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THE ULTRASTRUCTURE OF MACROPHAGE DIFFERENTIATION UNDER THE EFFECT OF YEAST RNA IN VITRO

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Indexing Words

electron microscope ; macrophage ; yeast RNA ; cell culture

Makoto TANAKA. *The Ultrastructure of Macrophage Differentiation under the Effect of Yeast RNA in vitro.* Kobe J. Med. Sci. 17, 161-184, December 1971.——The macrophages have been shown to play an important role in the immune response. To clarify the role of macrophages both in the phapocytosis and in the immune response, peritoneal macrophages were incubated and observed periodically after in vitro incubation with yeast RNA.

After the treatment with low concentration of yeast RNA (0.083 mg/ml), the cells showed marked growth and developed cytoplasmic processes, while the cells treated with the high concentration (0.166 mg/ml, 0.249 mg/ml) contracted remarkably and showed pyknosis.

Electron microscopic observations were performed in the two types of macrophages, one containing phagocytic vesicles and the other without any vesicles, after the treatment (0.083 mg/ml). The former showed the further development of phagocytic function with some retardation and did not exhibit any ribosomal activation. The latter, however, showed the activation of ribosomes and polysomes without being accompanied by phagocytosis.

Macrophages in different stages of cell differentiation differently responded to the same exogenous RNA in vitro and the only undifferentiated macrophages could be converted into receptor cells capable of enhancing ribosomal activity. The mechanism under which heterologous RNA is incorporated into macrophages was discussed.

INTRODUCTION

Metchnikoff described large mononuclear phagocytic cells as macrophages in contrast to polymorphonuclear cells as microphages in the last century, but surprisingly little has been known of the basic features of these cells. The macrophages are prominently distributed in the bone marrow, liver, spleen, lymph node, connective tissue and serous cavity. Nevertheless the origin of these cells remained uncertain and was frequently disputed. Some investigators believe that macrophages are derived from migration of monocytes from blood stream [12, 28, 51, 52]. Others insist that the cells originate from histiocytes which are essentially formed in the reticuloendothelial-system as defined by Aschoff and Kiyono [42]. Recently many investigators [10, 27, 30, 31, 32, 55, 56] demonstrate another possibility that the small lymphocytes transform into the macrophages.

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Many studies have been published on the responsibility of macrophages for cellular immunity as well as clearance mechanism and the relationship between macrophages and the immune competent cells has become progressively clarified in the last decade [20, 21, 45].

Fishman [1, 21] reported that antibody formation could be experimentally initiated in the lymph node cell culture by addition of purified ribonucleic acid fractions from antigenically stimulated macrophages and antibody response in the experiments was shown to be biphasic, consisting of an early phase of 19S IgM antibody and a second phase composed of 7S IgG antibody. In the experiments performed with the cells from rabbits chosen so that macrophages and lymph node cells came from animals of different allotypes, IgM antibody contained allotypic markers characteristic of the donor of the macrophages, while IgG antibody had allotypic specificity of the donor of the lymph node cells [1, 22].

The result obtained from these experiments suggests the possibility that the immunogenic activity of macrophage RNA can determine both allotypic marker of the light chain and antibody specificity of the immunogloblin in the lymph node cells. And it is generally accepted that the immunogenecity of the antigen taken up by macrophages is highly effective in inducing antibody synthesis [1, 8, 37, 53]. But the inhibition of the antibody response is also reported, attributed to the sequestration of antigen by macrophages [40]. The cause of these conflicting interpretations has not been explained and the difference between the stages of phagocytic cell differentiation was not taken into consideration in the experiments described above.

On the other hand, the addition of exogenous RNA to lymphocyte cultures stimulated with PHA resulted in considerable inhibition of blast formation. This inhibition was seen only when exogenous RNA was added at the time of RNA activation in PHA-stimulated lymphocytes prior to the blast formation [9, 44].

This indicates that biological effect of exogenous RNA on the host cells partially depends on the physiological status of the host cells [57].

The present study has been done to clarify the relationship between macrophages in different stages of differentiation and the effect of exogenous RNA. Two types of macrophages, one differentiated and the other not differentiated in the phagocytosis, were employed. Morphological changes of these two types of macrophages were light- and electronmicroscopically observed during the period in which the cells were incubated in vitro after the treatment of yeast RNA.

MATERIALS AND METHODS

Experimental animals ;

Guinea pigs of 6–10 weeks of age and weighing 300–350 g were used in all experiments.

Introduction of peritoneal cells with glycogen ;

Animals recieved an intraperitoneal injection of 10 ml glycogen solution

(2 mg/ml) (Daiichi Chemicals, Tokyo, Japan) 3 days before the collection of peritoneal cells.

Collection and counting of peritoneal exudate cells;

Animals were anesthetized with ether and recieved an intraperitoneal injection of 30 ml of Hanks' solution (Difco Laboratories, Michigan, USA) containing 5 units of heparin. Gentle massage was applied before harvesting cells from the peritoneal cavity. The cell rich fluid was aspirated with a syringe with needle and centrifuged for 7 min. at 800 r.p.m. in a sterile conical tube. The pellets were washed twice with Hanks' solution, centrifuged and resuspended in the incubation medium. The cells were counted with a haemocytometer and differential cell counts were performed by observing the smears of the pellet on slide glass stained with May-Giemsa stain.

Guinea pig in the normal, unstimulated stage yielded $1-5\times10^7$ peritoneal exudate cells, of which 50–70 per cent were macrophages. The animals recieved an injection of glycogen solution, 3 days before yielded $7-11\times10^7$ peritoneal exudate cells and after 4 hours incubation, 70–80 per cent of these peritoneal exudate cells showed the tendency to adhere on the glass surface. The cells adhering to the glass wall were large macrophages and a large percentage of medium-sized mononuclear cells, while the cells not adhering to glass wall were lymphocytes, a small percentage of medium-sized mononuclear cells and a few polymorphonuclear leukocytes.

Incubation of peritoneal macrophages;

Peritoneal macrophages were incubated at 37° C in the desired volume of Eagle's Minimum Essential Medium (MEM) (Nissui Seiyaku, LTD, Tokyo, Japan) containing 10 per cent inactivated fetal calf serum (Gibco, N. Y., USA) in a glass culture bottle (4 cm×8 cm, 5 cm×11 cm). Eagle's MEM were autoclaved at 15 pounds for 15 min.

Purification of yeast RNA;

Yeast RNA (Nakarai Chemicals, Kyoto, Japan) was purified with chroloform-gel method and determination of RNA was made by orcinol test. Purified RNA solution (0.5 mg/ml) in 0.15M sodium chloride and 0.0015M magnesium citrate was filtrated through millipore-filter.

Incubation of macrophages with yeast RNA;

Peritoneal exudate cells were incubated at 37°C for one hour with yeast RNA solution in Eagle's MEM. At the end of this period, the cells began to adhere to the glass wall. The cells were removed from the glass wall by gentle pipetting, collected by centrifugation at 800 r.p.m. for 7 min. and then resuspended in Eagle's MEM containig 10 per cent fetal calf serum.

Observation by light microscope;

Macrophages were incubated at 37°C in a culture bottle at the bottom of which

cover slips were previously fixed with egg albumen, and macrophages attached to the cover slips were then fixed by ethanol and stained with May-Giemsa stain.

Observation by electron microscope ;

Macrophages were removed from the glass wall by gentle scratching or pipetting without using trypsin, collected by centrifugation at 800 r.p.m. for 5 min. and washed twice with phosphate buffer solution. The clear supernatant fluid was discarded. The pellets were fixed with two ml of cold phosphate buffered 2 per cent 0s 0_4 at 0°C for 30 to 45 min.

The osmium-fixed cells were dehydrated and embedded in Epon. Thin sections were made with an ultra-microtome MT-1 (Iwan Sorval Inc. Conneticut, USA), stained with lead and uranyl ions, and observed with a Hitachi Electron Microscope HU-11D (Hitachi, LTD, Tokyo, Japan).

RESULTS

1) Macrophage cultivation and the concentration of yeast RNA

Macrophages were incubated for 4 hours at 37°C prior to the addition of yeast RNA. The cells of the coverslip surface consisted of macrophages and medium-sized mononuclear cells. The macrophages in monolayer were generally large and showed irregular contour with cytoplasmic processes. The cells were relatively uniform in their size as well as in the other morphological aspect. Three different concentrations of yeast RNA (0.083 mg/ml, 0.166 mg/ml, 0.249 mg/ml, final) were applied.

After one hour incubation the entire medium was replaced by the new one without RNA and the incubation continued The control cells were not exposed to RNA throughout the experiments. Macrophages, one hour after the treatment with yeast RNA, showed bipolar or tripolar in form with cytoplasmic extensions and the cell growth on coverslip was usually profuse. The nuclei were still round but some of them showed indentation in its contour. Vacuoles in the cytoplasm were visible in some cells treated with high concentrations of yeast RNA (0.166 mg/ml, 0.249 mg/ml, final). In control groups the cells were 15–20μ in diameter and round or oval in shape. Nuclei were mostly round and rarely showed indentations. At 6 hours after the treatment the cells showed remarkable growth in size with cytoplasmic processes (Fig. 1). Vacuoles in the cytoplasm are also observed in the case of treatment with large dosage of yeast RNA (Fig. 2). Macrophages in the control groups, however, showed less prominent cytoplasmic processes and no vacuoles in the cytoplasm (Fig. 3). At 12 hours after the treatment the cells exhibited a tendency to become compact, accompanied retraction of the cytoplasmic processes. The cells treated with the large dosage showed the marked reduction in size as well as the contraction of the cytoplasmic processes. At 24 hours after the treatment the cells treated with large dosage of yeast RNA contracted remarkablely and floated off from the coverslips into the medium (Fig. 4), but the cells treated with the small dosage showed rounded

contour indicating slight reduction in size and a few cells floated off from the coverslips. At 48 hours after the treatment the cells treated with the low concentration exhibited again cytoplasmic processes, but the nuclei of macrophages treated with the high concentration showed pyknosis and the majority of of cells floated off into the medium. At 72 hours after the treatment the cells exposed to the low concentration revealed cytoplasmic processes with enlargement of the cytoplasm, but the number of cells reduced in comparison with that at the bigining of incubation.

Comparing the effect of the low concentration of yeast RNA with that of the high concentration, the former induced the prominent cell growth accompanied by forming of the cytoplasmic processes, and the latter caused pyknosis and marked cytoplasmic reduction.

2) Observation by electron microscope.

A) Administration of yeast RNA (0.083 mg/ml) at the initiation of incubation.

The cells were spherical in shape and contained neither phagocytic nor pinocytic vesicles prior to the administration of yeast RNA. At 1 hour after the treatment the cells were round in shape. Indentation of the nuclei and dense rims of chromatin were frequently observed. Increase of the free ribosomes was not demonstrated but the rough surfaced endoplasmic reticulum (rER) was in moderate number observed (Fig. 5). The Golgi apparatus was poorly developed and scarcely seen. Vesicles caused by pinocytosis or phagocytosis were not observed. At 3 hours after the treatment the cell surface bacame irregular and villous projections developed. The major alterlation at this stage was the increase of ribosomes closely surrounding rER (Fig. 6). The nuclei were deeply indented. Nucleolus, Golgi apparatus and mitochondria showed no appreciable changes. At 6 hours after the treatment numerous villous processes from the surface were seen and the increase of cytoplasmic mass was evident. The nuclei varied in shape: some were deeply indented and the others spherical. Significant change occurred in ribosomes: ribosomes around rER decreased in number and free ribosomes appeared in the cytoplasm (Fig. 7). Many round lacunae outlined with the plasma membrane appeared in the cell periphery and small pinocytic vesicles were also noted. At 12 hours after the treatment villous projections well developed. Dilatation of rER cysternae and a moderate number of free ribosomes were observed (Fig. 8). The nuclei were generally indented and located at the periphery of the cytoplasm. Many vesicles outlined with the plasma menbrane and small pinocytic vesicles were seen but phagosomes were not demonstrated.

On the othe hand, numerous electron lucent microvesicles, and the enlarged Golgi apparatus composed with accumulation of both lamellae and vesicles in juxtanuclear region (Fig. 9) were observed in the macrophages of control groups cultivated for 3 hours. Ribosomes and rER poorly developed. The cells cultivated for 12 hours without administration of yeast RNA showed significant heterophagic function. Numerous phagosomes, coated vesicles and electron dense granules were evident (Fig. 10). Enlarged Golgi zone was always found in the hof of the nucleus.

At 24 hours after the treatment villous processes moderately increased and many large round lacunae outlined with the plasma menbrane were visible in the cell peryphery and small pinocytic vesicles in the cytoplasm. Moderate number of mitochondria were scattered in the cytoplasm. Golgi apparatus well developed in the juxtanuclear region. Clusters of ribosomes and polysomes were also present free in the cytoplasm (Fig. 11).

At this stage the cells of the control group were spherical in shape and a moderate number of villous processes were observed. Large electron opaque granules and an increase in size of Golgi apparatus were noted. Sometimes intracytoplasmic electron opaque vesicles showed a lamellated appearance resembling the early stage of myelin figure formation. Ribosomes, polysomes and rER poorly developed (Fig. 12)

At 48 hours after the treatment a moderate number of ribosomes, polysomes and rER were found in the cytoplasm. Villous processes, mitochondria and moderately developed Golgi apparatus were also observed but phagosomes were not demonstrated. At this stage intracytoplasmic organella showed no significant morphological changes in comparison with those of cells at 24 hours after the treatment (Fig. 13).

In the case of the cells treated with yeast RNA at the bigining of cultivation, transient accumulation of ribosomes closely around rER was seen at the early stages and then clusters of ribosomes or polysomes consistently appeared in the cytoplasm without any special locarization at the middle phase of cultivation. Phagosomes could not be demonstrated in any stage of incubation but pinocytic vesicles were observed.

B) Administration of yeast RNA at the 6th hour of incubation.

Macrophages in monolayer already contained pinocytic vesicles in the cytoplasm before the treatment with yeast RNA. At one hour after the treatment many phagocytic and pinocytic vesicles, moderate number of rER and clusters of ribosomes were scattered in the cytoplasm. Golgi apparatus moderately developed in the hof of the nucleus which located at one side of the cytoplasm (Fig. 14). At 6 hours after the treatment electron lucent vesicles and phagocytic vesicles containing foreign particles were constantly found. The nuclei were deeply indented. Free ribosomes or polysomes slightly increased in comparison with the state at one hour after the treatment (Fig. 15). At 12 hours after the treatment the prominent findings were the increase of pinocytic vesicles and transformaion of phagocytic vesicles to the lamellated figures. The decrease of free ribosomes and polysomes was also noted (Fig. 16).

From the observation described above, it was concluded that the heterophagic function of macrophages shown before the treatment was fairly inhibited by the treatment but afterwards developed again, and that pinocytic function was on the contrary not affected. On the other hand, rER as well as ribosomes around rER showed no essential changes by the RNA treatment.

DISCUSSION

Macrophages as well as microphages have the important function of participating in the phagocytosis of foreign particles such as bacteria, virus and miscellaneous foreign proteins. But macrophages, unlike microphages are not only active in the uptake and degration of foreign materials [38, 43, 54] but also able to interact with immunocompetent cells and induce them to produce specific antibody [11, 13, 14, 20, 21].

Foreign particles are taken up and degraded at similar rate both by macrophages and microphages. Macrophages, however, hold a small percentage of antigen in a form protected from normal rapid digestive process [28] and stable antigen fragments appear to be localized in an area not subjected to phagolysosome system. The immunogenecity of macrophages persists for prolonged period after the loss of most parts of antigen content [7, 37, 54].

It has been described by several investigators that structural units consisting of macrophages and surrounding lymphoid cells in the lymph nodes taken from immunized [animals will play some important role in the antibody formation [36, 58, 60] and that the penetration of RNA from macrophages into lymphocytes is demonstrated in vivo [21].

Non-immune spleen cells can be converted into antibody-forming cells by RNA extracted from the spleen of immunized animals [11, 13, 14]. Trnasplantation immunity is transferred from one animals to another by injecting autologous spleen cells incubated with the donor RNA extracted from the lymph node cells sensitized against skin homograft rejection [34, 35].

The experiments by Fishman [21, 22] documented that IgM and IgG antibody are produced in vitro by two kinds of immunogenic macrophage RNA, one of which is linked with antigenic fragment described by Askonas and Friedman [8, 23, 26], and the other of which is synthesized in macrophages after contact with antigen and not linked with antigenic fragment. These 2 kinds of RNA are responsible for IgM and IgG antibody respectively. In the experiments performed with cells from rabbits homozygous at B locus on the light polypeptide chain, IgM antibody has allotypic characteristics for the immunogloblin of the donor of the antibody producing cells [1].

In this antibody producing cells macrophage RNA can induce synthesis of immunogloblin that differs from their normal product in the B locus of the light chain. This type of RNA is considered to define both allotypic and antibody specificity.

Schoenberg [49] reported that direct cytoplasmic connection between the macrophages and surrounding lymphocytes or pasma cells exist morphologically and that through this connecting corridor cytoplasmic content may be transferred. This exchange is most likely concerned with the transfer of ribosomal particles and not antigenic molecules because the transfer of the electron dense or labeled particles used as atigen was not recognized.

Meanwhile, the influence of non-specific RNA on the cellular activities was also observed in vivo and in vitro [17, 18, 47]. Amos [2, 3, 4] reported that bacterial RNA could stimulate chick embryo fibrobrasts to synthesize the protein related antigenically to the donor of RNA; in Niu's experiments [39] Ehrlich ascites tumor cells of the mouse produced serum protein and lost anaplastic feature after the

treatment with normal liver RNA from a calf or a mouse.

As mentioned above, functional role of exogenous RNA is explained by transformed cellular activities in the host cells, but explanations about the utilization mechanism of exogenous RNA are not satisfactory obtained.

Autoradiographic study by Galand [24, 25] showed that the RNA taken up during incubation with the labeled yeast RNA was found in the nucleus and cytoplasm of the carcinoma cells. Comparing the distribution of radioactivity among four bases and nucleotides of the extracted RNA from the host cells with that of the labeled yeast RNA, it was shown that the RNA taken up by the host cells retained its original base composition without any marked changes of its properties. From the finding that exogenous RNA penetrated into the nuclei, Holubeck [29] proposed that added RNA could interfere with RNA synthesis from DNA template. Schwarz [50] observed the incorporation of labeled RNA into various cells including peritoneal mononuclear cells in vivo and in vitro. His study clarified that radioactivity was mostly detected at first in the pinocytic vesicles, then in the nucleoli and nuclei, and at last in the cytoplasm. From these studies it is proposed that the uptake of exogenous RNA by peritoneal mononuclear cells, embryonal cells as well as carcinoma cells is performed through pinocytosis and that RNA taken up may retain its original structure in these cells. According to Bachi's report [9], the blast formation of human lymphocyte stimulated by PHA is supressed by the addition of homologous RNA under certain conditions and exogenous RNA interferes with the passage of the newly-formed RNA and with the translation of information at the level of ribosomes.

RNA from yeast is also shown to suppress the blast formation of lymphocyte stimulated by PHA in tissure culture and to delay skin allograft rejection [44, 46]. Cohen [13, 14] suggested that activation of antibody producing cells after exposure to RNA from immunized animals is strain-specific, but Alexander [5] and Ramming [41] proposed the possibility of immune response mediated also by heterologous RNA.

On the other hand, in vitro antibody response is not unequivocally demonstrated during the incubation of antibody producing cells with macrophage RNA. And the immunogenecity of antigen ingested in macrophages is frequently disputed. Argyris [6, 7] demonstrated a rapid decrease of the immunogenecity of intracellular antigen during in vitro incubation. Comparing the persistence of immunogenecity of intracellular antigen phagocytised by macrophages in vitro with that in vivo, it was found that in the latter case the immunogenecity decreased much slower than in the former. Perkin [40] suggested that the immunogenecity of antigen was destroyed by macrophages because the immunogeecity of antigen decreased after the exposure to macrophages. Many investigators [1, 6, 8, 37, 53], however, reported that the phagocytosis of macrophages did not destroy the immunogenecity because antigens taken up by macrophages were highly effective in the immune response. And others said that macrophages did not play at least a negative role in the immunization process. These contradictory interpretations are due to the dose of the antigen, the number of antibody producing cells and the physiological status of macrophages [37, 57.] But the physiological status of the cells has not been satisfactorily investigated in the experiments described above.

Macrophages have the possibility to differentiate into the two types of function; activation of RNA production containing the code for synthesis of specific antibody and the active engagement in phagocytosis i. e. destruction of foreign particulate. The cells in RNA producing stage and in phagocytic stage can differently respond to the same stimuli. This indicates that macrophages differently respond to the immunological stimuli according to the state of cell differentiation. If this is so, it may be proposed that the response of macrophages containing no phagocytic vesicles could be different from that of the phagocytes i. e. macrophages containing phagocytic vesicles.

In this experiments, peritoneal macrophages were classified into two groups: the cells containing phagocytic vesicles and the cells without phagocytic vesicles. Fine structures of these two types of macrophages were electronmicroscopically observed during the period in which the cells were incubated after the treatment of yeast RNA. According to Cohn's report, ultrastructure of macrophages were easily affected by the change of the incubation medium [15, 16, 19]. In this study, morphological changes of macrophages treated with yeast RNA were carefully compared with that of the control and fine structural features of the treated macrophages were counted as significant. Observation by light microscope demonstrated that peritoneal macrophages revealed a remarkable growth accompanied by prominent cytoplasmic processes after the administration of the low concentration of yeast RNA (0.083 mg/ml) but that the high concentration (0.166 mg/ml, 0.249 mg/ml) caused pyknosis and cytoplasmic retraction.

According to the electron microscopic observations, the administration of yeast RNA (0.083 mg/ml) at the bigining of incubation of cells, which were not differentiated to exhibit phagocytic activity, caused to increase transiently ribosomes around rER at 3 hours later and a constant aggregation of ribosomes or polysomes in the cytoplasm thereafter. Many roun lacunae outlined with the plasma menbrane appeared prominently in the cell peryphery and small pinocytic vesicles were rarely seen. Phagosomes were not present and phagocytic activity were not observed in any stages of cultivation. However, in the case of the cells showing some differentiation in phagocytic activity, the phagocytosis of macrophages was fairly inhibited by the treatment of yeast RNA and it appeared again with some retardation compared with control. Aggregation of ribosomes or polysomes slightly increased but ribosomes around rER were not observed. The cells which have been already differentiated to show the phagocytic activity prior to the harvestry from the peritoneal cavity presented no recognizable changes at the ribosomal level by the treatment of yeast RNA.

From the experiments reported above, it can be concluded that undifferentiated macrophages show the accelerated activity of ribosomes and polysomes after the treatment of yeast RNA and that macrophages differentiated in phagocytic activity do not reveal the acceleration of ribosomal activity by the treatment. And that only the undifferentiated macrophages can develope into the receptor cells capable of accepting exogenous RNA and of enhancing the ribosomal activity in vitro is also suggested. As the conclusion, it can be proposed that the macrophages which actively produce RNA materials containing the code for synthesis of specific antibody [1, 6, 7, 8, 11, 13, 14, 20, 21, 22, 23, 26, 33, 40] are converted in vitro from the undif-

ferentiated macrophages and not from the differentiated ones which are known as phagocytes.

CONCLUSION

Peritoneal macrophages of guinea pig induced by glycogen solution were periodically observed with light and electron microscopes during in vitro incubation after the treatment with yeast RNA.

1) After the administration of small dose of yeast RNA (0.083 mg/ml) macrophages showed cell growth with prominent cytoplasmic processes but the cells treated with the larger dose (0.166 mg/ml, 0.249 mg/ml) revealed pyknosis, vacuolization of the cytoplasm and contraction of cytoplasmic processes.

2) After the treatment with yeast RNA (0.083 mg/ml) at the initiation of incubation, increase of ribosomes around rER was transiently seen at the early stage (3 hours later) and then increase of free ribosomes or polysomes was constantly found at the middle stages (6–24 hours later).

3) The cells stimulated at the level of the ribosomal or polysomal activity by the treatment of yeast RNA did not show the development of phagosomes.

4) The cells containing phagocytic vesicles did not reveal the enhancement of ribosomal activity.

5) The phagocytic function of the cells shown before the treatment, was fairly inhibited by the treatment and appeared again with some retardation.

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EXPLANATION OF PICTURES

Fig. 1. Peritoneal macrophages cultivated in vitro for 6 hours after one hour incubation with yeast RNA (0.083 mg/ml). The cells are spindle of triangular in shape. May-Giemsa stain ×150

Fig. 2. The cells cultivated for 6 hours after one hour incubation with the large dosage (0.166 m g/ml). The cells contain vacuoles in the cytoplasm. ×150.

Fig. 3. Control cells incubated for 6 hours without yeast RNA. Cytoplasmic processes poorly developed. ×150

Fig. 4. Macrophages incubated for 24 hours after treatment with large dosage (0.249 mg/ml). Cellular shrinkage and vacuoles in the cytoplasm are evident. ×150

Fig. 5. A peritoneal macrophage found in the one hour incubation which was treated with yeast RNA (0.083 mg/ml) at the initiation of incubation. This cell is spherical in shape and has the indented nucleus. A moderate number of ribosomes and rER

are seen in the cytoplasm. ×14,000

Fig. 6. Higher magnification of perinuclear region of a macrophage incubated for 3 hours after the administration at the begining of incubation. Numerous ribosomes juxtaposed with rER are shown. ×22,500

Fig. 7. A macrophage cultivated for 6 hours after the treatment. The cell is enlarged and cytoplamic projections are visible. Moderate number of mitochondria and rER are seen. Golgi apparatus is present in the hof of the deeply indented nucleus. ×12,000

Fig. 8. Portion of perinuclear region of a macrophage cultivated for 12 hours after one hour incubation with yeast RNA at the initiation of incubation. Aggregation of ribosomes is present. Partial dilatation of rER cysternae are visible. The cell surface is irregular and has many villous processes. ×17,500

Fig. 9. A macrophage incubated for 3 hours in Eagle's MEM containig 10 per cent fetal calf serum without RNA. Nucleus is indented. Golgi apparatus is seen in the juxtanuclear region. Electron lucent vesicles and microvesicles are abundant in the cytoplasm. Ribosomes and rER poorly developed. ×13,500

Fig. 10. Juxtanuclear region of a macrophage cultivated for 12 hours without RNA. Golgi apparatus is enlarged. Phagosomes, electron dense granules and coated vesicles are abundantly seen. ×15,000

Fig. 11. Portion of a macrophage cultivated for 24 hours with one hour initial incubation with yeast RNA. Moderate number of mitochondria and aggregation of ribosomes are present. Electron lucent vesicles outlined with the plasma membrane are seen at the peryphery. ×17,500

Fig. 12. A macrophage cultivated for 24 hours without RNA. The Golgi apparatus well developed. Laminated phagosomes are present in the peryphery. ×11,500

Fig. 13. Two macrophages cultivated for 48 hours with one hour initial incubation with yeast RNA. The cell surface is irregular and numerous projections are present. Ribosomes and rER are not numerous. No electron opaque vesicles are seen. ×7,000

Fig. 14. A macrophage administered with yeast RNA for one hour after 6 hour incubation. Nucleus is indented and located eccentrically. Numerous, electron opaque and lucent vesicles are seen. ×15,000

Fig. 15. A macrophage which was incubated for 6 hours prior to the administration of yeast RNA. Increase in size and in number of vacuoles is prominent comparing with Fig. 14. ×11,500

Fig. 16. A macrophage which was incubated for 6 hours prior to the treatment and cultivated for 12 hous after one hour inucubation with yeast RNA. Many electron lucent vesicles, large as well as small, are observed throughout the cytoplasm. Phagosomes show the appearance of the myelin figure. Ribosomes are very few. ×12,000

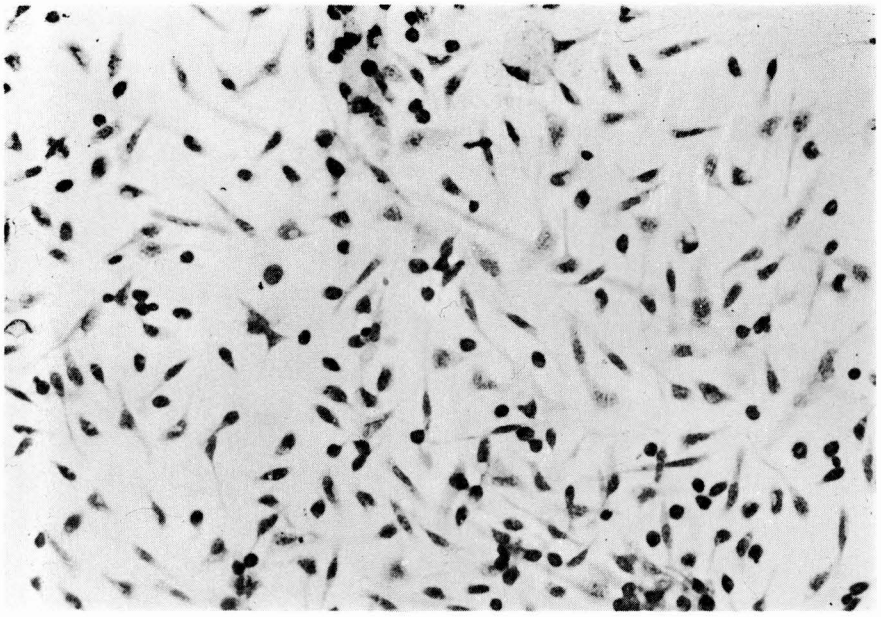


Fig. 1

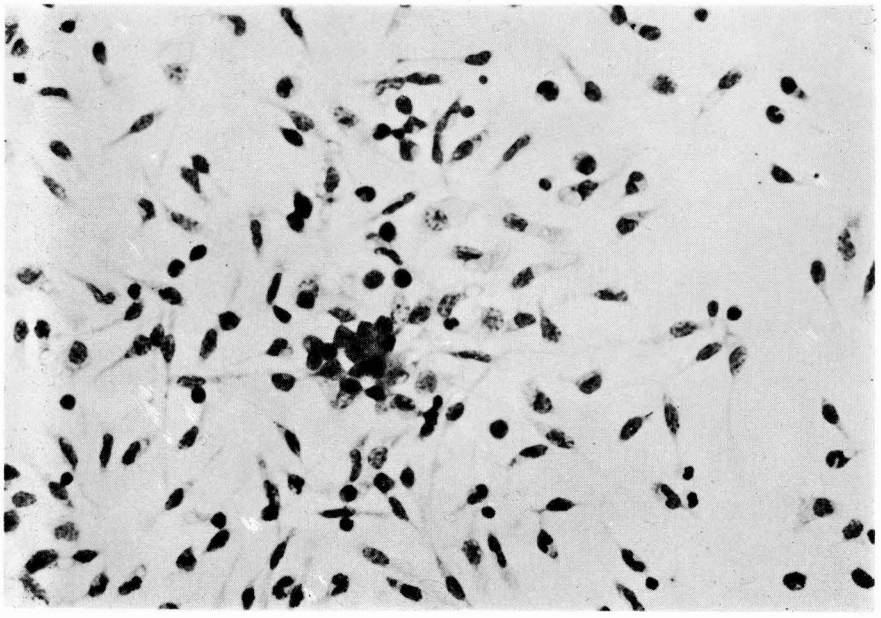
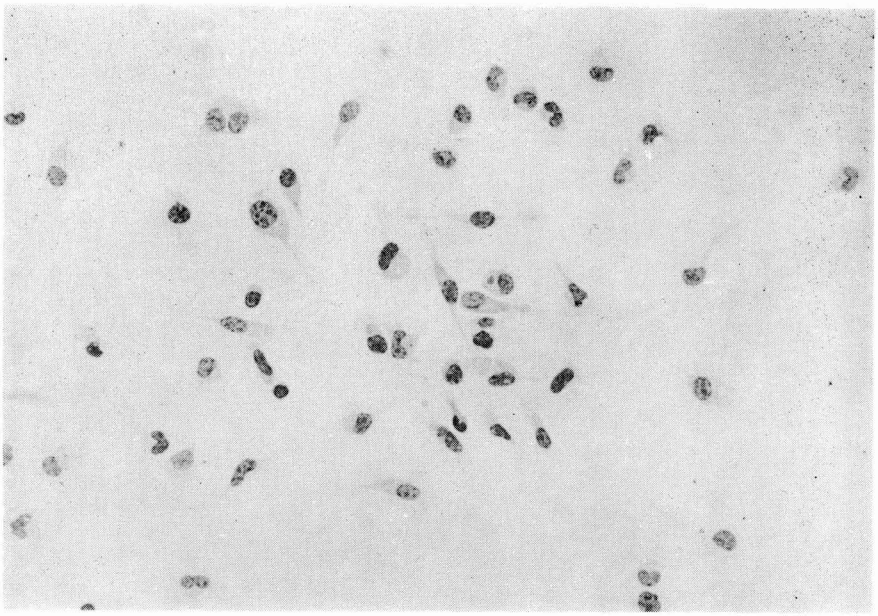


Fig. 2



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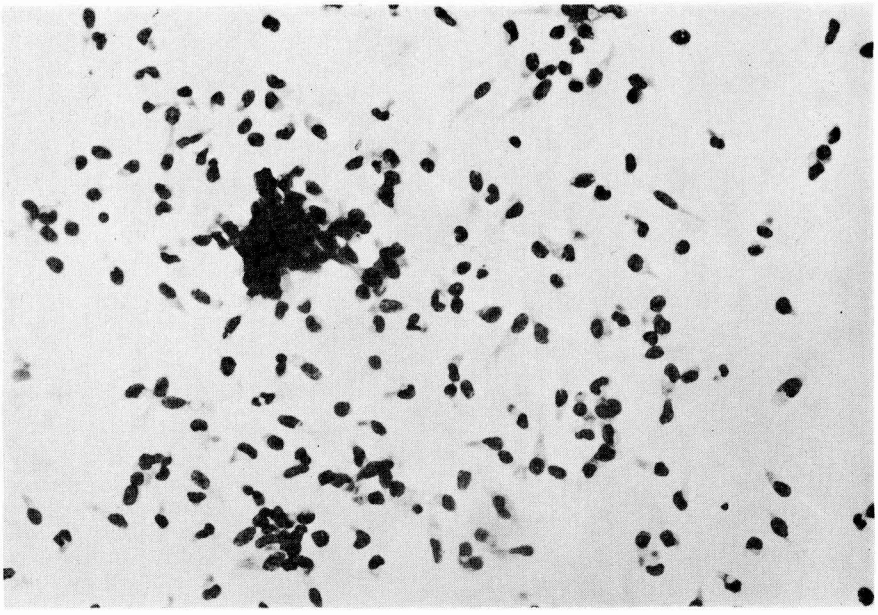


Fig. 4

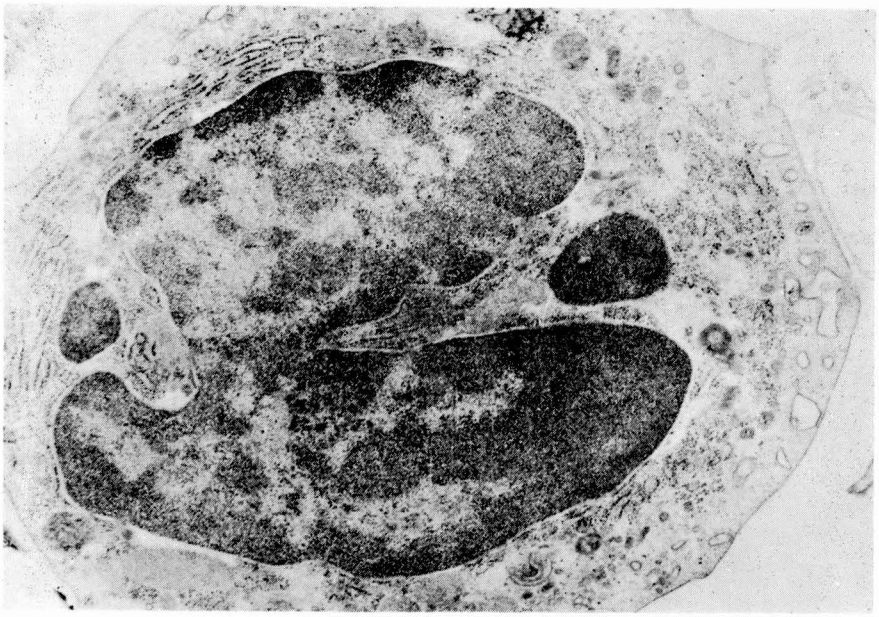
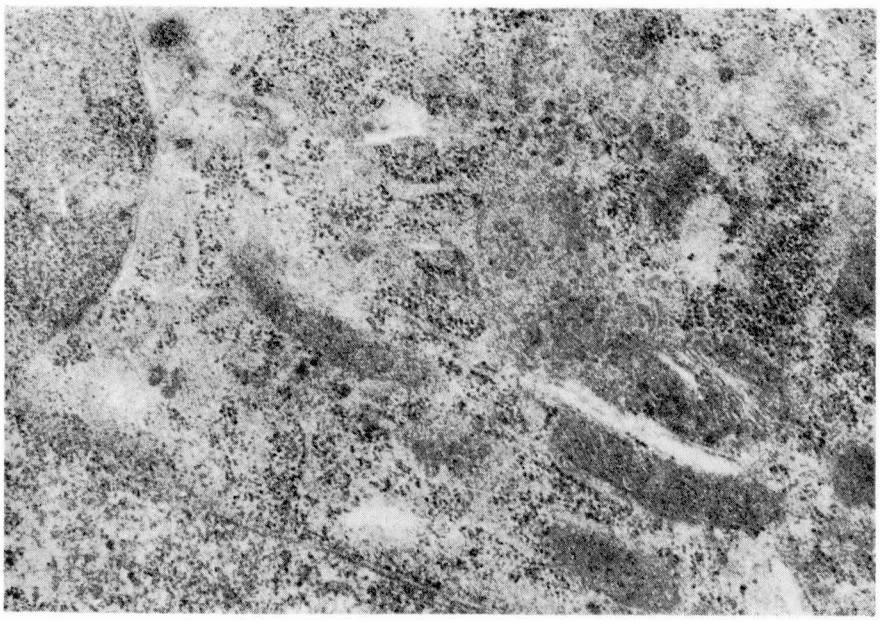


Fig. 5



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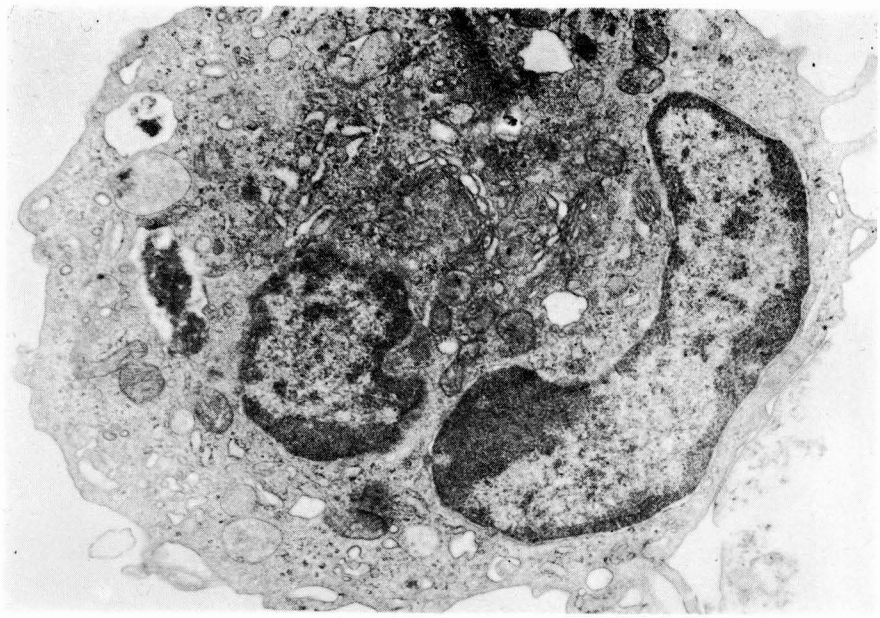
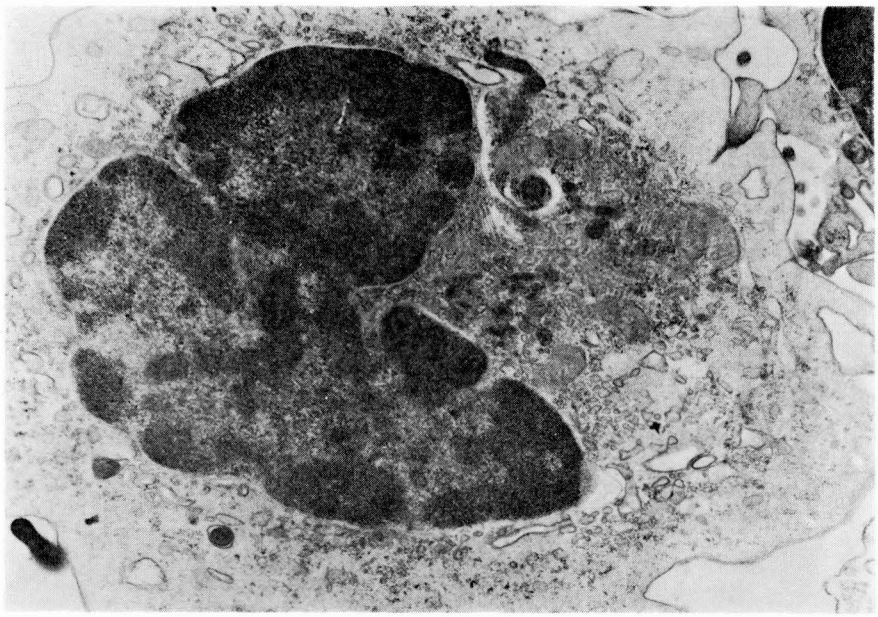


Fig. 7

Fig. 8



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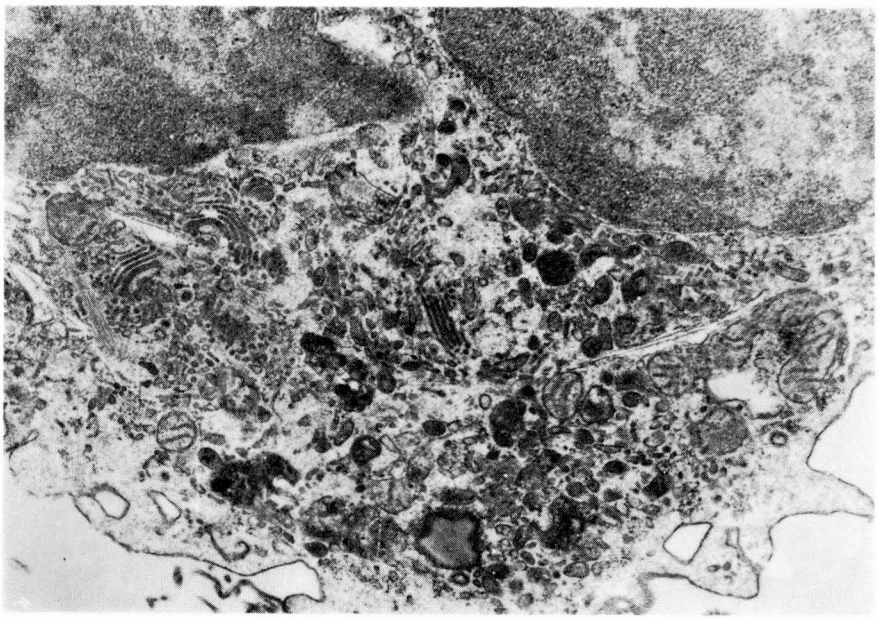

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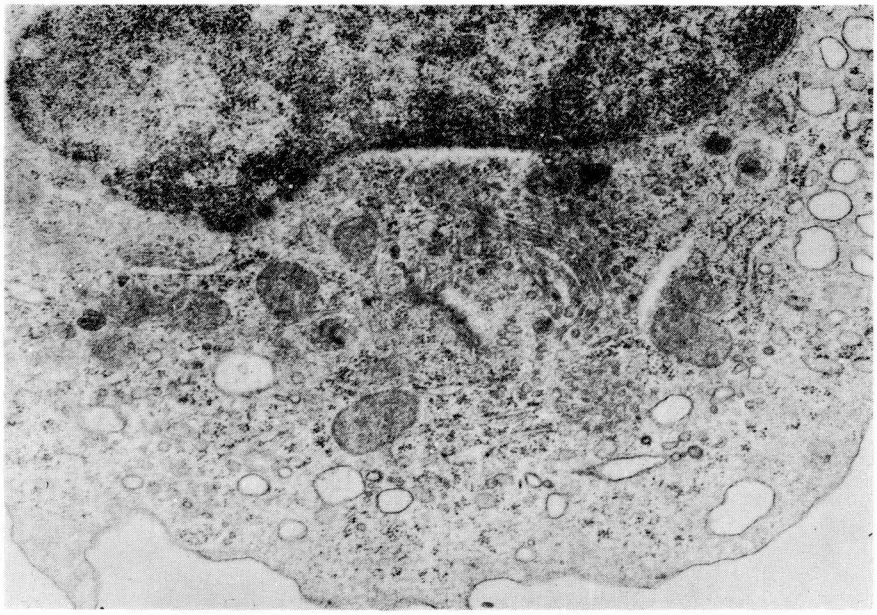

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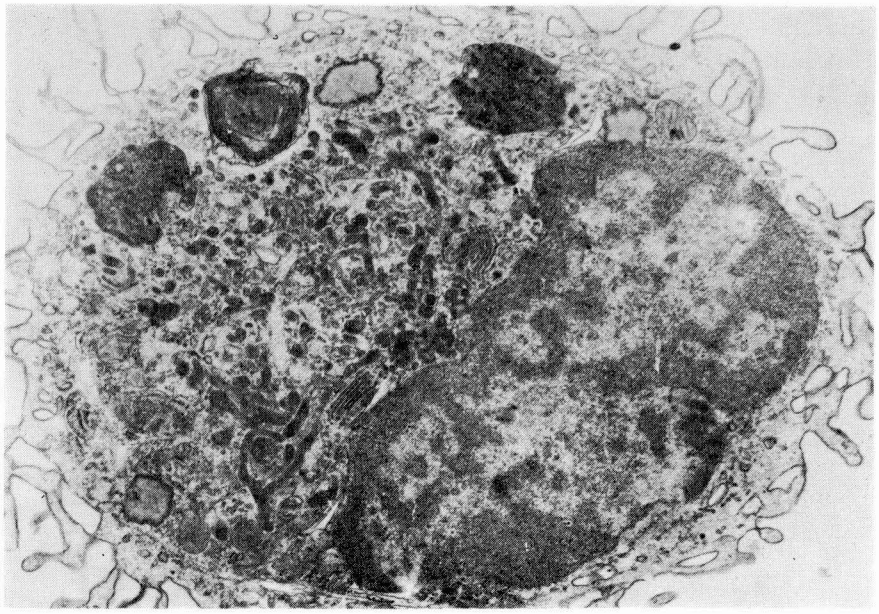

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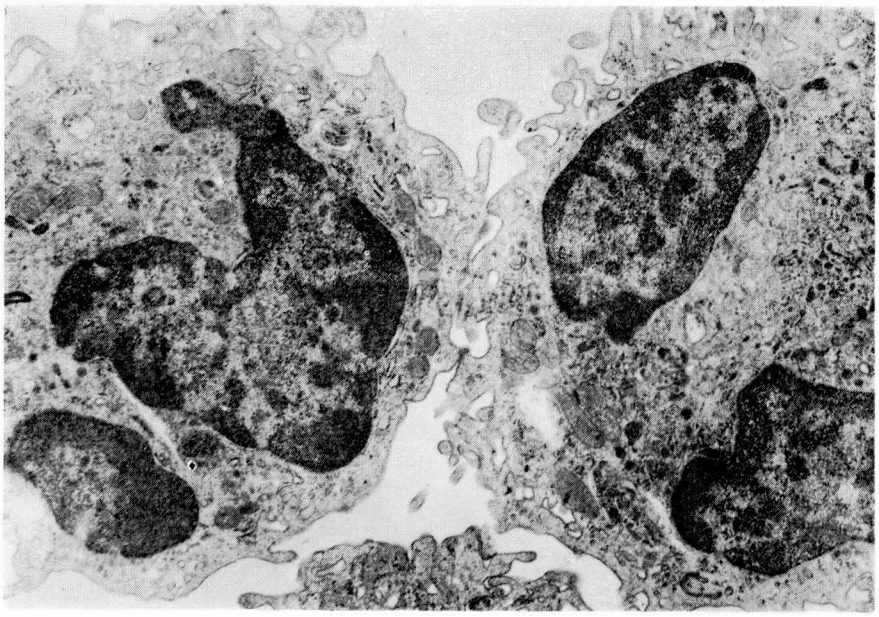
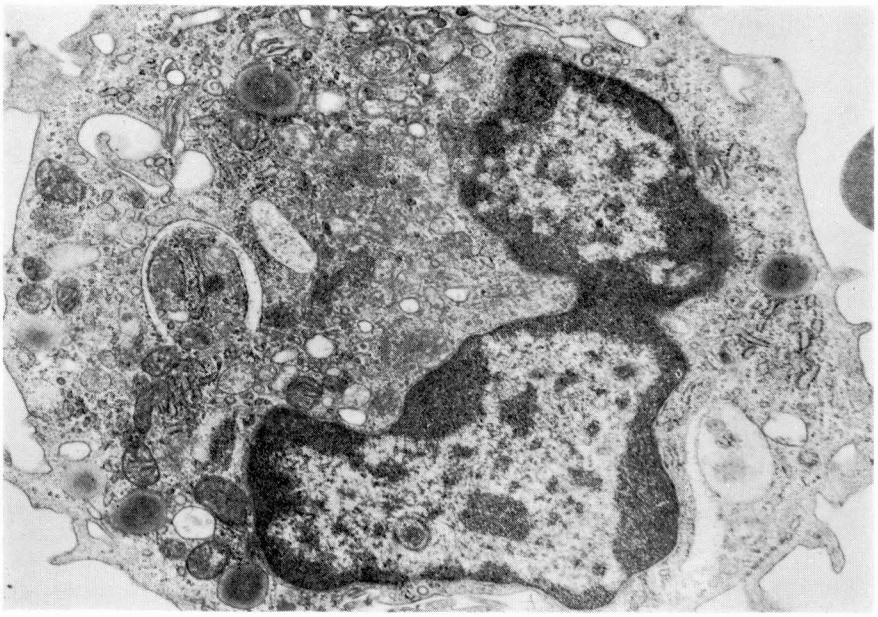


Fig. 13



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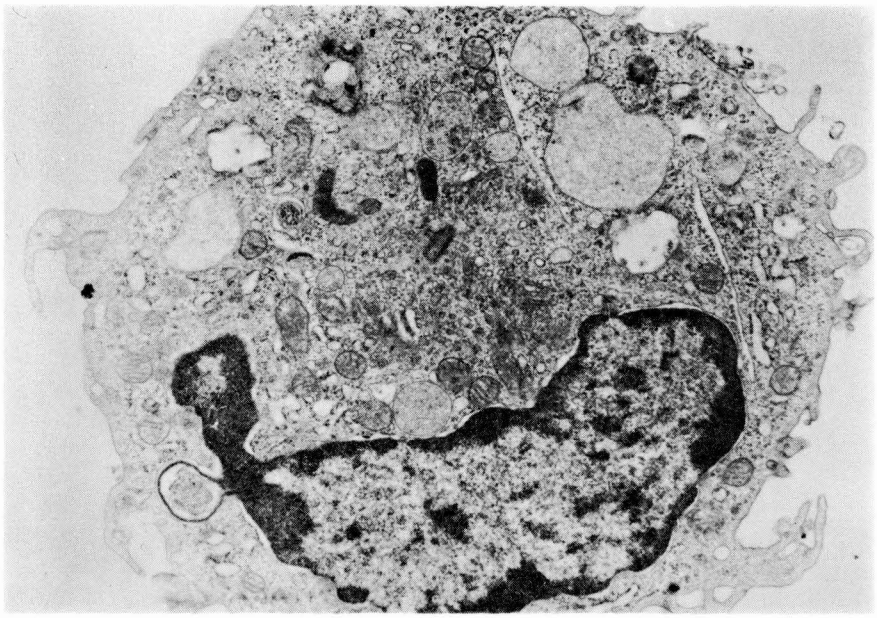

Fig. 15

Fig. 16