LIGHT AND ELECTRON MICROSCOPIC STUDIES ON THE LEUCOCYTES OF THE MEDAKA

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ABSTRACT - The blood cells of the medaka *(Oryzias latipes)* were examined by light and electron microscopy. Four main types of leucocytes, thrombocyte, lymphocyte, neutrophil, and monocyte, were identified by the method of May-Grünwald-Giemsa. These identifications were confirmed by electron microscopy. The major morphological characteristics of them were described.

INTRODUCTION

In spite of many light microscopic studies on teleost blood cells (see the review by Ellis, 1977), few studies have been carried out on their ultrastructure and function (Weinreb, 1963 ; Ferguson, 1976 ; Cannon *et al.*, 1980 ; Savage, 1983). Medaka *(Oryzias latipes)* has been used as a research animal in various disciplines, including physiology, developmental biology, genetics, immunology, and radiation biology. But there is little information on the morphology of its blood cells. The purpose of this study was to obtain a basic knowledge of the hematology of this species. In this paper, the morphological features of the leucocytes of medaka will be described according to observations made by light and electron microscopy.

MATERIALS AND METHODS

Animals

Adult males and females of the *d-rR* strain of *Oryzias latipes* were used. They were kept in a plastic aquarium (17×28×18cm), maintained at 22±3°C and fed Tetra-min daily.

Light microscopy

The fish were bled by transection of the caudal peduncle (without the use of anesthetics) and the fresh blood was smeared directly from the cut end of the fish body. After cutting open the abdomen of the fish, peritoneal fluid was also smeared directly from the opening.

For general identification the smears were fixed in absolute methanol and stained with May-Grünwald-Giemsa.

In order to identify the phagocytic cells, May-Grünwald-Giemsa stain was also used after intraperitoneal injection of 0.025ml of a 10% suspension of sheep red blood cells (SRBC ; Cappel Laboratories) and a 1% dilution of India ink (carbon particles).

For the cytochemical observations on the peroxidase content of the leucocytes, the o-tolidine (3, 3'-dimethylbenzidine) peroxidase reaction was carried out by a slight modification of Quaglino-Flemans' method (Quaglino and Flemans, 1958).

Electron microscopy

Peripheral blood cells were studied in the tissue sections of the heart and kidney. Peritoneal leucocytes were studied in the sections of the cell aggregates (=SRBC clot), which were found in the peritoneal cavity of the fish 2 to 3 days after intraperitoneal injection of SRBC. Small blocks of the heart, kidney, and SRBC clot were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer, pH 7.2 for 2.5 h at 0°C and postfixed in 1% OsO_4 in the same buffer for 2 h at 4°C. They were dehydrated in graded ethanols and embedded in Quetol 651 (Kushida, 1974, 1975). Ultrathin sectoins were cut on a Porter-Blum MT-2B ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with a JEOL JEM-100B transmission electron microscope operated at 80KV.

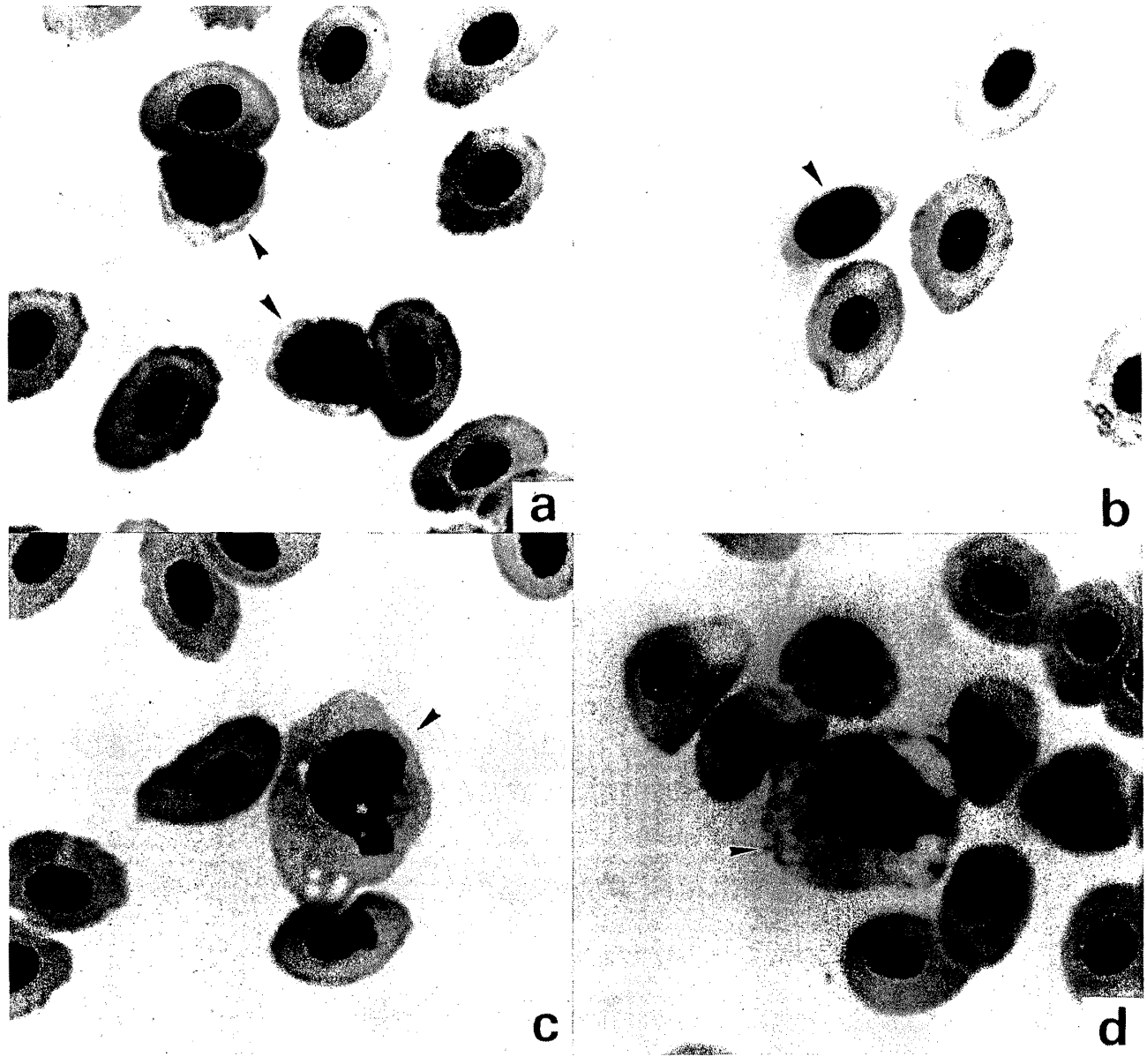

Fig. 1 Light micrographs of peripheral blood leucocytes (arrowheads) and erythrocytes. May-Grünwald-Giemsa staining. ×2000. a) Lymphocyte, b) Thrombocyte, c) Neutrophil, d) Monocyte.

The nomenclature of the leucocytes is based on the morphological criteria described in several previous papers (Weinreb, 1963 ; Ferguson, 1976 ; Ellis, 1976, 1977 ; Cannon *et al.*, 1980 ; Savage, 1983).

OBSERVATIONS

By light and electron microscopy, four types of leucocytes, *i. e.*, thrombocyte, lymphocyte, neutrophil, and monocyte, were distinguished (Fig. 1). Certain observations made by light microscopy are summarized in Table 1. Electron microscopic observations are as follows.

Thrombocyte (Fig. 2)

The thrombocyte was a small spindle-shaped cell with a large nucleus which occupied most of the cell. The nucleus contained dense chromatin and was often indented. Indentations of the plasma membrane were often observed. Large vacuoles were common, because the indentations of the plasma membrane sometimes appeared as such. Cytoplasmic organelles, endoplasmic reticulum, Golgi apparatus, mitochondria, and free ribosomes, were scarce. Bundles of microtubules were sometimes observed in the peripheral region of the cytoplasm.

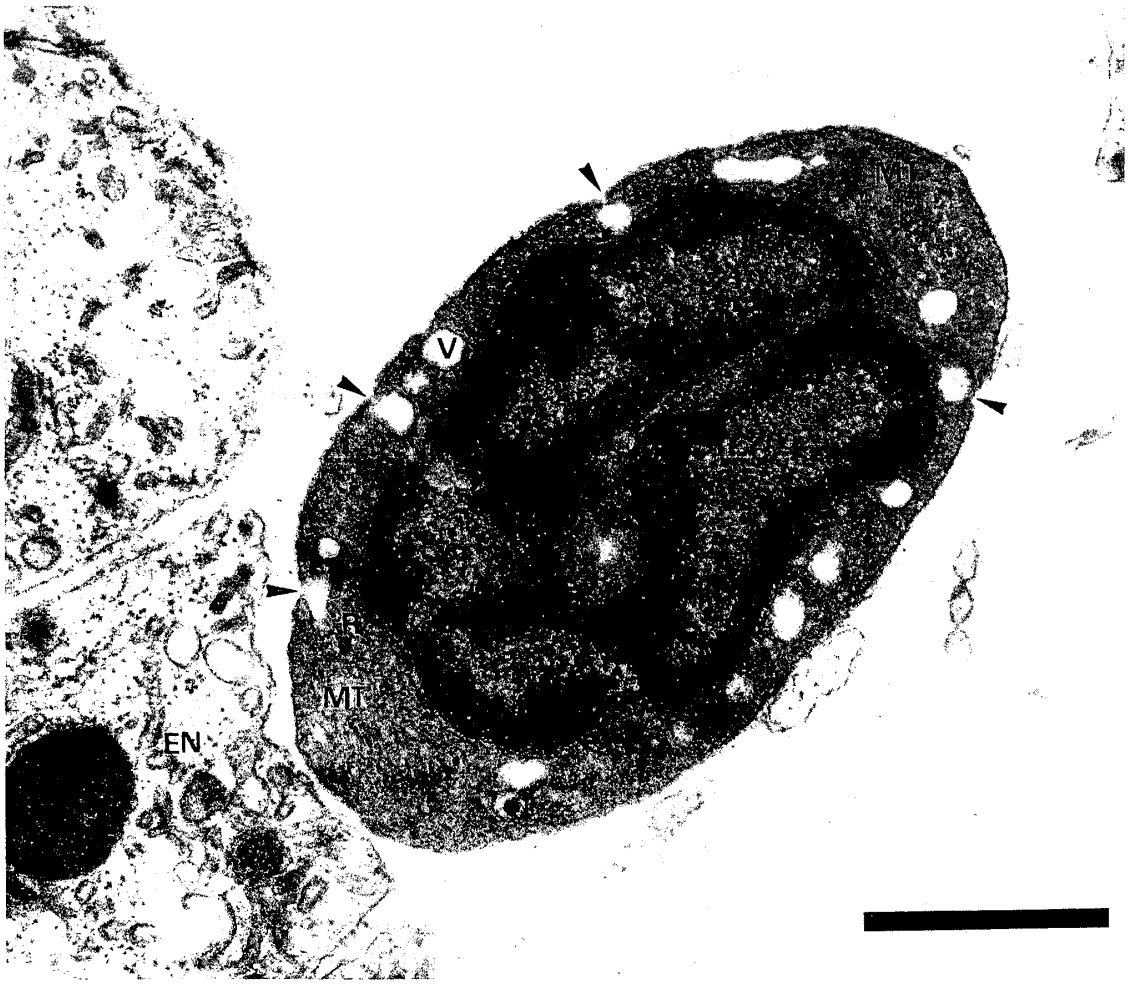

Fig. 2 An electron micrograph of a thrombocyte characterized by its bundles of microtubules (MT) and indentations of the plasma membrane (arrowheads). V, Vacuole ; EN, Endothelial cell of the heart. Bar indicates 1 μm.

Table 1 Light microscopic characteristics of the medaka leucocytes.

	May-Grünwald-Giemsa staining						
	Cell size (μm)	Cell shape	Cytoplasm	Nucleus shape	Nucleus	Peroxidase (o-Tolidine)	Phagocytosis (SRBC, carbon particles)
Thrombocyte	7-10	spindle-shape, ovoid	almost chromophobic, light grey	ovoid	condensed chromatin	−	−
Lymphocyte	6-9	round	blue	round	smooth-condensed chromatin	−	−
Neutrophil	7-20	ovoid	pinkish-grey	round, ovoid, irregular	rough-chromatin network	+	+
Monocyte	8-23	round, ovoid	blue, vacuolated	round, irregular, often deformed by vacuoles	rough-chromatin network	−	++

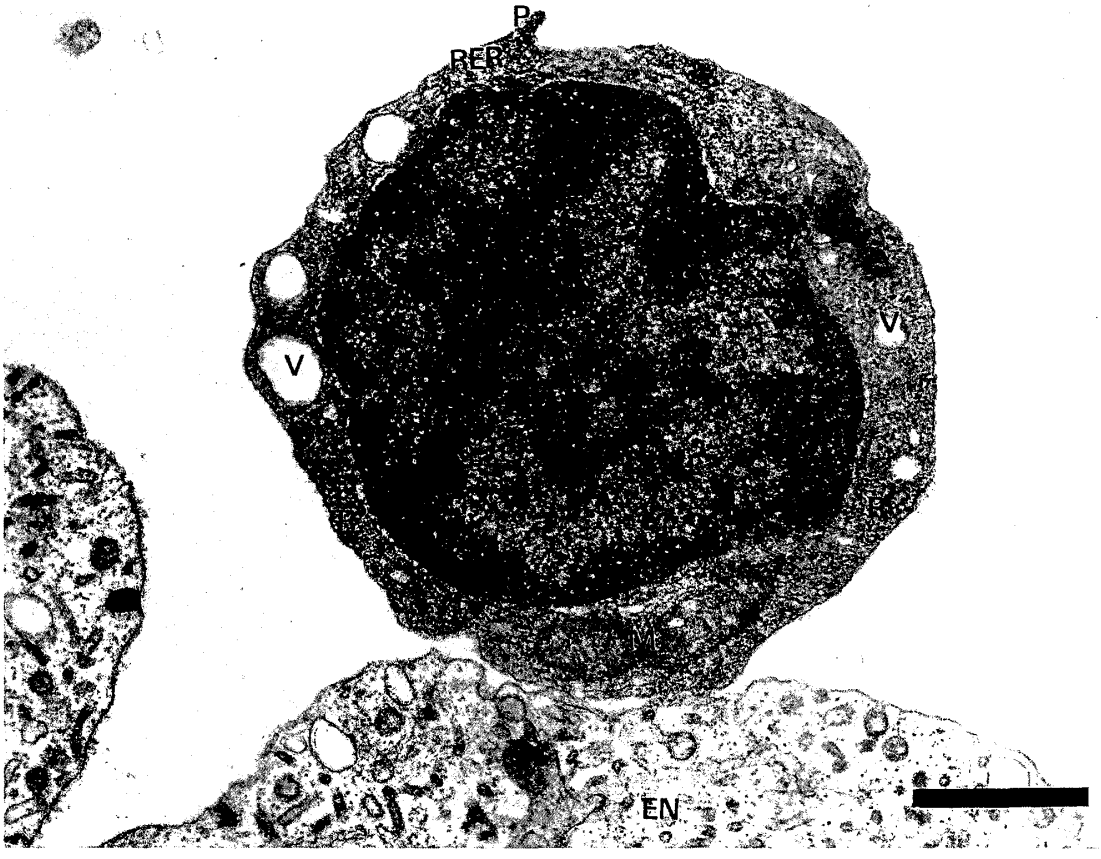

Fig. 3 An electron micrograph of a lymphocyte which contains a large dense nucleus and abundant free ribosomes (R). M, Mitochondria ; P, Pseudopodia ; RER, Rough endoplasmic reticulum ; V, Vacuole ; EN, Endothelial cell of the heart. Bar indicates 1 μm.

Lymphocyte (Fig. 3)

The lymphocyte was a small round cell and sometimes possessed pseudopodia on its surface. The round to slightly irregular nucleus contained heavily clumped chromatin and was surrounded by a small area of cytoplasm. The most prominent features of the cytoplasm were the abundant free ribosomes and the strands of the rough endoplasmic reticulum. Mitochondria and some vacuoles were also present.

Neutrophil (Fig. 4)

The neutrophil was large and sometimes irregular in outline. The nucleus was ovoid to horseshoe-shaped and often more varied in shape than that of the monocyte. Chromatin was moderately condensed on the nuclear membrane and clumped inside the nucleus. Golgi apparatus was occasionally visible. Rough endoplasmic reticulum was less prominent. Free ribosomes were scattered throughout the cytoplasm. Numerous vacuoles which appeared to be smooth endoplasmic reticulum and the specific granules were characteristic of the neutrophil. These granules varied in shape from round to elongated and often contained fibrillar materials.

Monocyte (Fig. 5)

The monocyte was a large cell with cytoplasmic pseudopodia varying in number and size. The nucleus was ovoid or kidney-shaped and often positioned off-center due to the abundant cytoplasmic organelles. Condensed chromatin was less prominent. Rough endoplasmic reticulum and free ribosomes were more abundant than those of the neutrophil. The most prominent features of the cytoplasm were the electron-lucent vacuoles and the phagosomes containing heterogeneous materials. Intraperitoneally injected carbon

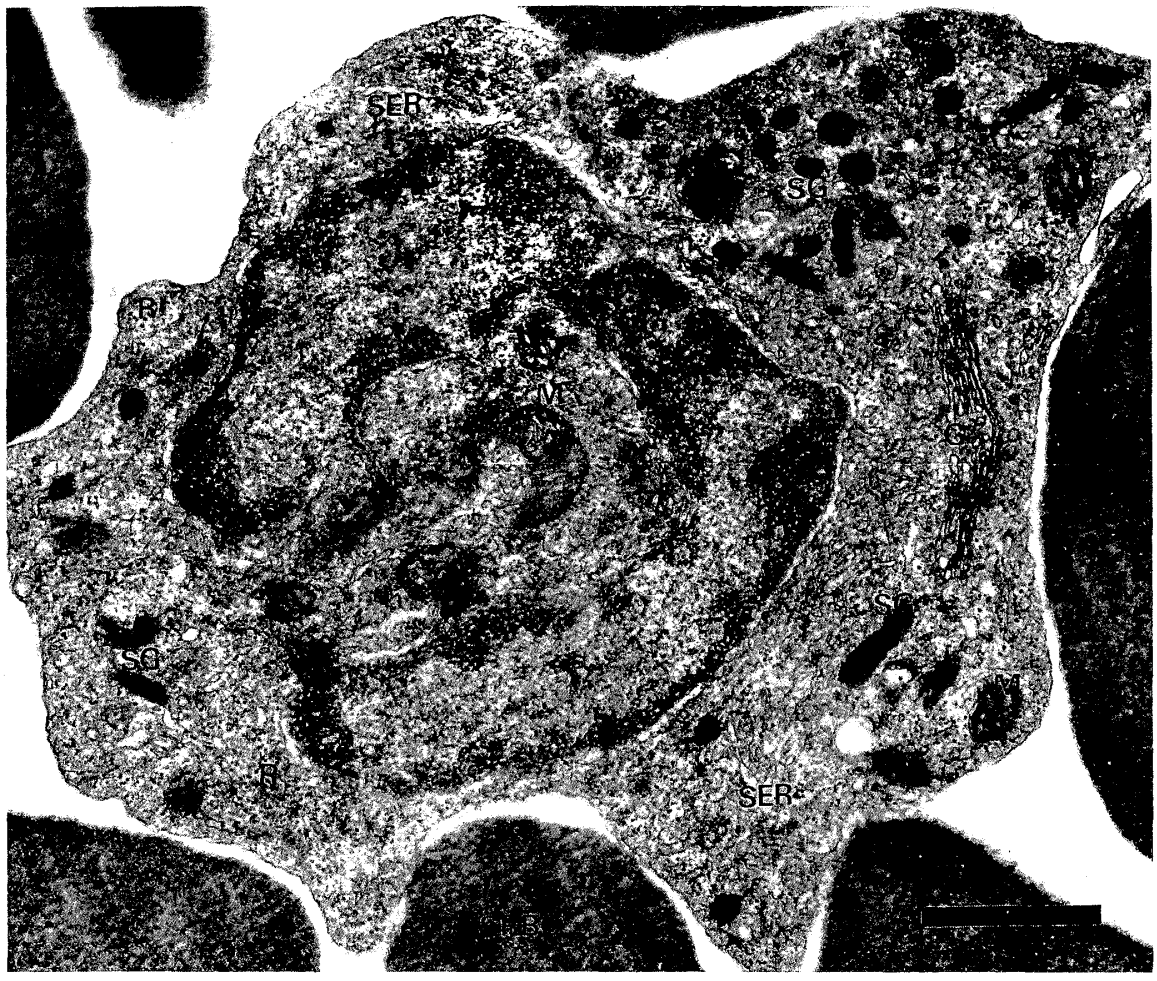

Fig. 4 An electron micrograph of a neutrophil characterized by its specific granules (SG) and a eccentric nucleus. G, Golgi apparatus ; M, Mitochondria ; R, Ribosome ; SER, Smooth endoplasmic reticulum ; SRBC, Sheep red blood cell. Bar indicates 1 μm.

particles were often observed in these phagosomes. Lysosomes were also visible.

DISCUSSION

In the blood cells of the medaka, four types of leucocytes, thrombocyte, lymphocyte, neutrophil, and monocyte, were distinguished.

The thrombocyte is ovoid to spindle-shaped and often groups together in the smear preparation. Like thrombocyte of other teleosts reported by many workers (see Ellis, 1977), the medaka thrombocyte has no granules stained with May-Grünwald-Giemsa. The most prominent feature of the ultrastructure of this cell is the indentations of the plasma membrane. This feature is similar to that of plaice, *Pleuronectes platessa* (Ferguson, 1976) and channel catfish, *Ictalurus punctatus* (Cannon *et al.*, 1980). Ferguson (1976) has reported the phagocytosis by the plaice thrombocyte, but we observed no phagocytic ability in the medaka thrombocyte in this study.

The lymphocyte can be easily recognized because of its morphological similarity to the mammals and other teleosts (Watson *et al.*, 1963 ; Weinreb, 1963 ; Ferguson, 1976 ; Ellis, 1976, 1977 ; Cannon *et al.*, 1980 ; Savage,1983). In this study, no attempt has been made to divide lymphocytes into populations of "small" and "large" cells. As previously reported, the medaka lymphocyte has no phagocytic ability.

The neutrophil is the only type of granulocyte observed in medaka. By electron microscopy, round to elongated granules have been observed in the cytoplasm of the neutrophil. These granules often contain fibrillar material resembling that of the neutrophil granule of plaice (Ferguson, 1976). Ellis (1976) has reported that the neutrophil

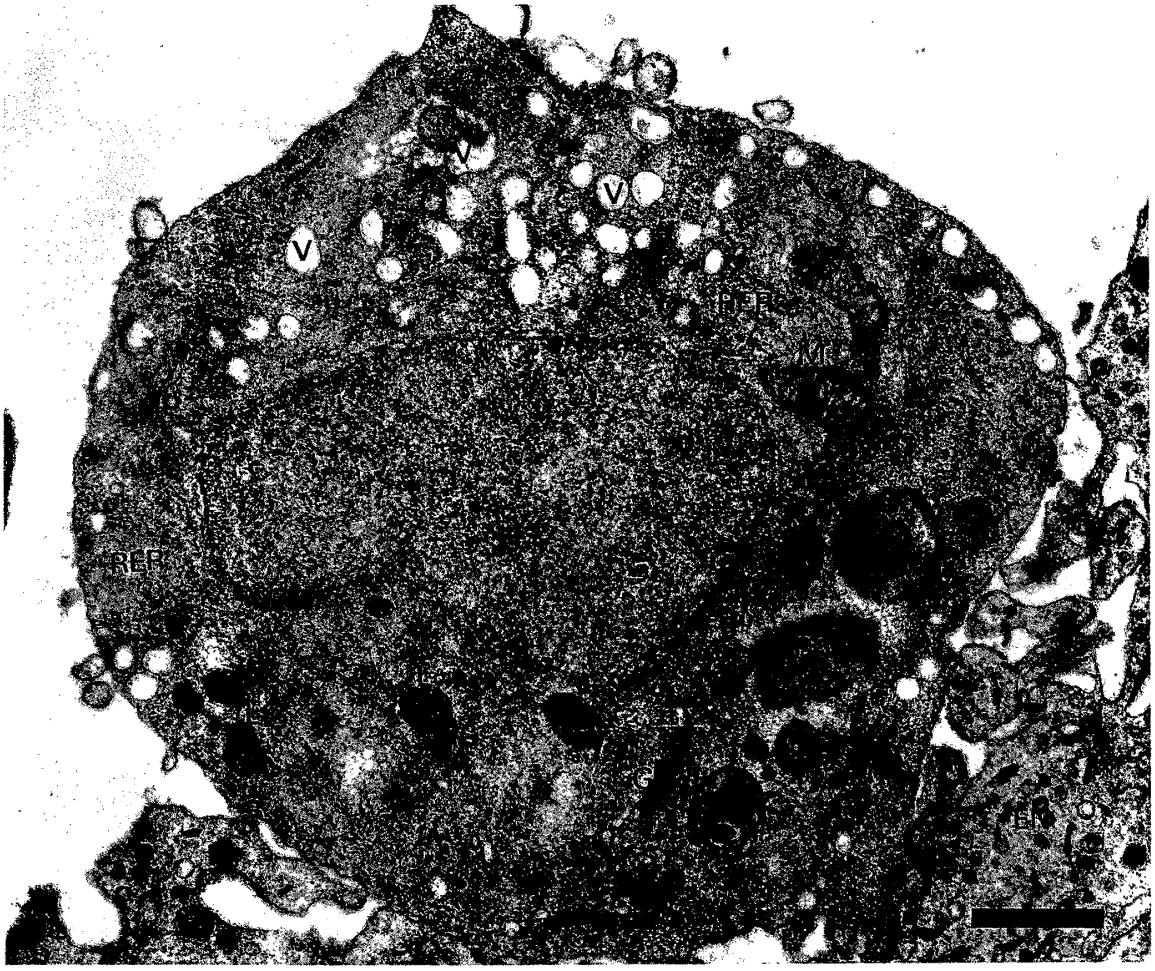

Fig. 5 An electron micrograph of a monocyte which contains large phagosomes (PS) and many vacuoles (V). G, Golgi apparatus ; L, Lysosome ; M, Mitochondria ; P, Pseudopodia ; RER, Rough endoplasmic reticulum ; EN, Endothelial cell of the heart. Bar indicates 1 μm.

of plaice had no phagocytic ability. In our observation, the medaka neutrophil rarely showed uptake of intraperitoneally injected carbon particles but did active phagocytosis of SRBC.

No attempt has been made to divide the basophilic phagocytes into categories of "monocyte" and "macrophage", and so these cell types are collectively termed "monocyte" in this paper.

The monocyte is the most active phagocyte in the leucocytes of medaka. Carbon particles and SRBC injected intraperitoneally are often observed in the cytoplasm of the monocyte. By electron microscopy, large phagosomes and a large number of vacuoles are the most prominent features of its cytoplasm. The ultrastructure of the medaka monocyte is similar to that of plaice (Ferguson, 1976) and channel catfish (Cannon *et al.*, 1980).

Granulocytes equivalent to the mammalian eosinophil and basophil have not been observed in the peripheral blood and the peritoneal fluid of medaka. But some indistinguishable blood cells were observed by electron microscopy. Further investigation of these cells is required.

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