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Electron Microscopic Features of Bone Marrow Cells in Korean Hemorrhagic Fever†

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=Abstract=In eight male patients with Korean hemorrhagic fever, electron microscopic observations were made on the bone marrow cells during the early stage of the illness with the following results. 1) Several vacuoles were found in the cytoplasm of the erythrocytes and normoblasts. 2) Plasmacytoid cells rich in the rough endoplasmic reticulum, but not in its granules nor in mitochondria, were increased in number. 3) Phagocytic activities were demonstrated together with well-developed Golgi apparatus in the histiocytes and granulocytes. 4) Most megakaryocytes revealed abundant cytoplasm, the peripheral zone of which was actively producing platelets.

Key word: *Korean hemorrhagic fever, Bone marrow, Electron microscope*

INTRODUCTION

The patients with Korean hemorrhagic fever caused by Hantaan virus show fulminating hemorrhagic manifestations and acute renal failure (Lee 1976; Lee *et al.* 1980). Profound thrombocytopenia and disseminated intravascular coagulation appear to play a major role in bleeding (Lee *et al.* 1983). The peripheral blood, in addition, reveals a characteristic leukemoid picture with increased numbers of the granulocytes and plasmacytoid lymphocytes (Lee *et al.* 1979).

The morphology of bone marrow cells in Korean hemorrhagic fever was the subject of several investigations with an attempt to elucidate the pathogenetic mechanisms of the characteristic blood features (Chi *et al.* 1976; Hong *et al.* 1976; Hullinghorst and Steer 1953; Kim 1972; Lee and Lee 1979; Lee *et al.* 1977; Lukes 1954; Moon *et al.* 1975; Park *et al.* 1973; Whang and Lee 1979). Despite these extensive studies with some fruitful results, however, there still remain some unsolved questions concerning the morphological changes of the bone marrow cells in this disease. This is in part due to scarcity of bone marrow observations on histologic sections, and in part due to a rapid change of the blood picture during the early stage of this illness (Lee *et al.* 1983). Electron microscopic observations on the bone marrow cells were even more difficult to find in the literature.

The aims of the present study were to observe the ultrastructural characteristics of bone marrow cells in Korean hemorrhagic fever, and to elucidate the pathogenetic mechanisms of the blood cell changes in the peripheral circulation through electron microscopic studies on the bone marrow cells which might show some additional findings unidentified under the conventional light microscopes.

MATERIALS AND METHODS

Bone marrow biopsy specimens were obtained from eight male patients with Korean hemorrhagic fever during the 1983 epidemic in Korea. Only those in febrile/hypotensive or early oliguric phases of the disease were selected. The ages of the patients range from 22 to 24 years. The diagnosis of Korean hemorrhagic fever was made from clinical

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findings and confirmed by the demonstration of Hantaan virus antibody.

Bone marrow biopsies were performed from the posterior iliac crest with a Jamshidi biopsy needle. Biopsy specimens were fixed in 2.5% glutaraldehyde fixative, and decalcified with 2.5% EDTA solution. The specimen was then postfixed with 1% osmium tetroxide, and dehydrated through graded ethyl alcohol. Following dehydration, the specimen was transferred into a plastic capsule and embedded in Epon mixture. Each Epon block was sectioned at 1 um thickness to find the optimal area, and ultrathin sections were cut with a glass or diamond knife on an ultramicrotome. The ultrathin sections were doubly stained with uranyl acetate and lead citrate, and then observed under the Hitachi H-500 or JEOL 100CXII electron microscope.

RESULTS

1. Erythroid series

A conspicuous finding observed in the erythroid precursors and red cells of the bone marrow was the demonstration of vaculoes in the cytoplasm. Polychromatic normoblasts shown in Fig. 1 have highly condensed chromatin in the nucleus (N), and reveal several vacuoles (V) in the cytoplasm which contains some mitochondria (M) as well. The vaculoes are clearly different from the agranulated vesicles normally seen at this stage of development of the erythroid cells. A red cell containing two vacuoles (V) is shown in Fig. 2. Dense particles (arrows) were observed in the center of each vacuole, but the exact nature could not be identified.

A basophilic normoblast in Fig. 3 shows about equal amounts of condensed chromatin and euchromatin. The cytoplasm, however, contains scanty polyribosomes and few cytoplasmic organelles.

2. Myeloid cells and histiocytes

The granulocytic cells revealed no significant abnormalities. A myelocyte shown in Fig. 4 has a nucleus (N) with the chromatin condensed along the inside of the nuclear envelope. The nucleus shows a sign of slight indentation indicating development to the metamyelocyte stage. A nucleolus (n) is discernible, and the cytoplasm contains numerous specific granules (G) some of which are still filled with granular materials. A few mitochondria (M) are observed near the nucleus.

A charateristic feature observed in the bone marrow was the demonstration of phagosomes in the granulocytes as well as in the histiocytes. A histiocyte with erythrophagocytic activity is shown in Fig. 5. Many red cells (R), some of which show evidence of degeneration, are noted in the cytoplasm. The cytoplasm also contains many Golgi apparatus (G) with numerous vesicles.

3. Plasmacytoid cells

The bone marrow of the patients with Korean hemorrhagic fever revealed an increase of plasmacytoid cells the whole cytoplasm of which is occupied by cisternae of the rough endoplasmic reticulum (Fig. 6). In general, these plasmacytoid cells resembled the mature plasma cells in the bone marrow. They contained abundant rough endoplasmic reticulum (R), arranged into lamellar and concentric formation giving these cells a unique appearance. However, the granules on the rough endoplasmic reticulum were not so abundant in number, and the mitochondria were also less in number and smaller in size than in the usual mature plasma cells.

A plasmacytoid cell shown in Fig. 7 reveals the characteristic concentric arrangement of the rough endoplasmic reticulum (R) and a few mitochondria (M) in the cytoplasm. A nucleolus (n) is clearly identified in the nucleus (N) along with rearrangement of the heterochromatin in a patchy, wheel-like pattern.

4. Megakaryocytes

It was not difficult to find the bone marrow area containing megakaryocytes suggesting a normal or even increased number of these cells. Most megakaryocytes showed multilobulated nuclei with peripheral condensation of chromatin, and abundant cytoplasm often with cytoplasmic fragmentation (Fig. 8). The cytoplasm of a megakaryocyte shown in Fig. 9 shows an abundance of ribosomes, profiles of the rough endoplasmic reticulum, vesicles (V), and some tubular structures. Platelet granules (G) are prominent, and demarcation membranes (arrows) are discernible. Peripheral zone of the megakaryocyte revealed platelets (P) actively budding from the main body of the cell (Fig. 10).

Some megakaryocytes, however, revealed scanty cytoplasm indicating that most cytoplasm had been denuded from the preceding liberation of platelets (Fig. 11).

DISCUSSION

Some characteristic blood pictures are observed during the early stage of Korean hemorrhagic fever.

They are manifested by an increase of the hemoglobin concentration, leukoerythroblastic features mimicking a leukemoid reaction, and profound thrombocytopenia with accompanying disseminated intravascular coagulation (Lee *et al.* 1979; Lee *et al.* 1983).

The increase of the hemoglobin concentration appears to be due to a loss of plasma through damaged capillaries (Earle 1954). The red cells are normochromic and normocytic, but mild anisocytosis is observed (Lee 1976).

The leukocyte count reveals a similar change to that of the hemoglobin concentration. Leukocytosis observed in the early phases of these patients reflects a leukemoid reaction. The peripheral blood shows an increased number of myeloid elements. Some patients show myelocytes and metamyelocytes, the number of which reaches a peak about one week after the onset of the illness. Thereafter, lymphoid elements predominate while one of the most conspicuous findings observed at this period is the occurrence of the so-called plasmacytoid lymphocytes in the peripheral circulation (Lee 1976; Lee *et al.* 1979).

Marked thrombocytopenia develops quite early in the course of this disease (Lee *et al.* 1983). The peripheral blood reveals some giant platelets which decrease in number as the disease progresses (Lee 1976; Moon *et al.* 1975).

The bone marrow smears show increased M/E ratios with increased numbers of plasmacytoid cells, undoubtedly the counterpart of the plasmacytoid lymphocytes in the peripheral blood, and histiocytes with phagocytic activities (Hong *et al.* 1976). The number of megakaryocytes were reported to be increased (Hullinghorst and Steer 1953; Lee *et al.* 1977; Lukes 1954; Moon *et al.* 1975; Whang and Lee 1979) or decreased (Chi *et al.* 1976; Hong *et al.* 1976; Lee and Lee 1979; Park *et al.* 1973) by different authors. But a recent observation on the histologic specimens of the bone marrow revealed an increase of the megakaryocytes in number, and in size as well, during the early stage of this illness (Park *et al.* 1985). This appears to be the first stage-adjusted observation made on bone marrow biopsy sections, and indicates a compensatory increase of megakaryocytopoiesis in the bone marrow together with the appearance of giant platelets in the peripheral blood.

Although the changes detected in the bone marrow may not be specific for Korean hemorrhagic fever, and might also be observed in a number of acute infectious diseases as well, particularly those caused by viruses, the more intense the changes in the peripheral blood, the more distinct are the changes found in the bone marrow of these patients (Smorodintsev *et al.* 1964).

In recent years, an increasing number of publications has appeared describing the usefulness of electron microscopy in the differential diagnosis of hematologic disorders. Concerning Korean hemorrhagic fever, however, only few reports are available to review from the literature on the ultrastructural features of the blood and bone marrow cells, and that in limited aspects only. Lee *et al.* (1977) described ultrastructural changes of the megakaryocytes in the bone marrow. Ko *et al.* (1983) carried out an electron microscopic study on the peripheral blood cells in this disease.

Ko *et al.* (1983) observed plasmacytoid changes of the lymphocytes, and suggested the presence of some virus-like particles in the cytoplasm of the lymphocytoid plasma cells during the oliguric phase. In the present study, however, we could not demonstrate any reliable evidence for the presence of virus particles in the bone marrow cells.

A significant finding observed in our patients, in sharp contrast to that by Lee *et al.* (1977), was the ultrastructural features of the megakaryocytes. Lee *et al.* (1977) recorded a marked decrease of the cisternal system in the cytoplasm and lack of cleavage lines and platelet formation. They described that most megakaryocytes had large and multilobulated mature nuclei, but the amount of the cytoplasm was much less than that of a normal mature megakaryocyte. They also reported that the peripheral portion of the cytoplasm was homogeneous and the plasma membrane was rather smooth without evidence of platelet formation. They found no virus particles in the megakaryocytes.

Although Lee *et al.* (1977) did not clearly mention the size of the megakaryocytes, a marked decrease of the cytoplasm should result in a decrease of the size of the megakaryocytes in bone marrow. An increase of the megakaryocyte volume, however, has been observed in thrombocytopenic patients due to a reduction in platelet survival. They include those with idiopathic thrombocytopenic purpura, secondary immune thrombocytopenia, platelet consumption (Harker and Finch 1969), and Korean hemorrhagic fever (Moon *et al.* 1975; Park *et al.* 1985) as well, casting some doubt on the reliability of the previous report on the megakaryocyte changes by Lee *et al.* (1977).

In our patients, most of the megakaryocytes contained abundant cytoplasm with rich cytoplasmic organelles. Platelet granules were prominent, and demarcation membranes were evident. Active budding of the platelets was also demonstrated in the peripheral zone of the cytoplasm. Although some megakaryocytes revealed scanty cytoplasm, they probably reflect the preceding active formation of the platelets. The previous report by Lee *et al.* (1977) seems to have overemphasized these cells, and indicates the inherent difficulty to represent the whole picture of the bone marrow with an electron microscopic observation of so limited area. We believe that all these findings argue against the possibility of megakaryocytopoietic failue or faulty maturation of the megakaryocytes in Korean hemorrhagic fever.

Another interesting finding observed in the present study was the ultrastructural features of the plasmacytoid cells in bone marrow. They resembled mature plasma cells in general. But the granules on the rough endoplasmic reticulum were not so abundant in number as in the usual mature plasma cells. The mitochondria were also not so abundant in number nor so large in size whereas the normal plasma cells have the greatest number and the largest size of the mitochondria in bone marrow (Takefuda 1974). These features appear to indicate blunted secretory activity of these cells. Nevertheless, they more closely resembled the plasma cells in ultrastructural features than the lymphocytes, although they are traditionally called plasmacytoid lymphocytes in the peripheral blood.

Erythrophagocytic activities were demonstrated in the histiocytes and in the granulocytes, as well. More phagosomes, mostly in the form of degenerated red cells, could be observed under the electron microscope than under the light microscope. Abundant Golgi apparatus reflected active phagocytic activities of these cells.

Several vaculoes were observed in the erythroid precursor cells and mature red cells. Similar features were also recorded in red cells of the peripheral blood by Ko *et al.* (1983). They also described that virus-like particles were identified in the vaculoes. Although similar vacuoles with a dense particle were also found in the red cells of our patients as shown in Fig. 2, we do not have any additional evidence to suggest that they were virus particles. The vacuoles may indicate some damage to the erythroid precursor cells during their developmental stages.

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= 국문초록 =

한국형출혈열환자 골수세포의 전자현미경 소견

서울대학교 의과대학 내과학교실 · 암연구소* · 병리학교실**

이문호 · 박선양 · 장연복 · 김승택 · 이영열 · 김병국 · 김노경 · 김창식* · 김용일**

한국형출혈열 초기 환자 8명에서 골수세포의 전자현미경적 관찰을 시도하여 다음과 같은 소견을 얻었다.

1) 적혈구 및 정적아구의 세포질에서 다수의 공포가 관찰되었다.

2) 조면 소포체는 풍부하나 그 표면의 과립이나 mitochondria는 적은 형질세포양세포들이 증가되어 있었다.

3) 조식구와 과립구들에서는 Golgi체가 발달되어 있었으며 왕성한 식작용의 소견이 관찰되었다.

4) 대부분의 거핵세포들은 세포질이 풍부하였으며 세포질의 주변부위에서는 혈소판 생성이 활발하였다.

LEGENDS FOR FIGURES

Fig. 1. Polychromatic Normoblasts. The nuclei have highly condensed chromatin in thick strand form. Few polyribosomes and mitochondria (M) are identified, but the nuceolus is not discernible. Note the several vacuoles (V) in the cytoplasm which has already attained considerable density (x 12,000).

Fig. 2. Red Cell. The cytoplasm contains two vacuoles (V) in which dense particles (arrows) are discernible (x 12,000).

Fig. 3. Basophilic Normoblast. The nucleus has about equal amounts of condensed chromatin and euchromatin. The cytoplasm, however, contains scanty polyribosomes and few cytoplasmic organelles (x 13,800).

Fig. 4. Myelocyte. The chromatin in the nucleus (N) is condensed along the inside of the nuclear envelope. The nucleus shows a sign of slight indentation indicating development to a metamyelocyte. A nucleolus (n) is discernible, and the cytoplasm contains numerous specific granules (G) and some mitochondria (M) (x 12,500).

Fig. 5. Histiocyte. Note many red cells (R), some of which show evidence of degeneration, in the cytoplasm. The cytoplasm also possesses several well-developed, vesiculated Golgi apparatus (G) and numerous small, round or oval mitochondria (M) (x 6,200).

Fig. 6. Plasmacytoid Cells. The bone marrow revealed a sheet of plasmacytoid cells in some areas. Note that the whole cytoplasm is occupied by cisternae of the rough endoplasmic reticulum (R) (x 6,000).

Fig. 7. Plasmacytoid Cell. Note the concentric arrangement of the rough endoplasmic reticulum (R) characteristic of plasma cells. However, the granules on the rough endoplasmic reticulum are not so abundant in number, and the mitochondria (M) are also less in number and smaller in size than in the usual mature plasma cells (N; nucleus, n; nucleolus) (x 10,900).

Fig. 8. Megakaryocyte. The nucleus shows multilobulation with peripheral condensation of chromatin. The cytoplasm, a portion of which is fragmented (arrows), is abundant, and rich in cytoplasmic organelles (N; nucleus) (x 2,800).

Fig. 9. Cytoplasm of a Megakaryocyte. Note an abundance of ribosomes, profiles of the rough endoplasmic reticulum, vesicles (V), and some tubular structures. Platelet granules (G) are prominent, and demarcation membranes (arrows) are discernible (N; nucleus) (x 13,400).

Fig. 10. Peripheral Zone of a Megakaryocyte. Note the newly formed platelets (P) actively budding from the peripheral zone of the cytoplasm (x 13,400).

Fig. 11. Megakaryocyte. Scanty cytoplasm in this megakaryocyte indicates that most cytoplasm has been denuded from the preceding liberation of platelets (x 6,000).

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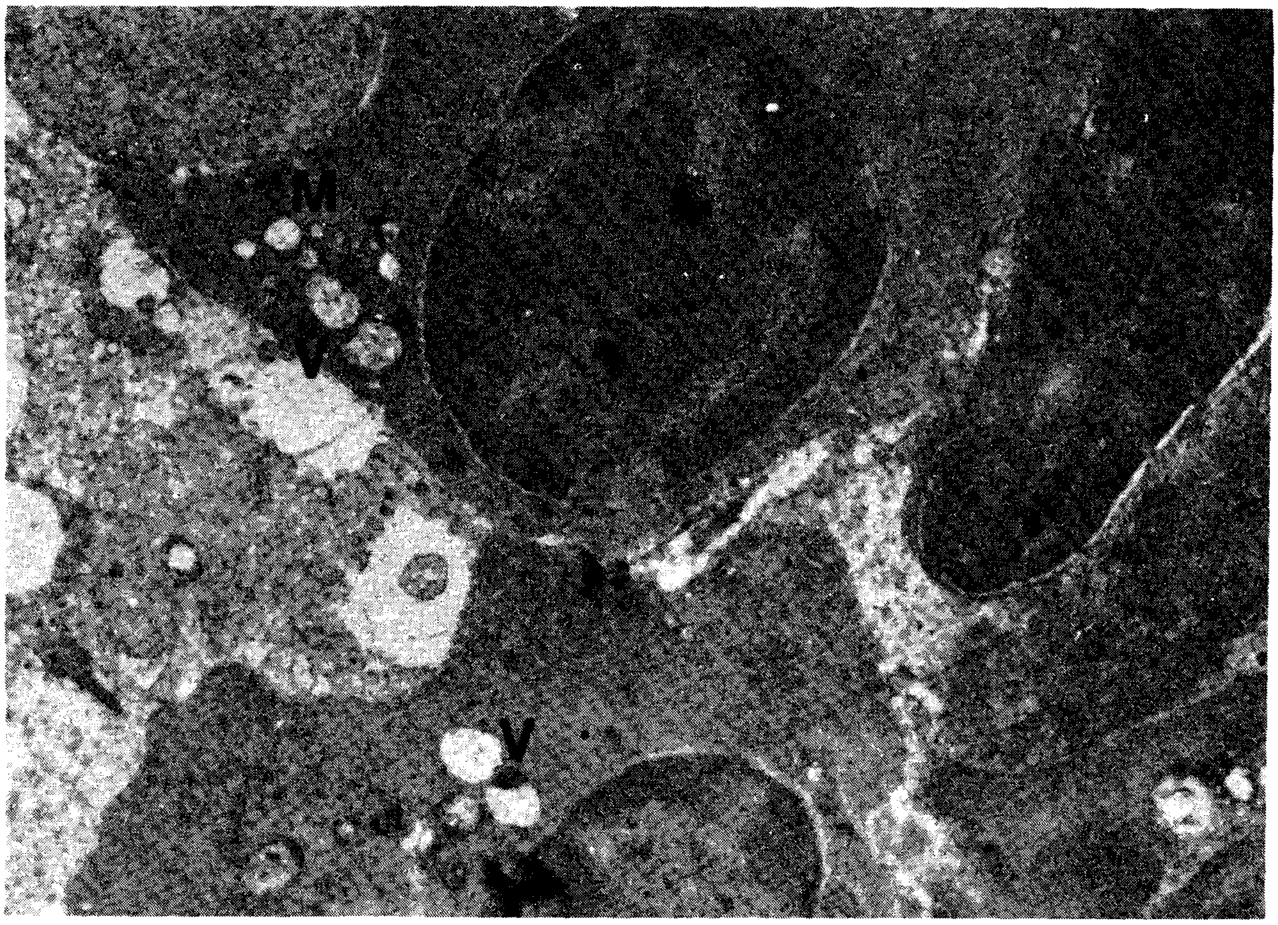

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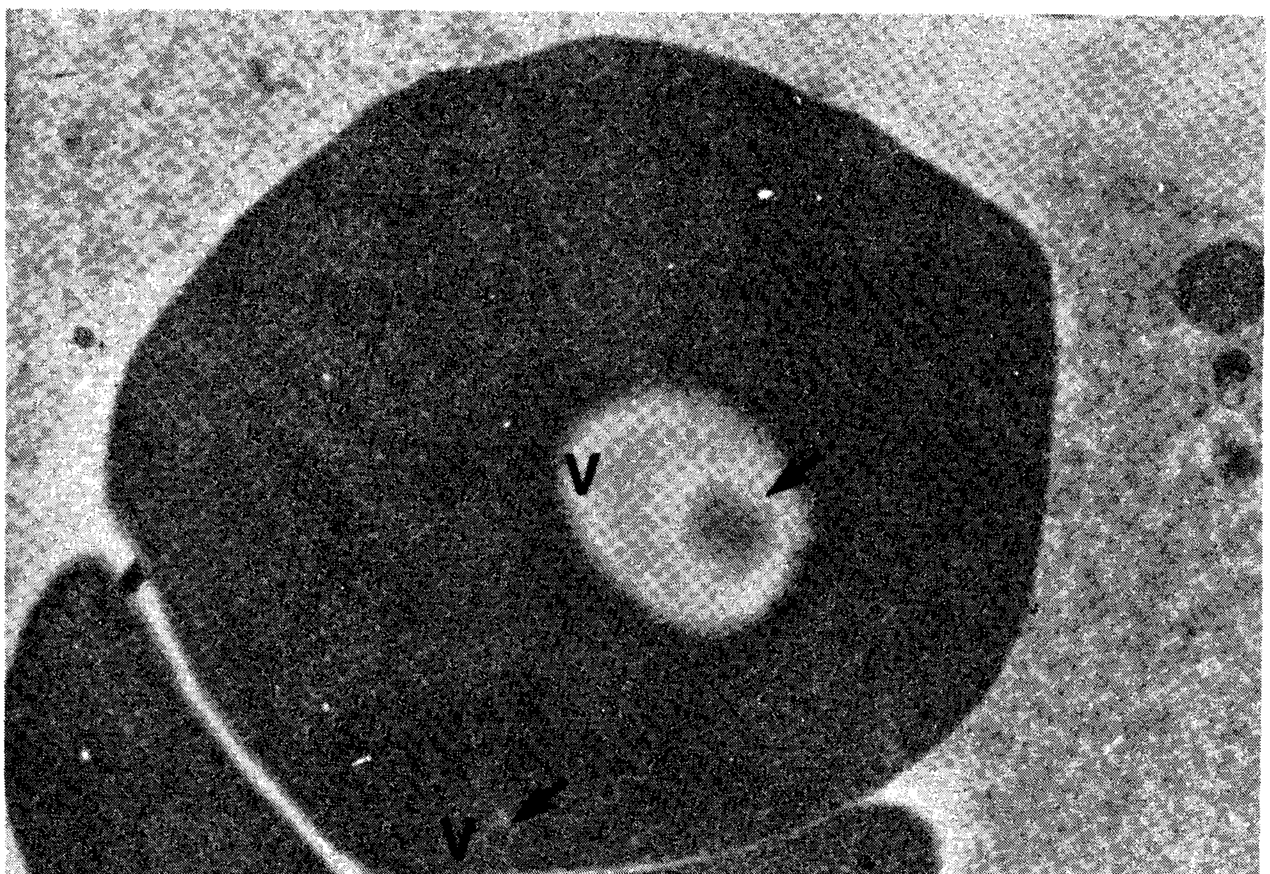

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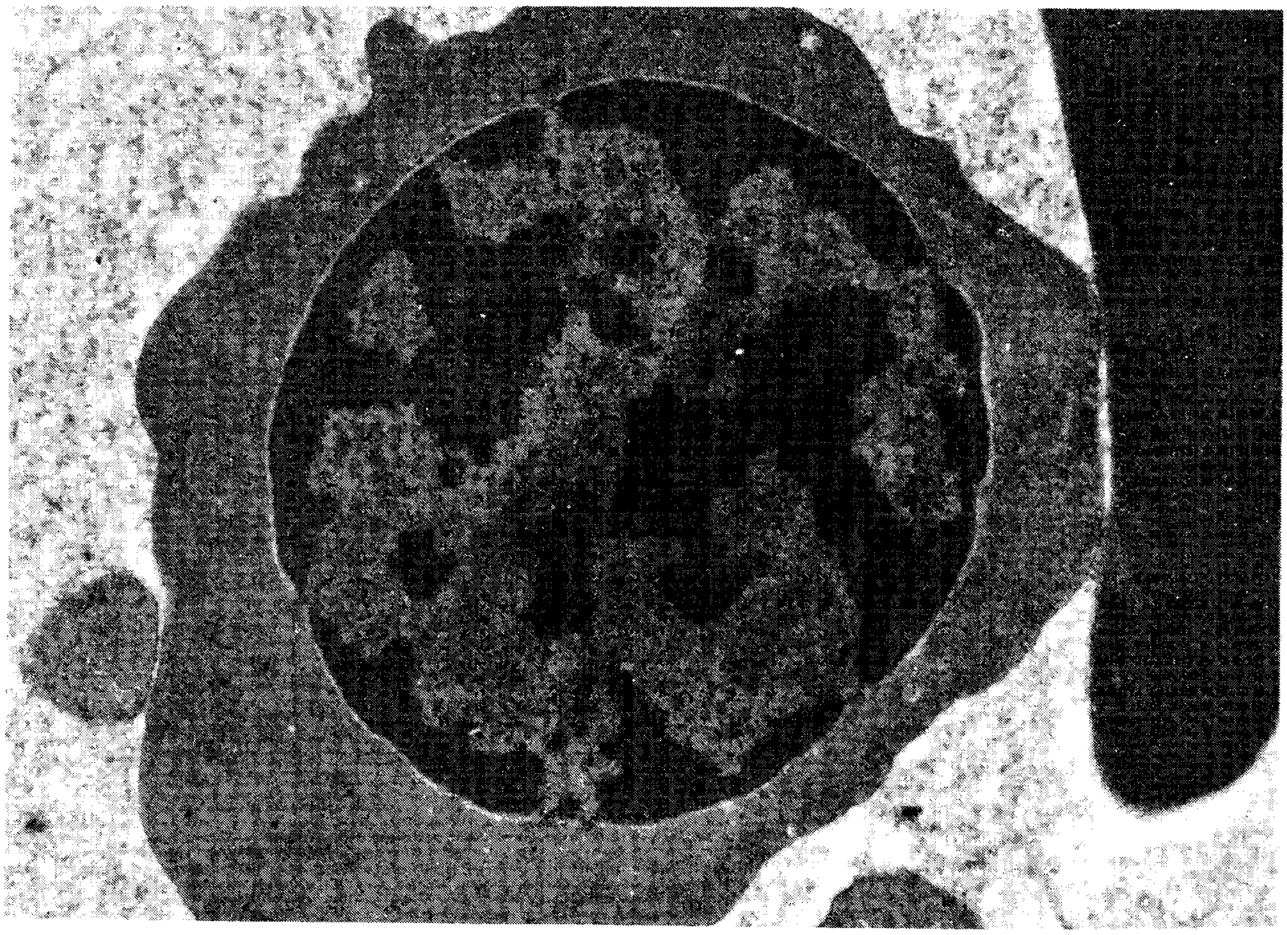

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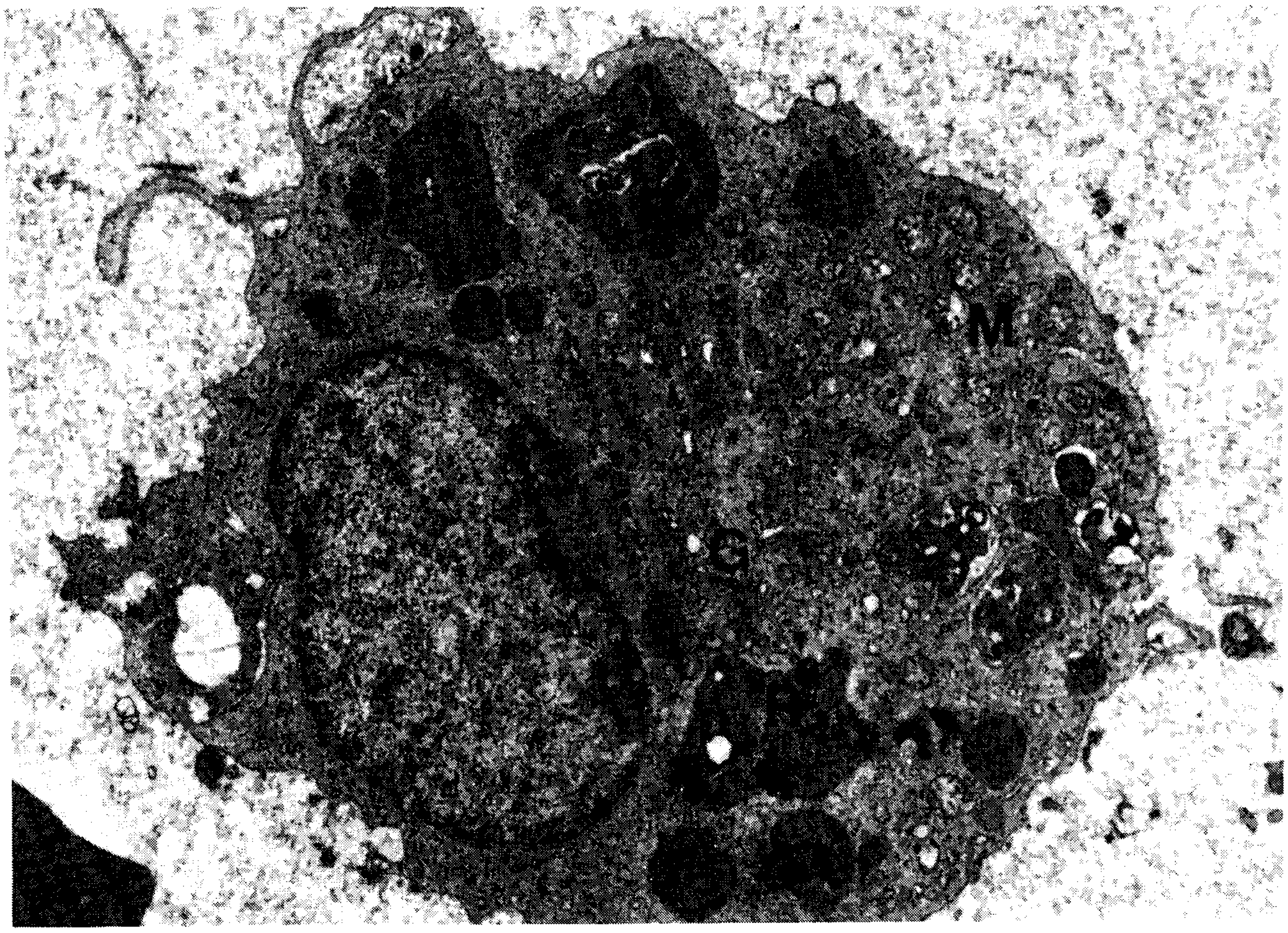

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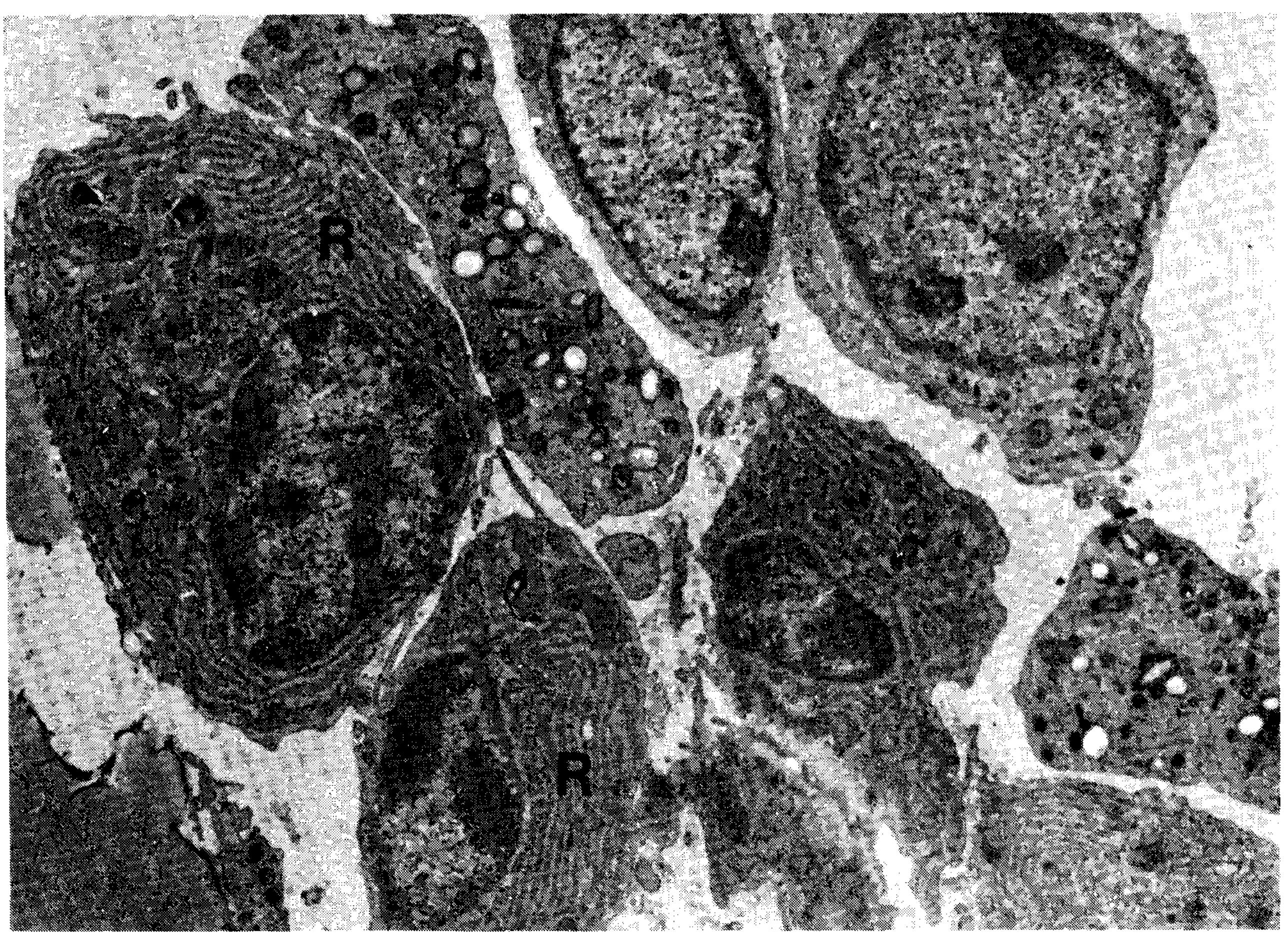

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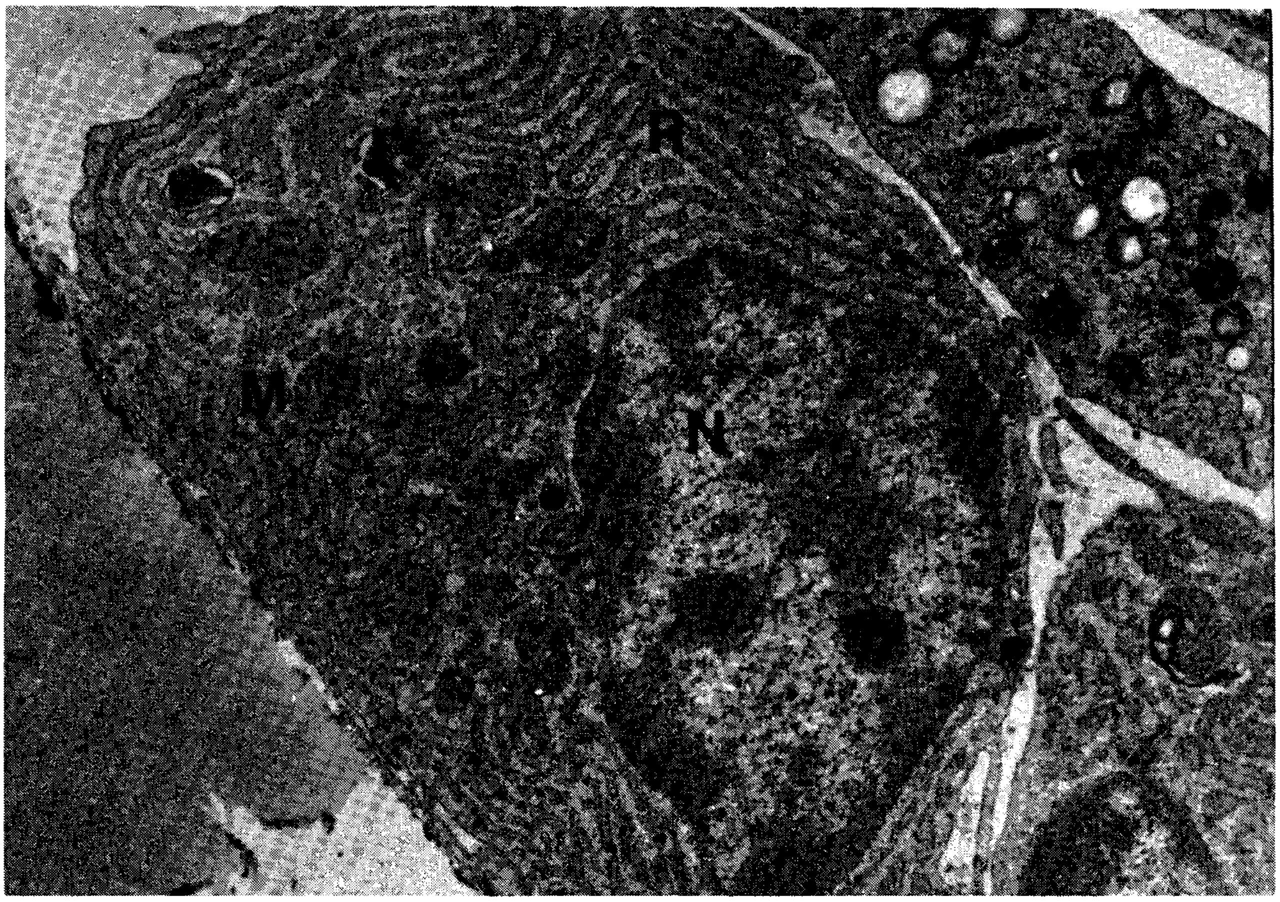

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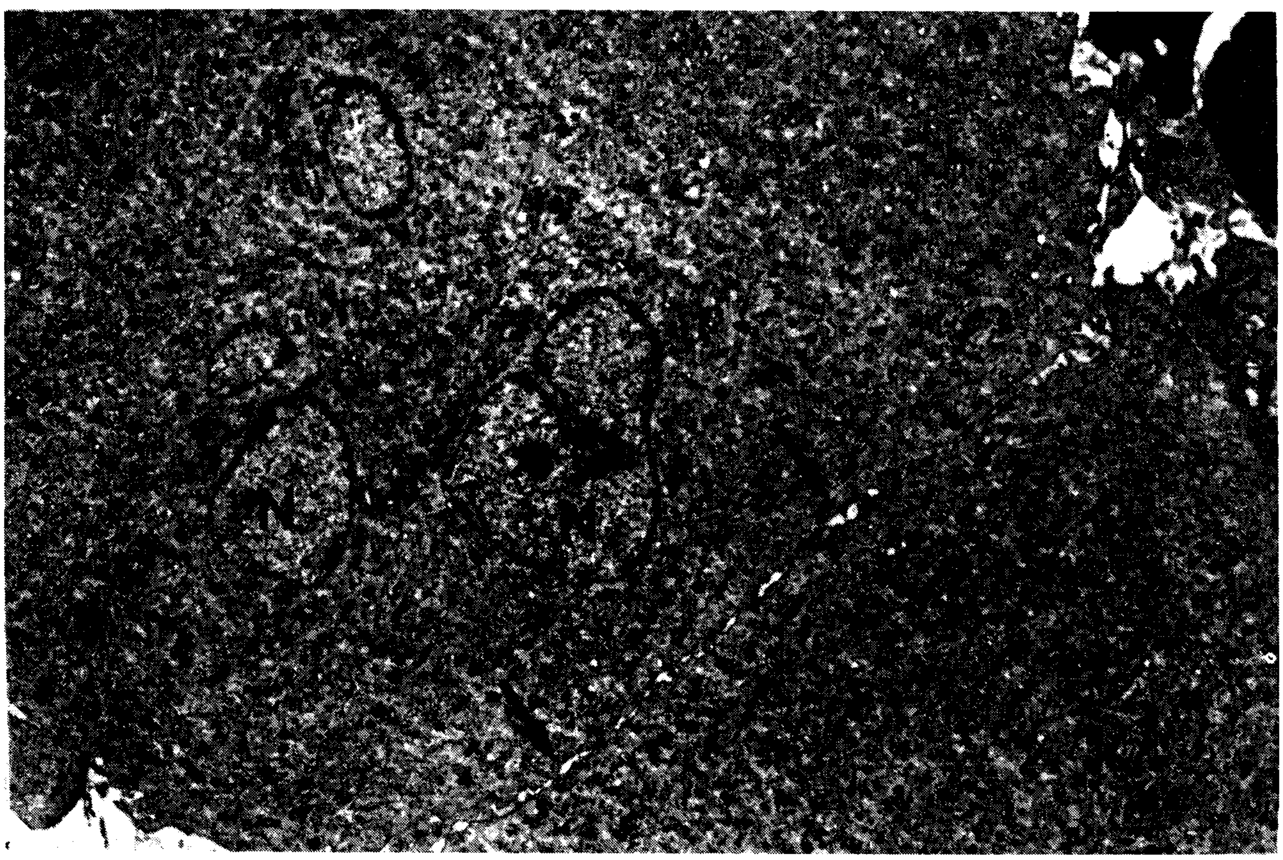

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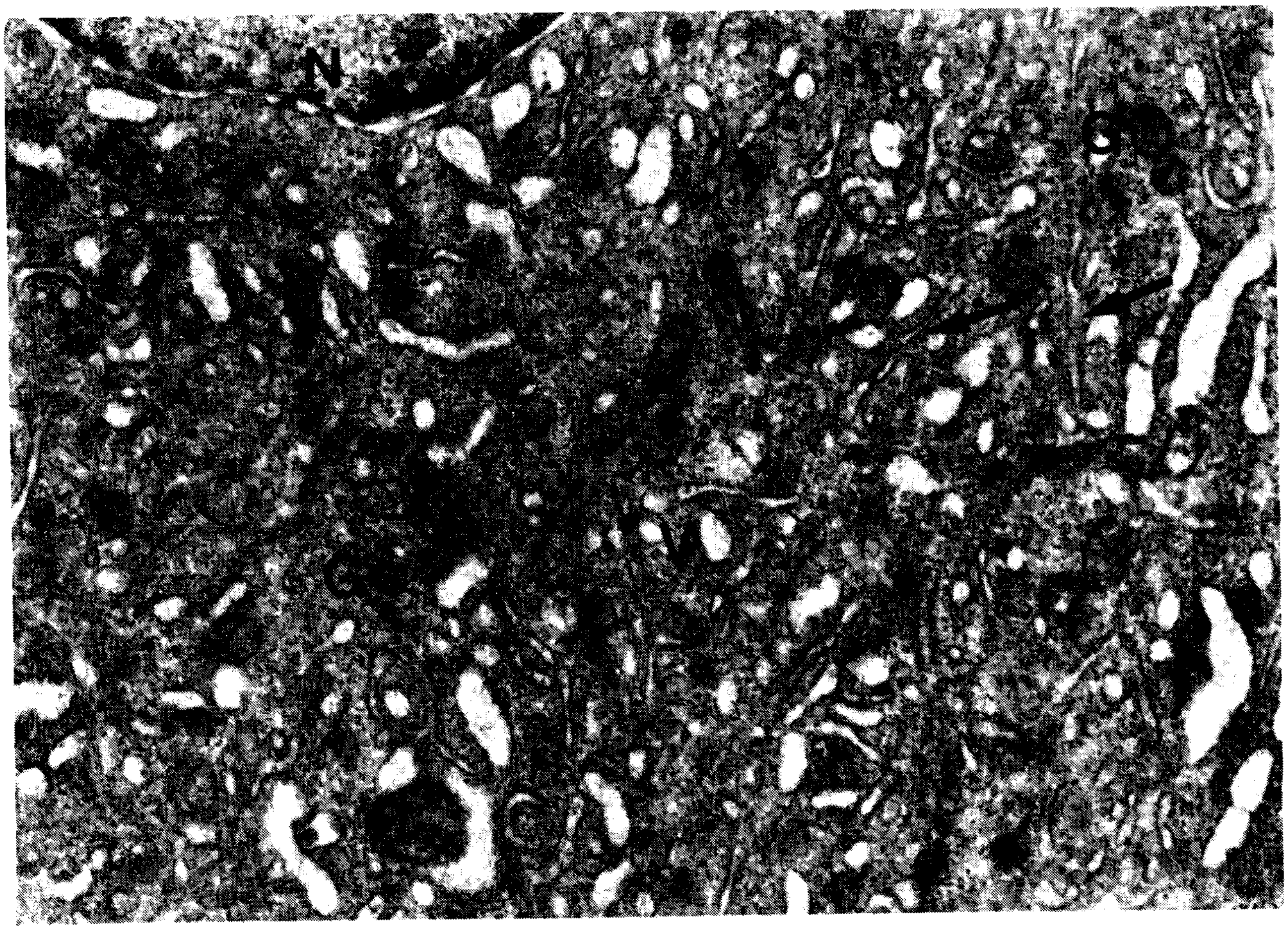

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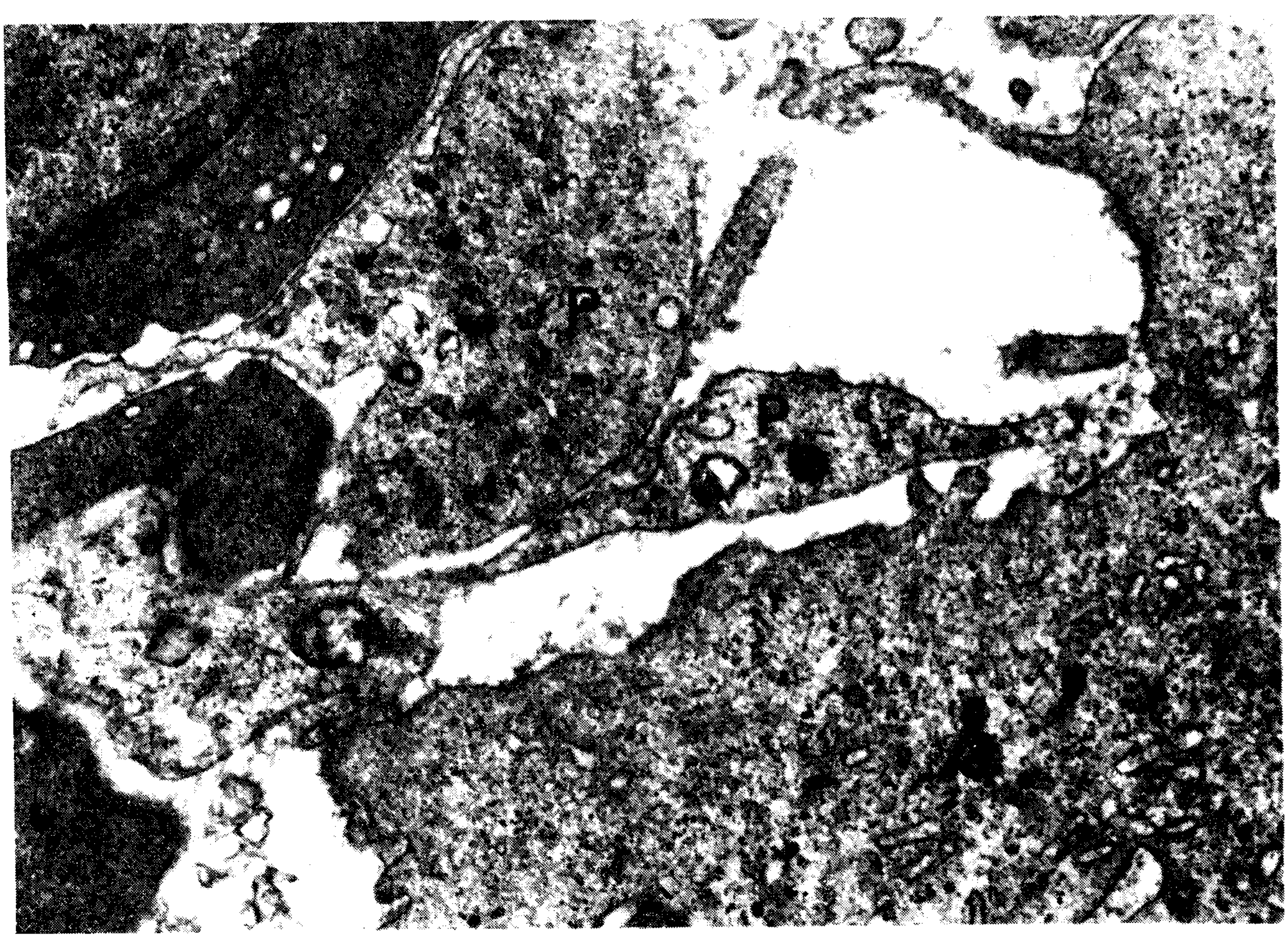

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