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ELECTRON MICROSCOPIC STUDIES ON THE PHAGOCYTOSIS OF EHRLICH ASCITES TUMOR CELLS

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Takayoshi FUJITA. *Electron Microscopic Studies on the Phagocytosis of Ehrlich Ascites Tumor Cells.* Kobe J. Med. Sci. 13, 249-266, December 1967——The purpose of this study was to investigate the phagocytosis of the pigment of benzidine yellow series by Ehrlich ascites tumor cells. The pigment emulsions were aseptically injected intraperitoneally into normal mature female mice on 4th and 5th day after preliminary intraperitoneal inoculation of Ehrlich ascites tumor cells. On the 7th day after tumor transplantation ascites cells were obtained, fixed in osmic acide, dehydrated with alcohol, embedded in Methacrylate or Epon 812 and serial ultra thin sections of these materials were observed with the electron microscope. The administered pigment particles congregated in granules which were scattered within the cytoplasm of the Ehrlich ascites tumor cells at several sites. Almost pigment granules were surrounded by a single layer of limiting membrane. The phagocytized pigment particles were digested as time went on, and some times the granules became similar to microbody or mitochondria with double membrane structure. These results suggest the mechanism of phagocytosis and digestion of Ehrlich ascites tumor cells.

INTRODUCTION

Although the picture of phagocytosis of red cells, fat drops, and tissue fragments by human tumor cells was occasionally noted through an optical microscope, no systemic study is yet available on this subject. In experimental tumors, on the other hand, phagocytosis of india ink by the tumor cells which are thought to be derived from the reticuloendothelial cell such as Usubuchi's sarcoma, Hirosaki's sarcoma and Takeda's sarcoma has been reported. However, no systemic studies are available concerning the phagocytosis of epithelial tumor cells such as Ehrlich ascites tumor cells. In our department, phagocytosis of some kind of dye in a high proportion by Ehrlich ascites tumor cells was detected. And the pigments of benzidine yellow series were subjected to phagocytosis in the highest proportion. About 5 pigments obtained by partial changes of chemical structure in the pigment of benzidine yellow series, the picture of phagocytosis of these pigments by Ehrlich ascites tumor cells was electron microscopically studied in order to elucidate the difference in the attitude to phagocytosis according to the difference in the chemical structure.

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MATERIALS AND METHODS

Emulsion of 5 kinds of pigments of benzidine yellow series with variations in the chemical structure were injected intraperitoneally into normal mature female mice (Strain DDN) on the 4th and 5th day after preliminary intraperitoneal transplantation of approximately 10^6 Ehrlich ascites tumor cells. On the 7th day after the transplantation, the ascites was obtained for electron microscopic study.

Table 1. Synthesis of benzidine yellow pigment.

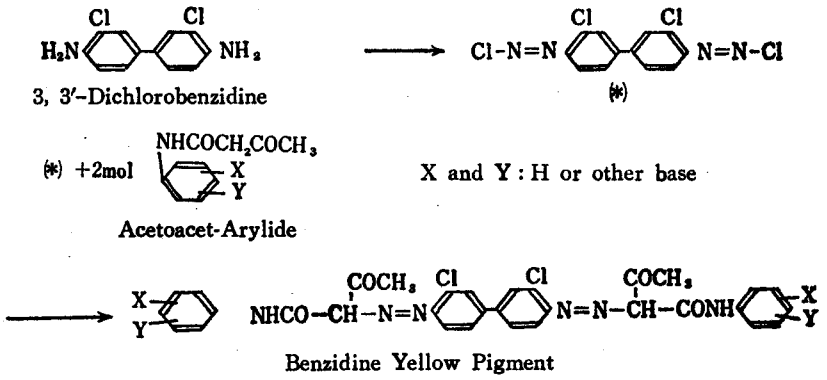
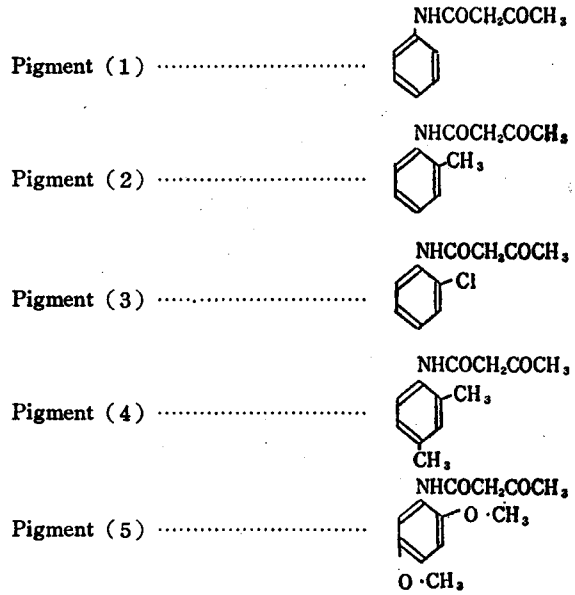


Table 2. Benzidine yellow derivatives obtained by utilizing various acetoacet-arylide compounds.



The pigments of the benzidine yellow series used in this experiment were synthesized through the reaction between the tetrazo derivative of 3-3'-dichlorobenzidine with acetoaceto-arylide (Table 1). Various pigments of benzidine yellow series may be obtained by substituting members of the benzidine nucleus of the acetoaceto-arylide structure. Table 2 summarized the 5 compounds of the acetoaceto-arylide series used in the present experiment. These pigments are insoluble in water and the solvents used to disperse these are summarized in Table 3.

Table 3. Solvent

Solvent (a)	— Polyoxyethylene laurylether.
Solvent (b)	— Polyoxyethylene nonylphenolether.
Solvent (c)	— Conjugated material of naphthalene sulfonic acid and formaldehyde.
Solvent (d)	— Polyoxyethylene alkylether.
Solvent (e)	— Polyoxyethylene alkylphenolether.
Solvent (f)	— Mixture of solvent (c) and (e). (c) : (e) = 1 : 4

In 0.1% aqueous solution of solvents, pigments were mixed in 0.5% proportion to prepare an aqueous emulsion, only pigment (5) was mixed in 1.5%. Thus pigment (1) was dissolved in solvent (a), (b), (c), (d) and (e), pigment (2), (3) and (4) in solvent (a), (b) and (c), and pigment (5) in solvent (c), (e) and (f). Two-tenths ml of these emulsion was intraperitoneally injected into mice on 4th and 5th day after the transplantation of Ehrlich ascites tumor cells. Seventeen kinds of experiment were carried out in total of 340 animals.

In cases with marked phagocytosis (1)-(b), (1)-(e), (2)-(b), (4)-(a), (4)-(b), (5)-(e) and (5)-(f), the ascites cells were fixed with osmium acide, embedded in Methacrylate or Epon 812, to be prepared into serial ultra thin sections for the stereoscopic study of the picture of phagocytosis.

RESULTS

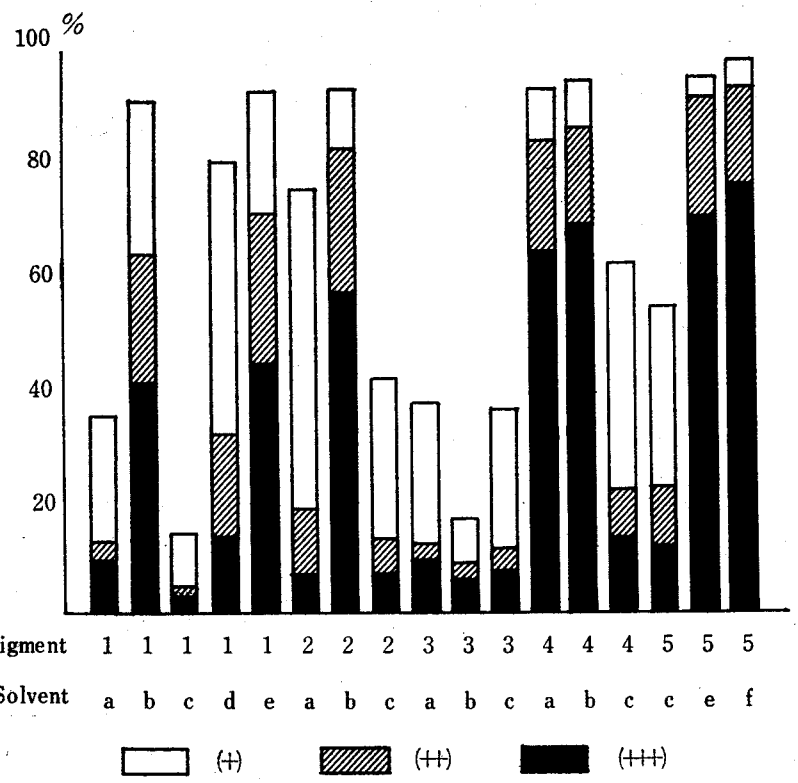
(1) Table 4 summarized the phagocytizing activity of Ehrlich ascites tumor cells against pigments of benzidine yellow series. The difference was noted according to chemical structure of the pigment and the solvent. Even the same pigment was used, phagocytizing activity was different rate when various solvents were used.

(2) Electron microscopic findings on phagocytosis of pigments of benzidine yellow series by Ehrlich ascites tumor cells.

i) Electron microscopic findings of each pigment.

Each pigment is noted in the electron microscopic picture as a substance with high electron density, rod or needle shaped, with the sized of 20 mμ × 150 mμ.

Table 4. Phagocytizing activity of Ehrlich ascites tumor cells.



ii) Electron microscopic findings of Ehrlich ascites tumor cells with phagocytosis of pigments.

The administered pigment particles congregated in granules which were scattered within the cytoplasm of the Ehrlich ascites tumor cells at several sites. The electron density of granules was considerably high, and the diameter was 0.5μ to 2μ. The granules in the cytoplasm were electron microscopically quite variable. Some were surrounded by a single layer of limiting membrane and densed pigment particles were found within, while others were also surrounded by a single layer of limiting membrane but part of dense granule showed vacuolation (Fig. 1).

In the case of (1)-(b), (2)-(b) it was remarkable that the internal structure became almost empty, and the limiting membrane at the place of the presence of foreign body became indistinct, and pigment particles with high electron density were adherent only at the site of the limiting membrane (Fig. 2).

In the case of (4)-(a), the administered pigment particles were seen in granules of high electron density within the structure of the limiting membrane. At the site, in agreement with the size of pigment particles, spot-like or rod-like crystal forms with low electron density are interspersed. In such instance, the space inside of the limiting membrane was either filled with substances with high

electron density or with substances with low electron density except for a small amount of electron-dense substance within the limiting membrane (Fig. 3).

A part of the pigment granules surrounded by the limiting membrane showed a low electron density assuming a lamellar structure within the limiting membrane. At times the myelin-like structure was noted (Fig. 4). Such myelin-like structure occasionally became giant sized (3μ to 4μ in diameter). In such a case, the cytoplasm invaded the inside where pigment particles were probably present surrounded by the limiting membrane, resulting in the formation of myelin-like structure (Fig. 5).

The state of administered pigment particles adhering to the surface of cell membrane was not found in our present experiment. In general no marked enlargement of the smooth surfaced endoplasmic reticulum in the cytoplasm was seen. However, in cells with slight process on the surface of the cell membrane, a considerable dilatation of the smooth surfaced endoplasmic reticulum was seen (Fig. 6).

Although the structure of mitochondria was almost normal, pigment granules of low electron density which underwent phagocytosis showing a lamellar structure simulate the picture of the deposition of pigment particles within the structure of the mitochondria (Fig. 4).

These granules which underwent phagocytosis were commonly found near the Golgi's apparatus, but were unrelated with the Golgi's apparatus. No definite evidence of intra-nuclear localization was obtained.

DISCUSSION

Details on electron microscopic studies of Ehrlich ascites tumor cells were reported by Friedlaender et al. (1956), Selby et al. (1956), Adams et al. (1957), Wessel et al. (1957), Yasuzumi et al. (1958), Wolf et al. (1959), and Löblich et al. (1960). According to these reports processes covered by a membrane with the width of approximately 70 mμ are found on the surface of cell membrane of Ehrlich ascites tumor cells. Neutral fat with high electron density is occasionally found within the cytoplasm. Such neutral fat was also noted in my experiment. The number of mitochondria was relatively small and most of them were found around the nucleus. Some showed a distinct double structure of crystal while others shows an indistinct appearance and occasionally vacuolization. Similar results were obtained in my experiment. The endoplasmic reticulum of Ehrlich ascites tumor cells was relatively few in number and the presence adjacent to the cell membrane is extremely rare. According to Selby et al. (1956) tumor cells do not perform phagocytosis or pinocytosis. The Golgi's apparatus was far smaller than that in glandular cells. Since these findings show a considerably good agreement with my electron microscopic findings in Ehrlich ascites tumor cells, further electron microscopic observation was carried out on the pigment granules which underwent phagocytosis and the findings associated with it.

As stated previously, no studies are yet available concerning the phagocytosis of tumor cells especially the epithelial tumor cells. A comparative study will therefore be carried out with the mode of phagocytosis by phagocytic cells. Three modes of phagocytosis were considered in the phagocytic cells. The first is the intake of

foreign body into the cytoplasm by a pseudopod already present or a pseudopod formed upon the cell membrane approached by the foreign particle. The second is the intake through invagination by physicochemical difference from the cell membrane to foreign body which adheres to the cell membrane. The third is the intake through an opening of the smooth surfaced endoplasmic reticulum in the cell membrane as Palade (1955) and Hanaoka (1956) pointed out.

Fenn (1921) presented the surface tension theory on the mechanism of phagocytosis by the cells. According to Fenn, the physical surface tension including the phagocyte, foreign body, and the fluid in which both of these are present plays a role in the intake of foreign body. Although no consumption of biochemical energy appears to occur in phagocytosis according to this theory, Senda (1961) presumed the necessity of energy consumption probably through ATP. Concerning the electron microscopic picture on such occasion, Senda is of the opinion that the smooth surfaced endoplasmic reticulum does not stream into the pseudopod and appear to be unrelated with phagocytosis, while the membrane of the phagocytic granule is probably derived from the cell membrane. According to Uchino (1957), however, the smooth surfaced endoplasmic reticulum is opened to the cell membrane upon phagocytosis of the foreign body and the foreign body is probably taken up through this opening. Other electron microscopic studies on phagocytosis of foreign body were conducted by Sampaio (1956) using colloidal ThO_2, Uchino (1957) using india ink and sepia melanin, Dempsy (1955) using silver particles, and Goodman (1956) using bacteria. According to the electron microscopic study on phagocytes against india ink and sepia melanin by Arai (1960), no new pseudopod is formed for the uptake of foreign body in histiocytes, reticuloendothelial cells and monocytes.

As the mechanism of phagocytosis, Bennett (1956) presented the membrane flow theory. A new formation of cell membrane in one place and destruction on another place would cause a kind of stream of cell membrane. Substances outside of the cell membrane might enter the cytoplasm along this stream. The foreign body which thus entered the cytoplasm from the cell membrane is surrounded by the cell membrane at the site of invagination. A foreign body may thus be present in the cytoplasm surrounded by a membranous structure. However, if the cell membrane and the smooth surfaced endoplasmic reticulum was continuous as Palade (1955, 1956) and Fawcett (1955) stated, it would be possible to postulate that the foreign body outside of the cell membrane enters the cytoplasm from the smooth surfaced endoplasmic reticulum which has an opening at the cell membrane.

Carr (1962) injected liver oil into mice subcutaneously or intraperitoneally to study the picture of phagocytosis of the phagocyte, demonstrating a finding called "appositional phagocytosis". According to him, the cytoplasm of the phagocytes adjacent to the liver oil became as electron-dense as the liver oil itself, followed by the disappearance of a part of the cell membrane. The mass of liver oil then gradually moved into the phagocyte, some being surrounded by the limiting membrane, and some not surrounded. In my experiment, since no finding of adherence of pigment particle to the cell membrane was obtained, the state of pigment particle on the cell membrane surface is not clear. However, in normal Ehrlich ascites

tumor cells, the smooth surfaced endoplasmic reticulum is very scanty, supporting the concept of invagination of part of cell membrane for the intake of foreign body as the mechanism of phagocytosis in Ehrlich ascites tumor cells.

Generally speaking, it is accepted that Ehrlich ascites tumor cells do not carry out phagocytosis. However, the reason for the phagocytosis of the pigment by Ehrlich ascites tumor cells in my experiment should be explained. The pigment of benzidine yellow series used in the present experiment is all insoluble in water. As the solvent, surface active agents were therefore used. The surface active agents are adsorbed to the surface thereby showing surface activity to decrease the surface tension and to facilitate the adsorption of the particles at the surface. When these properties are taken into consideration, the tumor cell membrane appears to adsorb the pigment particle more readily. The difference of the behavior according to the chemical structure of the pigment may represent the difference in chemical property on the cell membrane.

Arai (1960) and Yamori (1962) studied the phagocytic granules in the phagocytes. These phagocytic granules possess a limiting membrane of single layer and the foreign body is found within the granule. In my experiment, the pigment particles which underwent phagocytosis were found within the granules, surrounded by a single layer of limiting membrane, although the difference between tumor cells and phagocytes is present. In phagocytes a non-specific increase of such granules is noted. These granules were called lysosome by DeDuve et al. (1955) and Novikoff et al. (1956), microbody by Rhodin (1954), cytosome by Linder (1959), H-granule by Kajikawa et al. (1960) and segresome by Tanaka (1961).

According to the experiment of Arai (1960) the india ink particles in the granules within the phagocytes were standing along the limiting membrane. In the results of my experiment, pigment particles were standing along the limiting membrane within the dilated membranous structure. Also in the experiment of Arai (1960) using india ink, the limiting membrane of the phagocytic granule disappeared as time passed, the granules mutually fused, and occasionally differentiated into double membranous structure. In my experiment, findings indicating the disappearance of the limiting membrane and the phagocytic granules showing double membranous structure were occasionally noted.

In my experiment, myelin-like structure was occasionally noted among the large objects which underwent phagocytosis within the tumor cells. When these were examined in detail, the membranous process invaded the phagocytized objects from their wall to divide it. This apparently corresponds to the type 3 of the digestive process according to Yamori (1962). According to the experiment of Arai (1960), the phagocytic granule consisting of phagocytized india ink particle gradually shows a structure resembling microbody as time goes on and some become indistinguishable from mitochondria showing double membranous structure. In my experiment, the phagocytized granules within the tumor cells showed the forms closely resembling microbody and mitochondria.

Amano (1960) distinguished the following 3 types of proliferation of the virus related to the tumor cells. (1) RNA type virus proliferates on the surface of the cell membrane as particles but not within the cytoplasm. (2) DNA type virus such

as rabbit mucous tumor proliferates within the cytoplasm. (3) There are some such as shope papilloma virus which proliferates within the nucleus as particles. General virus is also said to infect and proliferate within the tumor cells. According to Bang (1955), viruses such as Ectromelya etc. infect and proliferate within the Ehrlich ascites tumor cells. Although no particles appearing like virus particles were demonstrated in the present experiment, the pigment particles invaded the tumor cells might have entered the cells as virus did. No details, however, are yet clarified at present.

According to the experiment of Artmann et al. (1965), administration of cholchicin caused the appearance of inclusion body within the nucleus of the hepatic cells, probably due to plasma inclusion. In my experiment, serial ultra thin sections failed to reveal pigment particles within the nucleus. The possibility of demonstration of pigment particles within the nucleus as the result of plasma inclusion according to Artmann should also be considered.

CONCLUSION

1) Since some pigments of benzidine yellow series were phagocytized by Ehrlich ascites tumor cells in a high per centage, the picture of phagocytosis and digestion was studied electron microscopically.

2) Difference in chemical structure of pigments and solvents had influence on the phagocytizing activity.

3) Upon the phagocytosis, surface active agent facititated the adsorption of pigment particles to the cell membrane.

4) The phagocytosis probably took place through the invagination of the cell membrane.

5) The phagocytized pigment particles congregated in granules which were surrounded by the limiting membrane were 0.5μ to 2μ in diameter. When myelin-like structure was seen, the diameter was 3μ to 4μ.

6) The phagocytized pigment particle was digested as the time went on, and some times phagocytic granule was resembling microbody or mitochondria with double membrane structure.

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REFERENCES

1. ADAMS W. R. and PRINCE A. M.
J. Biophys. Biochem. Cytol. 1957. 3. 161/170
An electron microscope study of the morphology and distribution of the intracytoplasmic "Virus-like" particles of Ehrlich ascites tumor cells.

2. AMANO S.
Saibo Kagaku Shimpozium. 1961. 11. 23/37
Nucleo-cytoplasmic interrelationship with special reference to some virological problems.

3. ARAI S.
Sapporo Ishi. 1960. 17. 299/318
Electron microscopic studies on the phagocytosis of the reticuloendothelial cells.

4. ALTMANN H. W.
Beitr. path. Anat. 1965. 131. 355/394
Über hepatozelluläre Mitosestörungen und Kerneinschlüsse nach wiederholten Colchicingaben.

5. BANG F. B.
Ann. Rev. Microbiol. 1955. 9. 21/44
Morphology of virus.

6. BENNETT H. S.
J. Biophys. Biochem. Cytol. 1956. 2 (suppl.). 99/103
The concepts of membrane flow and membrane vesiculation as mechanisms for active transport and ion pumping.

7. CARR I.
J. Pathol. Bacteriol. 1962. 83. 443/448
Appositional phagocytosis.

8. DeDUVE C., PRESSMAN B. C., GIANETTO R., WATTIAUX R. and APPELMAN F.
Biochem. J. 1955. 60. 604/617
Tissue fractionation studies. 6. Intracellular distribution patterns of enzyme in rat liver tissue.

9. DEMPSEY E. W. and WISLOCKI G. B.
J. Biophys. Biochem. Cytol. 1955. 1. 111/118
Use of silver nitrate as vital stain, and its distribution in several mannalian tissues as studied with electron microscope.

10. FAWCETT D. W.
J. Natl. Cancer Inst. 1955. 15 (suppl.). 1475/1502
Observations on the cytology and electron microscopy of hepatic cells.

11. FENN W. O.
J. Gen. Physiol. 1921. 3. 439/464
The Phagocytosis of solid particles.

12. FRIEDLAENDER H. and MOORE D. H.
Proc. Soc. Exptl. Biol. Med. 1956. 92. 828/831
Occurrence of bodies within endoplasmic reticulum of Ehrlich ascites tumor.

13. GOODMAN J. R. and MOORE R. E.
J. Bacteriol. 1956. 71. 547/556
Electron microscopic study of phagocytosis of staphylococcus by human leucocytes.

14. HANAOKA M.
Nippon Ketsueki Gakkai Zasshi. 1956. 19. 341/358
Phasemicroscopic findings of corpuscles — Golgi's Apparatus and endoplasmic reticulum.

15. KAJIKAWA K.
J. Electron Microscpy. 1950. 8. 50/53
A characteristic granular component of histiocytes of the mouse skin.

16. LINDNER E.
Zschr. f. Zellforsch. 1957. 45. 702/746
Die submikroskopische Morphologic des Herzmuskels.
17. LÖBLICH H. J. and LANDSCHÜTZ C.
Z. Krebsforsch. 1960. 63. 269/283
Vergleichende elektronenoptische Untersuchungen am Ehrlich- und Sa I-Aszites-tumor.
18. MIYASAKI K. and MASUTANI S.
Beitr. path. Anat. 1966. 133. 101/123
Elektronenmikroskopische Untersuchungen über Fremdkörperspeicherung in den Kernen und in den Zytoplasmen der Aszitestumorzellen.
19. MIYASAKI K., SUZUKI A. and FUJITA T.
Proc. Japan Cancer Ass. 24th Ann. Meet. 1965. 46/
Effects of solvents and chemicals for the phagocytosis of Ehrlich ascites tumor cells, and their electronmicroscopic observations.
20. MIYASAKI K., SUZUKI A. and FUJITA T.
Ninth International Cancer Congress. 1966. 35/
On the phagocytizing activity of Ehrlich ascites tumor cells.
21. MIYASAKI K., SUZUKI A. and FUJITA T.
Cancer Res. 1967. 27. 426/435
On the phagocytizing activities of Ehrlich ascites tumor cells.
22. NOVIKOFF A. B., BEAUFAY H. and DeDUVE C.
JBBC. 1956. 2 (suppl.). 179/184
Electron microscopy of lysosome-rich fractions from rat liver.
23. PALADE G. E.
J. Biophys. Biochem. Cytol. 1955. 1. 567/582
Studies on the endoplasmic reiiculum. II. Simple dispositions in cells in situ.
24. PALADE G. E.
Ibid. 1956. 2 (suppl.). 85/98
The endoplasmic reticulum.
25. RHODIN J.
Thesis. Karolinska Institutet. Stockholm. 1954. 1/76
Correlation of ultrastructural organization and functions in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney.
26. SAMPAIO M. M.
Anat. Rec. 1956. 124. 501/518
The use of thorotrast for the electron microscopic studies of phagocytosis.
27. SELBY C. C., BIESELE J. J. and GREY C. E.
Ann. N. Y. Acad. Sei. 1956. 63. 748/773
Electron microscope studies of ascites tumor cells.
28. SENDA N.
Proc. Japan. J. Reticulo-endothelial System. 1961. 1. 15/25
Phagocytosis of leucocytes.
29. TANAKA H.
Ibid. 1961. 1. 45/57
Electron microscopic study on the vital staining, comparatively with phagocytosis.
30. UCHINO F.
Nippon Ketsueki Gakkai Zasshi. 1957. 20 (supple.). 63/75
Electron microscopic studies on phagocytes with special reference to the relationship between phagocytosis.

31. WESSEL W. and BERNHARD W.
Z. Krebsforsch. 1957. 62. 140/162
Vergleichende elektronenmikroskopische Untersuchungen von Ehrlich- und Yoshida-Aszites Tumorzellen.

32. WOLF L.
Schweiz. Z. Allgem. Pnthol. Bacteriol. 1959. 22. 474/489
Elektronenmikroskopische Untersuchungen an Ehrlich Aszites Tumor Zellen

33. YAMORI T., SASAKI M., MORI S. and MATSUURA S.
Saishinigaku. 1962. 17. 1022/1032
Electron microscopic findings of reticuloendothelial cells.

34. YASUZUMI G. and SUGIHARA R.
Cancer Res. 1958. 18. 1167/1170
A comparative electron microscopic study on Ehrlich Ascites Cells, Yoshida Sarcoma Cells, and Human Cancerous Peritonitis Ascites Cells.

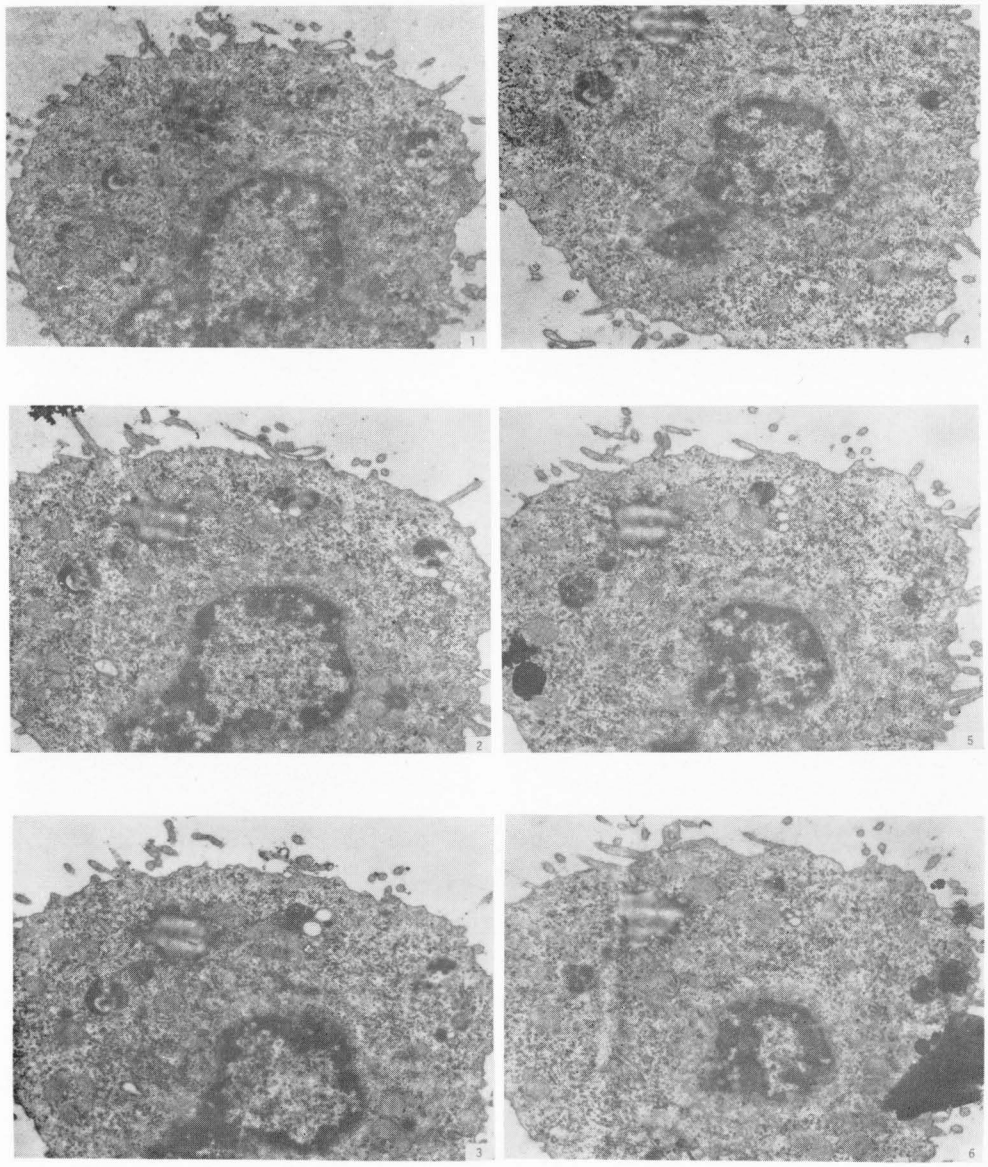

Fig. 1

Pigment (4)
Solvent (a)

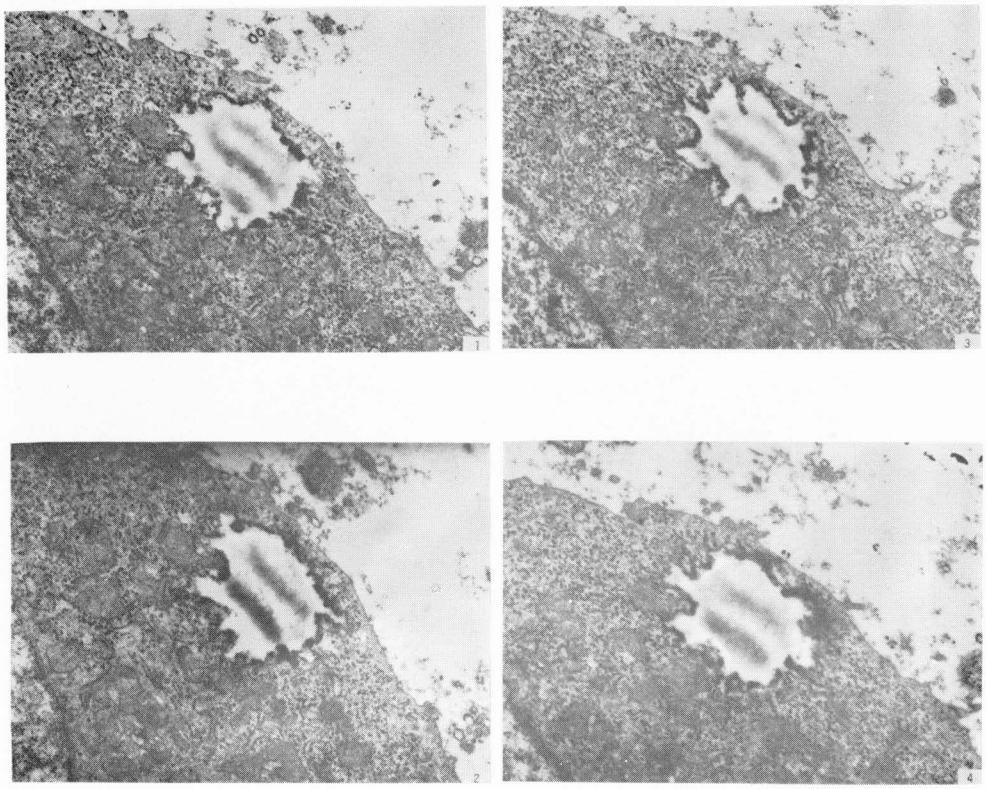

Fig. 2

Pigment (1)
Solvent (b)

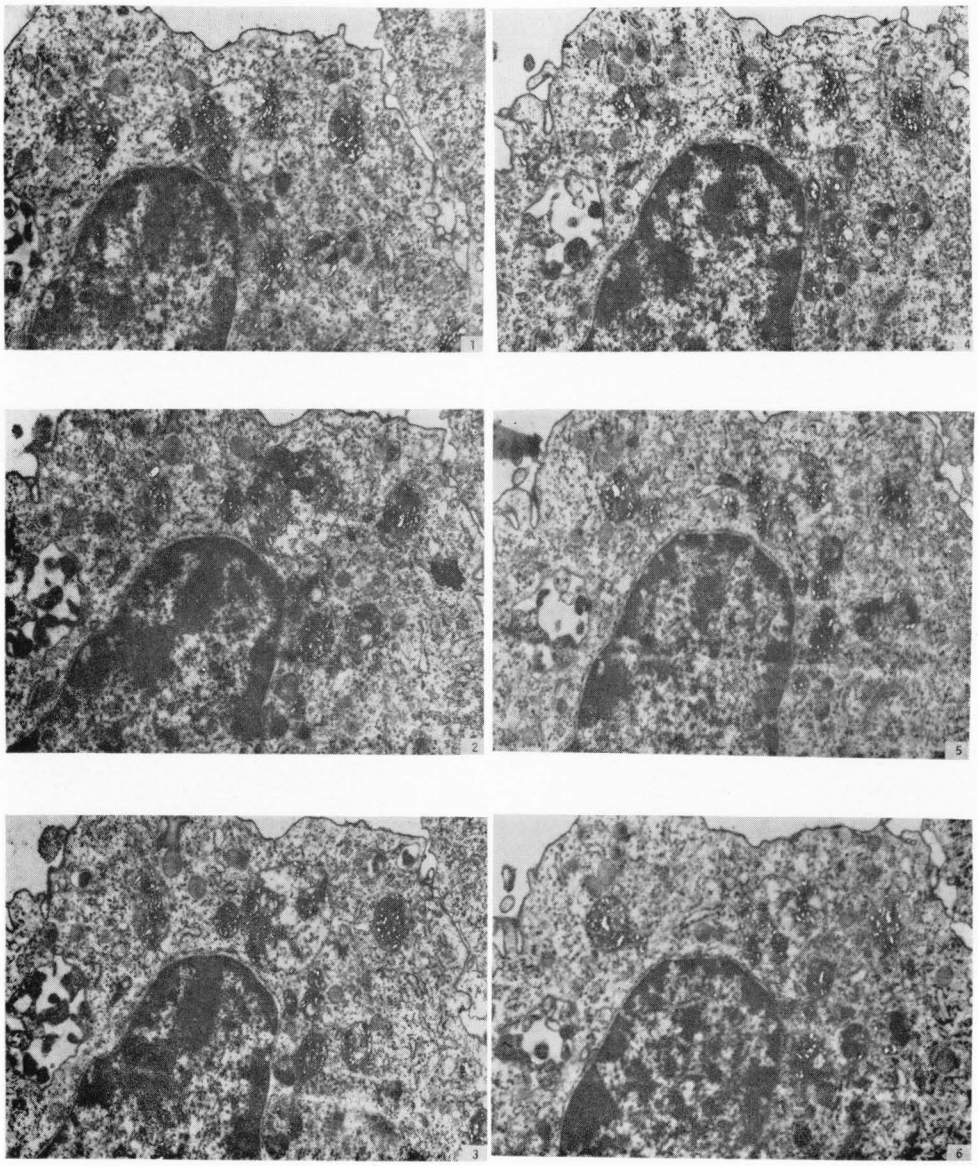

Fig. 3

Pigment (4)
Solvent (a)

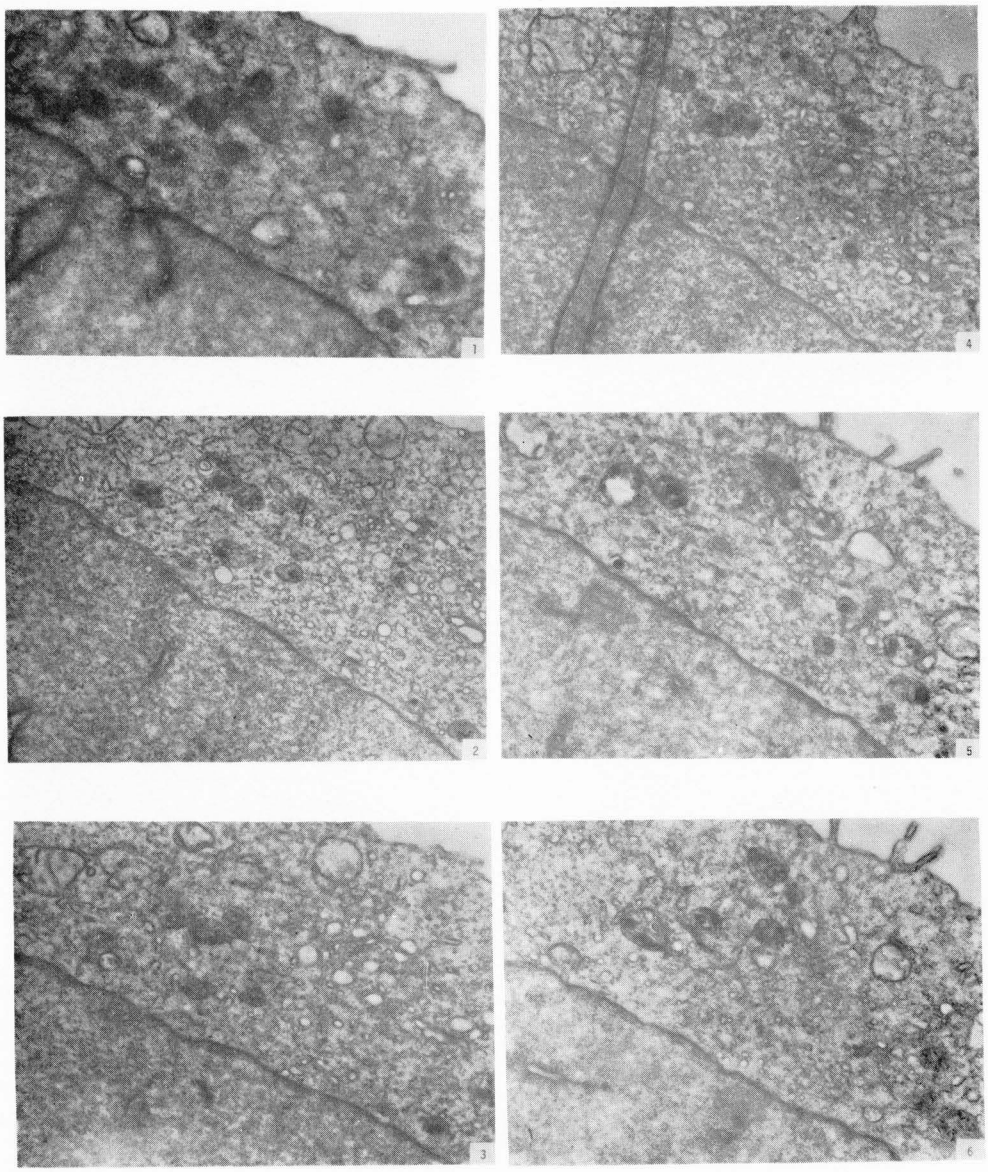

Fig. 4

Pigment (5)
Solvent (e)

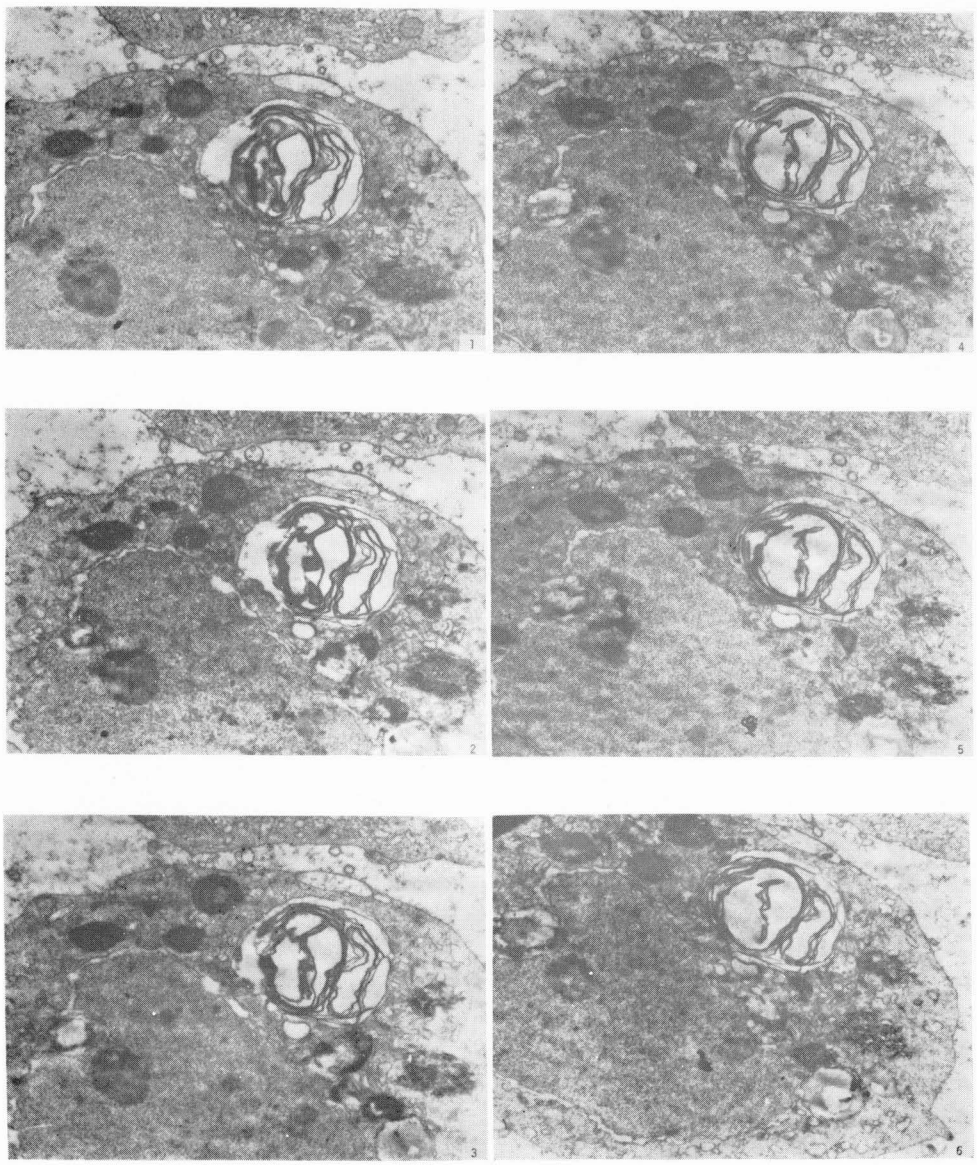

Fig. 5

Pigment (4)
Solvent (a)

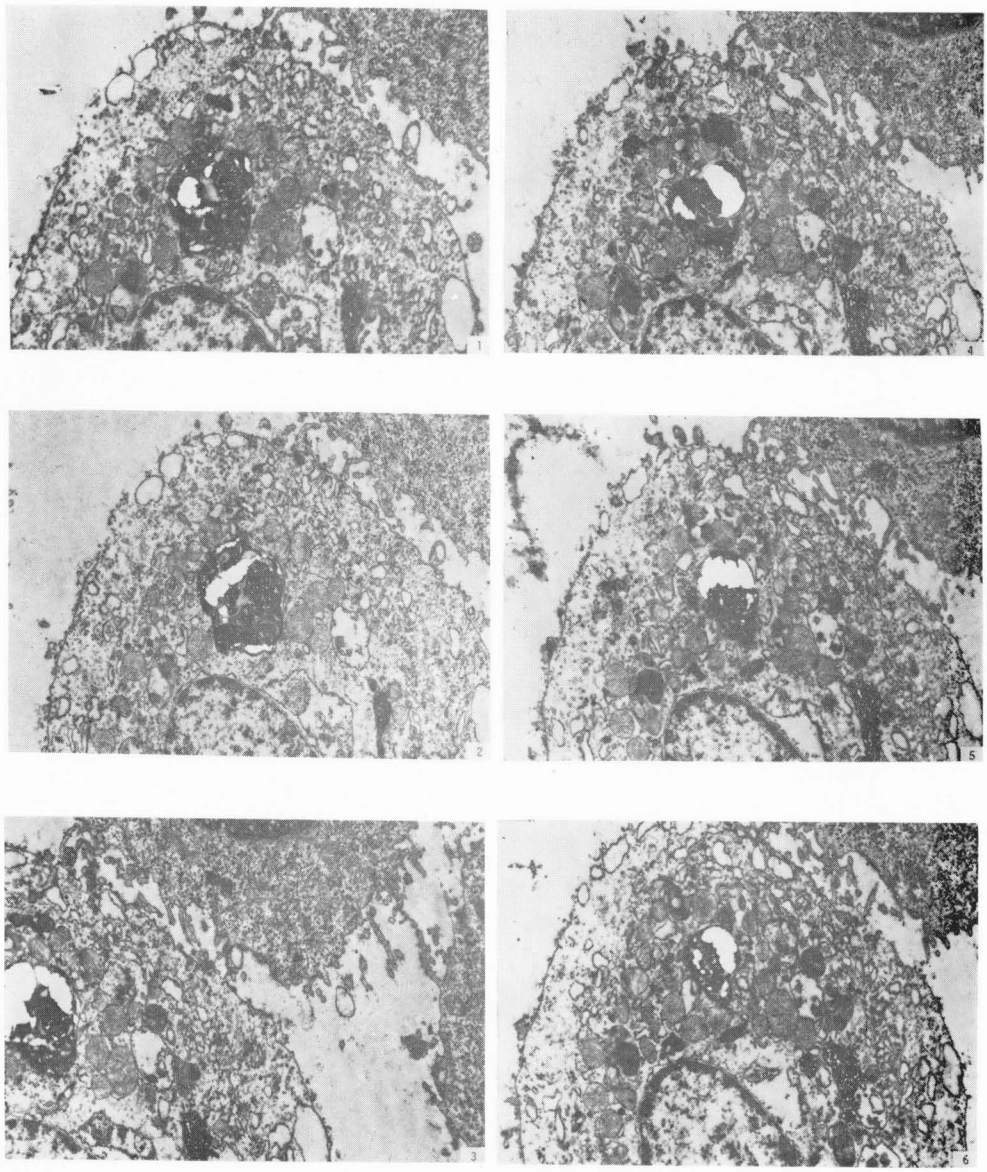

Fig. 6

Pigment (4)
Solvent (a)