi complex in the heterophils of quails. The shape of these granules were round, varying in diameter from 100 to 150  $\mu$ . These cored granules were divided into two types on the basis of electron density of their cores. SONODA & KOBAYASHI

(1970) observed that the neutrophils of canine peripheral blood had granules with dense cores. In the bone marrow of rabbits, the dense cored vacuoles were formed from the concave face of the Golgi complex by budding in the progranulocytes of the neutrophils (BAINTON & FARQUHAR 1966). Although BAINTON & FARQUHAR (1966) found dense cored vesicles, the authors found two types, that is, one having a dense core and the other a light core. In Japanese quails, mature heterophils in the peripheral blood frequently had small cored granules around the Golgi complex.

Neutrophil granules have been divided into two types by BAINTON & FARQUHAR (1968), that is, electron dense granules and electron light granules. They stated that electron dense granules equate azurophil granules and electron light granules equate specific granules. In Japanese quails, heterophil granules were divided into two types, but it is not clear whether azurophil granules equate dense granules or light granules.

It is well known that the fine structure of the eosinophil granules are very variable according to the difference of animal species. On the basis of the observations reported by YAMADA & SONODA (1970), the fine structures of their specific granules were grouped into the following four types viz., 1) granules containing middle plates as seen in those of humans (WATANABE 1956, OSAKO 1959, MILLER, HARVEN & PARADE 1966, HIRSH & FEDORKO 1968), in rabbits (OSAKO 1959, ZUCKER-FRANKLIN & HIRSH 1964) and in some species of rodents (MILLER, HARVEN & PALADE 1966), 2) the ones with middle trunks composed of a very characteristic lamellar structure as seen in those of cats (OSAKO 1959, WATANABE 1956), 3) the ones with two or three thick stratified concentric structures as seen in those of dogs (SONODA & KOBAYASHI 1970), 4) and homogeneous dense granules without any internal structure as seen in those of horses (OSAKO 1959) and chickens (OSAKO 1959, EMBERGS & KRIESTEN 1968, DHINGRA, PARRISH & VENZKE 1969, MAXWELL & TREJO 1970). In chickens, the eosinophil granules had a medium degree of electron density, and were round or oval in shape (DHINGRA, PARRISH & VENZKE 1969).

In the present observations of the quail's eosinophils, the specific granules were divided into two types on the basis of their electron densities. Namely, type I eosinophil granules were homogeneously compact, high density granules without any internal structure. Type II eosinophil granules were homogeneously compact and moderately dense without any internal structure. Type II eosinophil granules had clear space encompassed with a unit membrane in the granules. It has been clarified that the intragranular clear space was thought to be cytoplasmic invagination. The same structure was observed in chicken eosinophils by MAXWELL & TREJO (1970). Type I granules of quail eosinophils

were easily transformed with a variety of fixatives. In the double fixation with 2.5% glutaraldehyde and 1% OsO<sub>4</sub> in Millonig's buffer, the outline of type I eosinophil granules were the most preserved of all methods used.

Small cored granules were very rarely found in the eosinophils of the quails, although these cores showed the same electron density as type I eosinophil granules. It is conceivable that small cored granules are immature eosinophil granules. Small myelin-like structures were rarely observed on the periphery of the type I eosinophil granules, but it is not clear whether this is in an artifactual structure or a normal one.

The following characteristics may be used to differentiate a heterophil from an eosinophil: The heterophil has two types of granules, viz., type I granules have a high electron density and the shape is, in general, typically fusiform in structure. Type II granules have a light electron density and the shape is round generally. The eosinophil also has 2 types of granules, although type I granules have a very high electron density and the shape is usually round, and type II granules are of moderate electron density, round in shape. The intra-granular clear space is observed in type I heterophil granules, whereas it is observed in type II eosinophil granules. Small cored granules are observed more frequently in the heterophils than the eosinophils.

When comparing the authors' observations on quails with the observations reported already on chickens, the differences in the ultrastructures of the heterophils and the eosinophils between quails and chickens were clarified. The heterophil of the quail fundamentally resembles that of the chicken, although occasionally small cored granules were observed in the quail. As for the eosinophil, quails had two kinds of specific granules, one having a very high electron density and the other a moderate density, whereas chickens had only one kind of specific granule.

### Summary

The fine structures of the heterophils and the eosinophils of the peripheral blood obtained from nine clinically healthy Japanese quails were observed under an electron microscope.

The results thus obtained are summarized as follows:

Heterophils:

- 1) The heterophils were roundish cells with small pseudopodia about 6-7  $\mu$  in diameter.
- 2) The specific granules were fundamentally divided into two types on the basis of their electron densities. The type I granules were dense, and were filled compactly with homogeneous materials at a high electron density. The type II

granule had a light electron density, also filled with homogeneous materials.

3) The shape of type I granules was, in general, a typically long fusiform structure with pointed ends, although round and oval granules were also observed. It is reasonable that most of the type I granules had fusiform structures, because various transitional forms from long fusiform to round forms also were observed. The size of type I granules varied considerably measuring 0.3–3.3  $\mu$  by 0.4–0.7  $\mu$ .

4) Type II granules were usually round or oval, occasionally short rod-like in shape, having length and diameter of 0.3–1.2  $\mu$ .

5) In some granules, particular internal structures were observed.

6) Small cored granules were frequently observed around the Golgi complex, and the peripheral portion of the cytoplasm. These granules were round measuring 100–150 m $\mu$  in diameter.

Eosinophils :

1) The general shape of the eosinophils was round or oval, their size being about 6  $\mu$  in diameter.

2) The specific granules of the eosinophils were classified into two types on the basis of their variable densities.

3) Type I granules were homogeneously compact having very high electron dense materials. In general, the granules of this type were round in shape measuring 0.8–1.5  $\mu$  in diameter.

4) Type II granules were homogeneously compact and moderately dense. Generally, the granules of this type were round, oval or short rod-like structure measuring 0.6–0.9  $\mu$  in diameter.

5) Small cored granules were very rarely observed in the cut planes of any sections of the eosinophils.

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## 摘 要

ニホンウズラ (*Coturnix coturnix japonica*) は近年、実験動物として賞用されているが、血液細胞の微細構造に関する報告にはいまだ接することができない。そこで著者らはウズラ血液細胞の微細構造を明らかにするために本研究を企てた。第 I 報として、その染色性から光顕的に類似している好異球と好酸球について報告する。

材料は 9 羽の臨床的に健康と思われる雄の成熟ウズラを用い、後頭部切断によって得た血液 1.5 ml に対して 0.5 ml の血液凝固防止剤 (1.5% EDTA-2K 水溶液と 1.5% EDTA-3K 水溶液の等量混合液) を混和し、2 段階遠沈により buffy coat を作り、1%  $\text{OsO}_4$  の単固定、2.5% glutaraldehyde と 1%  $\text{OsO}_4$  の混合固定または 2.5% glutaraldehyde と 1%  $\text{OsO}_4$  の二重固定を行ない、次のごとき成績を得た。

- 1) 好異球は直径約 6~7  $\mu$  で、類円形を呈し、細胞表面には少数で、小さな偽足様突起

を有し、まれに葉足をも有していた。

2) 好酸球は直径約  $6\mu$  の類円形の細胞で、偽足様突起はごく少数認められた。

3) 好異球の特殊顆粒は2型に大別された。すなわち、電子密度の高い顆粒 (I型) と、電子密度の低い顆粒 (II型) であった。I型顆粒はその多くのもが紡錘形として認められた類円形のものも認められたが、それらは紡錘形のもの断面と考えられた。これらの顆粒は、ほぼ  $0.6\sim 3.3\mu \times 0.4\sim 0.7\mu$  の大きさを示した。II型顆粒はその多くが円形または類円形であったが、しばしば、短桿状のものも認められた。これらの大きさは直径約  $0.3\sim 1.2\mu$  であった。

4) 好酸球の特殊顆粒もまた2型に大別された。すなわち、I型顆粒は電子密度が非常に高く、直径約  $0.8\sim 1.5\mu$  のほぼ円形であった。II型顆粒は中等度の電子密度で、大多数のものが類円形を示し、直径約  $0.6\sim 0.9\mu$  であった。

5) 前述の特殊顆粒のほかに芯 (core) を持つ小顆粒が好異球、好酸球ともに Golgi 装置の周囲および細胞質の辺縁近くに散在性に認められたが、好異球においてより高頻度であった。

6) 好異球と好酸球において、細胞小器官の発達程度には明らかな差異は認め得なかった。

以上のことより、好異球と好酸球はそれらの特殊顆粒の形状およびそれらの電子密度より容易に区別することができた。

## Explanation of Plates

### Plate I

- Fig. 1.** Heterophil. The heterophil granules are of two kinds. The type I granules (dense granules) are dominant and they are fusiform, short rod-like, oval or round in shape. The type II granules (light granules) are small number and they are round or oval in shape. The pseudopodias are observed.  $\times 16,800$ , fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .
- Fig. 2.** Heterophil. The micrograph shows a higher magnification of Fig. 1. The Golgi complex and the centriole are observed between the nuclear lobes. The type II granules have distinct double limiting membrane. The one of the type I granules (arrow) has intragranular clear space which is similar to the cytoplasm. The limiting membrane of the type I granules are not distinguishable in this figure, but in the type II granules, the limiting membranes are observed clearly.  $\times 39,000$ .

### Plate II

- Fig. 3.** Heterophil. The heterophil granules are almost round or oval in the both types. The lobopodia is shown in one side of the cytoplasm. The mitochondria are small and a few.  $\times 14,400$ , fixed with  $\text{OsO}_4$ .
- Fig. 4.** Heterophil. The micrograph shows a higher magnification of Fig. 3. The type I granules are surrounded with distinct double limiting membrane. The nuclear lobe has a nuclear thread. The one of the type I granule has a cleft-like structure (arrow).  $\times 33,000$ .

### Plate III

- Fig. 5.** Heterophil. This heterophil has 2 nuclear lobes. The ill-developed Golgi complex and centriole situate between the nuclear lobes. The small cored granules are scattered around the Golgi complex and peripheral region of the cytoplasm. The arrow shows fusiform shape of the type II granules.  $\times 16,800$ , fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .
- Fig. 6.** Heterophil. The nucleus is lobulated into 3 lobes. This heterophil has the type I granules only. These granules show various shapes. The small cored granules are observed randomly throughout the cytoplasm.  $\times 16,800$ , fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .

### Plate IV

- Fig. 7.** Heterophil. One of the type I granules has a cleft like structure (arrow). The margin of this granules is wavy.  $\times 78,000$ , fixed with  $\text{OsO}_4$ .
- Fig. 8.** Heterophil. One of the type I granules has a number of clear spots (arrow).  $\times 78,000$ , fixed with  $\text{OsO}_4$ .
- Fig. 9.** Heterophil. This type II granule shows a filamentous structure.  $\times 78,000$ , fixed with  $\text{OsO}_4$ .

### Plate V

- Fig. 10.** Heterophil. Note that the type II granule is a short rod-like in shape and is surrounded by distinct double limiting membrane (arrow).  $\times 39,000$ , fixed with  $\text{OsO}_4$ .
- Fig. 11.** Heterophil. The various shape of the type I granules are observed. One of the type I granules has a large clear space which is similar to the cytoplasm. This space contains a small cored granule. The small cored granules are observed also in the cytoplasm.  $\times 39,000$ .

fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .

### Plate VI

- Fig. 12.** Heterophil. Micrograph showing around the Golgi area. The centriole situates beside the Golgi complex. The small cored granules are scattered around the Golgi complex.  $\times 48,000$ . fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .
- Fig. 13.** Heterophil. This micrograph is similar to the finding of Fig. 12, but the Golgi complex is not seen. Many microtubules are observed around the centriole.  $\times 48,000$ . fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .

### Plate VII

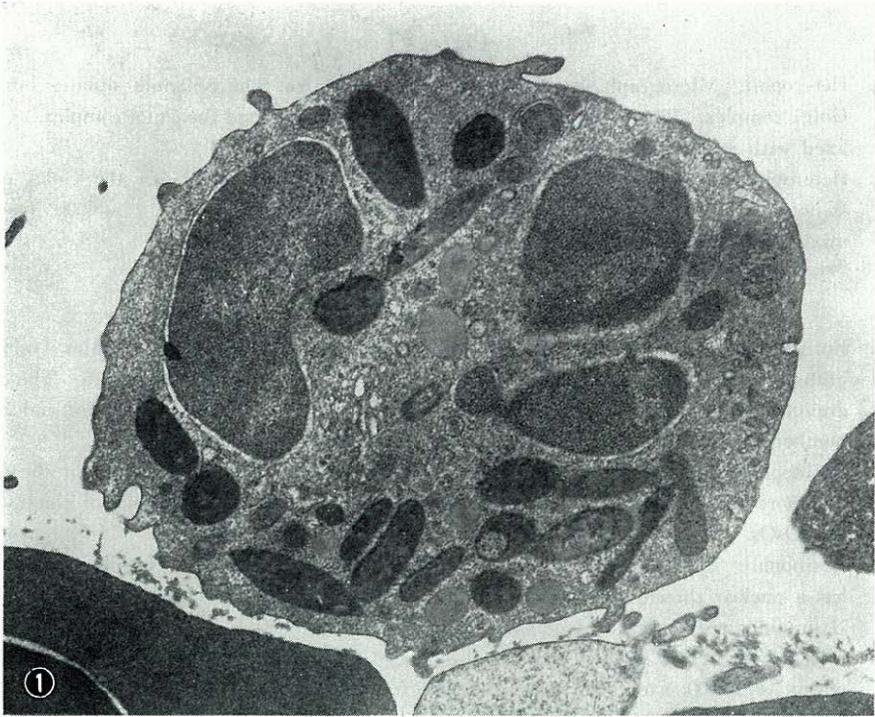
- Fig. 14.** Eosinophil. The cytoplasm has two types granules. The type I granules (very dense granules) show very irregular outline. Compare with Figs. 14, 15 and 16. The type II granules (moderately dense granules) are round in shape. A Golgi complex and a small number of the mitochondria are observed.  $\times 12,000$ . fixed with  $\text{OsO}_4$ .
- Fig. 15.** Eosinophil. The outline of the type I granules are more smooth than that in the Fig. 14, but more irregular than that in the Fig. 16.  $\times 12,000$ . fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .
- Fig. 16.** Eosinophil. Three nuclear lobes are in the plane of this section. One of the nuclear lobes has a nuclear thread. The type I granules are round or quadrilateral-like form. The type II granules are round, oval or short rod-like in shape. Some of the type II granules have intragranular clear space. The rough-surfaced endoplasmic reticulum scatter in the cytoplasm.  $\times 12,000$ . fixed with glutaraldehyde followed with  $\text{OsO}_4$ .

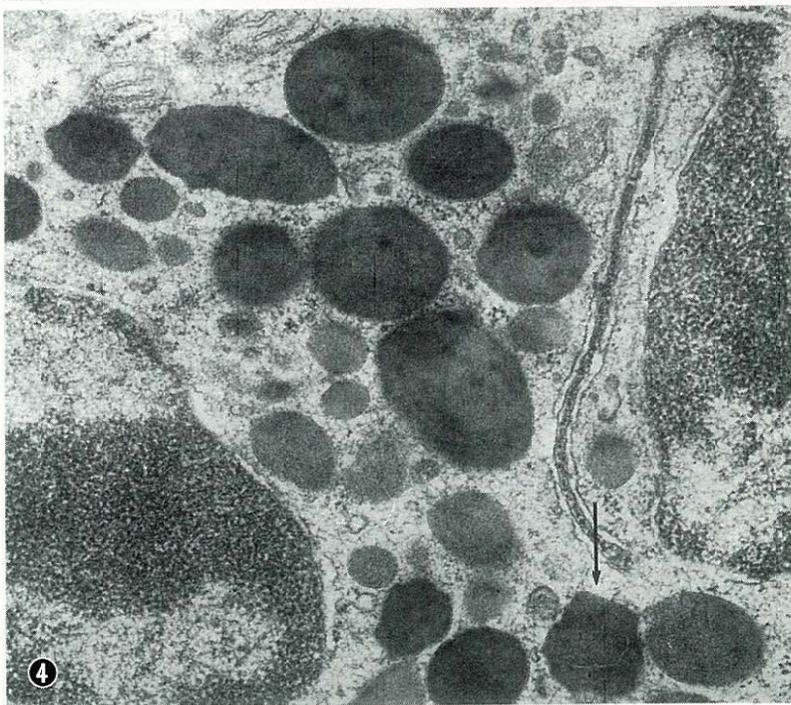
### Plate VIII

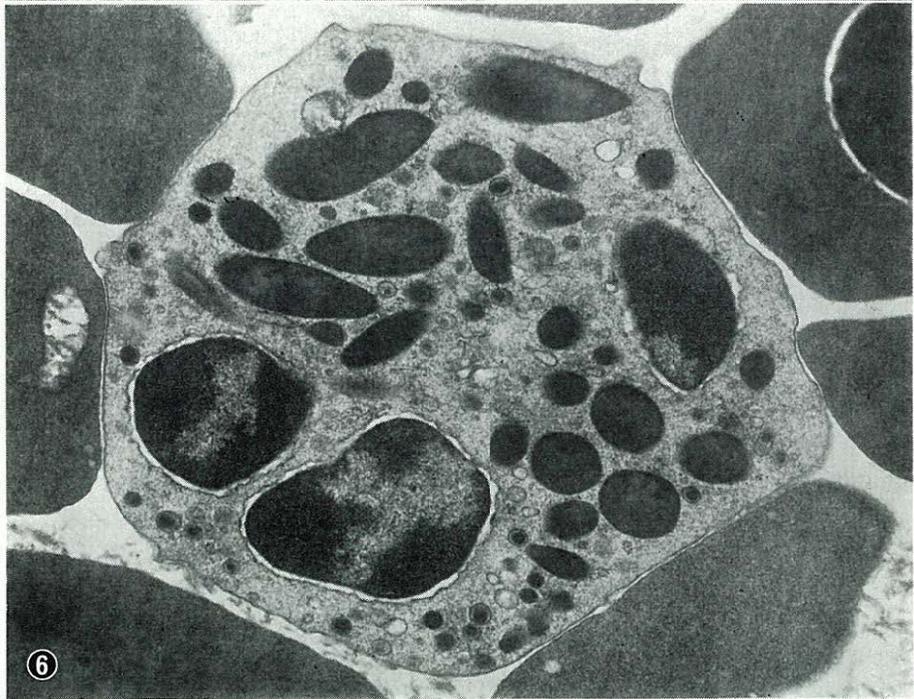
- Fig. 17.** Eosinophil. The type I granules are enveloped with distinct unit membrane. These granules contain fine and dense granular materials. The Golgi complex develops relatively and the numerous Golgi vesicles are found around the lamellae.  $\times 48,000$ . fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .
- Fig. 18.** Eosinophil. The type I and type II granules are observed in the cytoplasm. The type I granules are homogeneously compact and very dense materials without any internal structures, but very small number of the granules have a small myelin-like structure in the peripheral portion (short arrow). One of the type II granules has cytoplasmic invagination in the plane of section (long arrow).  $\times 24,000$ . fixed with glutaraldehyde followed with  $\text{OsO}_4$ .

### Plate IX

- Fig. 19.** Eosinophil. A large number of the small cored granules are observed in the peripheral portion of the cytoplasm. Some of them are elongated form and have 2 dense cores.  $\times 39,000$ . fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .
- Fig. 20.** Eosinophil. The small cored granules are observed beside the Golgi complex. The microtubules situate around the centriole.  $\times 39,000$ . fixed with mixture of glutaraldehyde and  $\text{OsO}_4$ .

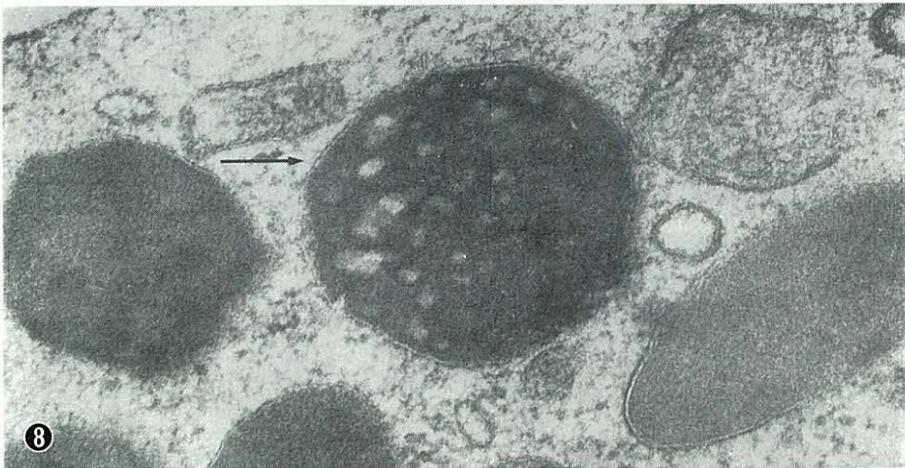
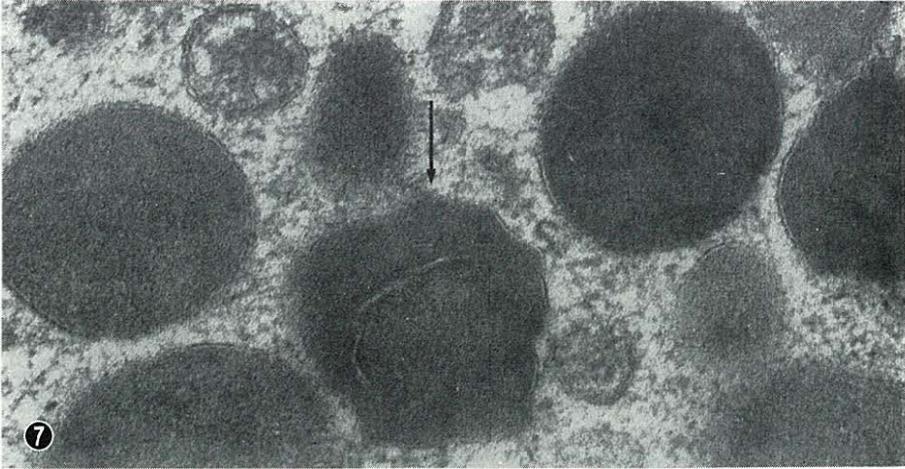


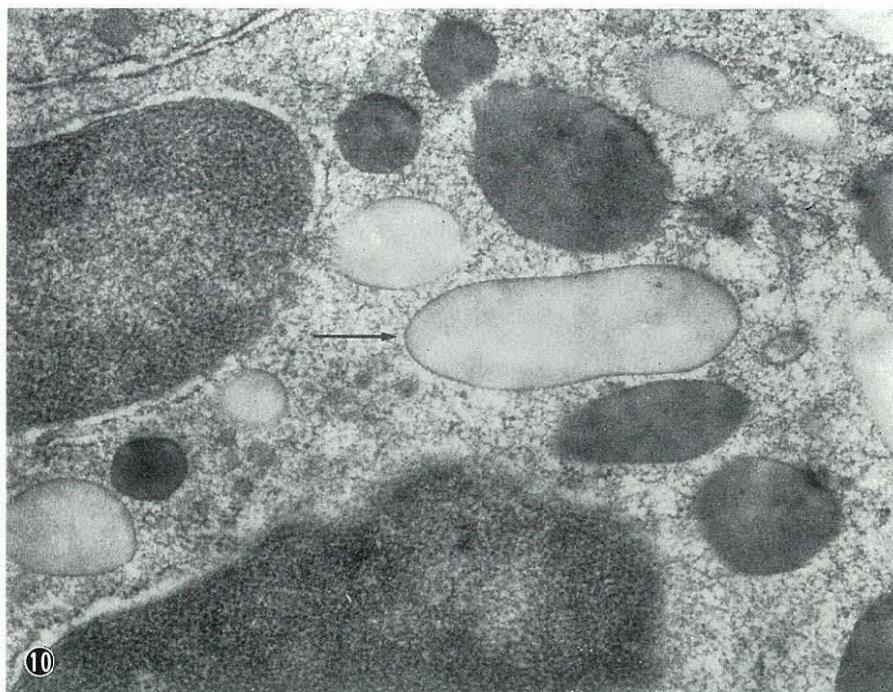




YAMADA, J. *et al.*

Plate IV





YAMADA, J. *et al.*

Plate VI

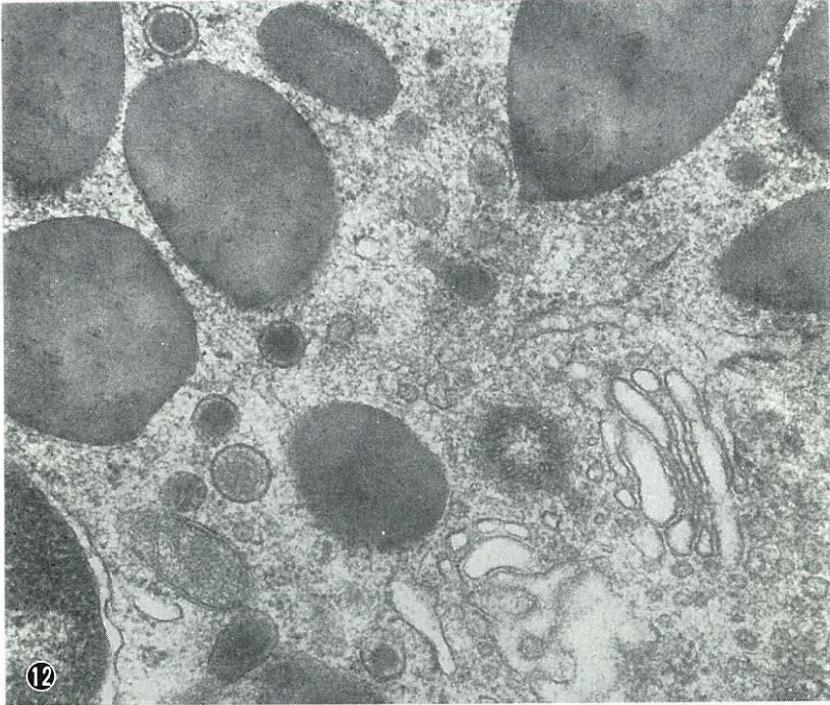
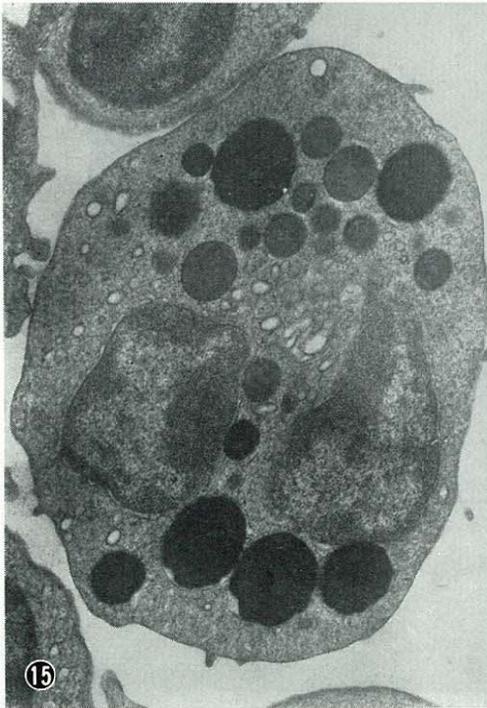


Plate VII

YAMADA, J. *et al.*



YAMADA, J. *et al.*

Plate VIII

