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Electron Microscopic Study on Detoxication Effect of Red Ginseng Extract on Mouse Liver Injury induced by Methyl Mercury

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메틸水銀으로 損傷된 생쥐 肝臟에 대한 紅蔘 抽出物의 解毒效果에 관한 電子顯微鏡的 硏究

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抄 錄

생쥐 肝細胞의 微細構造에 미치는 메틸水銀의 毒性的 影響과 이에 대한 紅蔘 抽出物의 解毒效果를 電子顯微鏡的으로 연구하였다.

對照群의 肝細胞에 비하여 메틸水銀만을 投與한 群에서는 核의 일부가 崩壞되고, mitochondria의 膨大, cristae의 收縮 내지 損失 및 다수의 대형 腔胞의 出現이 일어나고 glycogen의 量이 감소되었다.

메틸水銀과 紅蔘 抽出物을 併行 投與한 群에서는 메틸水銀만을 投與한 群에서보다 mitochondria의 膨大 程度가 적고 腔胞의 크기와 수가 감소되었으며 正常 細胞와 비슷한 상태로 나타났다.

Introduction

Methyl mercury absorbed generally through respiratory organ, gastrointestinal tract, and skin (Tunnicliff and Wood, 1973; Vallee and Ulmer, 1972) is bound with hemoglobin in the red blood cells and circulates in this form for months (Arena, 1986). It passes easily into the cell membranes due to its lipid solubility(Fang and Fallin, 1976; Lau and Sarkar, 1979). It is distributed mainly liver, kidney, and central nervous system, then has toxic influences on these organs (Donaldson and Gubler, 1978). The accumulation of a large amount of mercury after methyl mercury administration also produces a variety of morphological alterations in these organs (Valek, 1965; Ware *et al.*, 1974).

Meanwhile, ginseng is absorbed rapidly in the gastrointestinal tract and distributed to many organs, such as the liver, kidney, brain, stomach, and lung (Han, 1974). Brekhman and Dardymov (1969) reported adaptogenic theory that ginseng increases resistance of a living body under various undesirable conditions non-

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specifically. Ginseng promotes synthesis of protein in the liver and is helpful to rapid hepatic regeneration when the liver is injured by various causes (Bae, 1978). Furthermore, ginseng prevents the accumulation of toxic materials by speeding up the metabolic rate of toxic materials (Hong *et al.*, 1979). However, the detoxifying mechanism of ginseng has not been clearly defined.

In this study, the detoxifying effect of red ginseng extract against toxicity of methyl mercury to fine structures of mouse liver was investigated by means of electron microscopy.

Materials and Methods

Experimental animal

Female ICR mice, 20~30 g, were used and divided into three groups (control, group I and group II). Ten animals per group were housed in cage at 20~25°C and permitted free access to food (Samyang Laboratory Chow) and water.

Treatment

Control group was administered orally with distilled water only for 23 days. Group I was treated with methyl mercury chloride of 15 mg/kg, b.w./day by oral administration with animal feeding needle for 23 days. Group II was administered with red ginseng extract of 200 mg/kg,b.w./day for 7 days before mercury treatment, and then treated with methyl mercury chloride of 15 mg/kg,b.w./day and red ginseng extract of 200 mg/kg,b.w./day as post-treatment. The red ginseng extract containing crude saponin of 150 mg/g was manufactured by the Korean office of monopoly.

Electron microscopic observation

The excised mouse liver was diced into one cubes, immediately fixed in 2% paraformaldehyde-2.5% glutaraldehyde mixture buffered with 0.1M phosphate (pH 7.4) for 2 hours, postfixed in 1% osmium tetroxide in the same buffer for 1 hour (Palade, 1952), dehydrated in graded ethanol and acetone, and embedded in Epon 812 (Luft, 1961). Ultra-thin sections, made by LKB III Ultramicrotome, were stained with uranyl acetate and lead citrate(Reynolds, 1963), and examined under JEM-100B type electron microscope.

Results and Discussion

Hepatocyte of control group showed a large nucleus containing a prominent nucleolus. The chromatin was scattered throughout the nucleoplasm and the periphery of nucleus showed typical rough endoplasmic reticula (Figs. 1, 2). There were numerous microvilli on the base surface of the cell, which projected into the space of Disse and promote rapid metabolic material exchange by increase of surface area (Fig. 2). Numerous mitochondria contained normal cristae (Fig. 3). The glycogen granules were distributed widely in the cytoplasm. And there were lysosomes and numerous microvilli near bile canaliculus. The desmosomes, symmetrical plaques between two adjacent cells, were observed near bile canaliculus (Figs. 1, 2). Microbody showed a spherical body with dense core (Fig. 3), which is responsible for detoxifying action such as decomposition of hydrogen peroxide toxic to cellular life (Lentz, 1971).

Hepatocyte of group I showed appearance of many large vacuoles and partial rupture of nucleus (Fig. 4). Mitochondria were swelled to the whole volume increase considerably. Matrix with a low electron density and cristae shrinked near inner membrane of mitochondria were observed(Figs. 4, 5). Such mitochondrial change also was observed in case of the necrosised hepatic cells of rat treated with $HgCl_2$ (Gritzka and Trump, 1968; Trump *et al.*, 1965) or CCl_4

(Ashworth *et al.*, 1963; Leduc and Wilson, 19 58). Considering that normal mitochondria enable hepatic cell to use the energy for synthesis of metabolic material (Lentz, 1971), the degenerations of mitochondria would suggest the abnormal hepatic function. Glycogen granules were seldom and this phenomenon might be caused by the toxicity of methyl mercury. That is, the enzyme activity for synthesis and decomposition of glycogen in carbohydrate metabolism was inhibited by the methyl mercury toxicity. Trump *et al.* (1965) reported that toxic material administered to mouse induces the decrease of glycogen content during the hepatocyte necrosis.

In hepatocyte of group II, swelling level of mitochondria was diminished and the vacuoles were decreased in size and number compared with those of group I. From this event, it would be assumed that the red ginseng extract enabled damaged liver cells to be recovered around the control. This is supported by the report that the ginseng is helpful to rapid hepatic regeneration of injured liver due to toxic materials (Bae, 1978). Yu (1977) also reported that the morphological alterations of mouse liver treated with ginseng and carbon tetrachloride are milder than liver treated with carbon tetrachloride only and there is no cytoplasmic ballooning in the hepatic cells.

Summary

Detoxication effect of red ginseng extract against toxicity of methyl mercury on ultrastructure of mouse liver was studied by electron microscopy.

The hepatocyte of methyl mercury treatment group showed partial rupture of nucleus, mitochondrial swelling, decrease of glycogen content, and appearance of a great number of large vacuoles.

While, the hepatocyte of methyl mercury-red ginseng extract treatment group showed slight mitochondrial swelling and decrease of vacuoles in size and number than those of methyl mercury treatment group.

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Figure Legends

Fig. 1. Electron micrograph of hepatocyte from control mouse with normal nucleus(N), nucleolus (Nu). Glycogen granules(Gl), mitochondria(M) with cristae(Cr), rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum(SER) are distributed throughout the cytoplasm. Lysosomes(Ly) are seen near the bile canaliculus(BC) and microvilli(Mv) are toward the bile canaliculus. Arrowhead indicates desmosome(D). ×9,000.

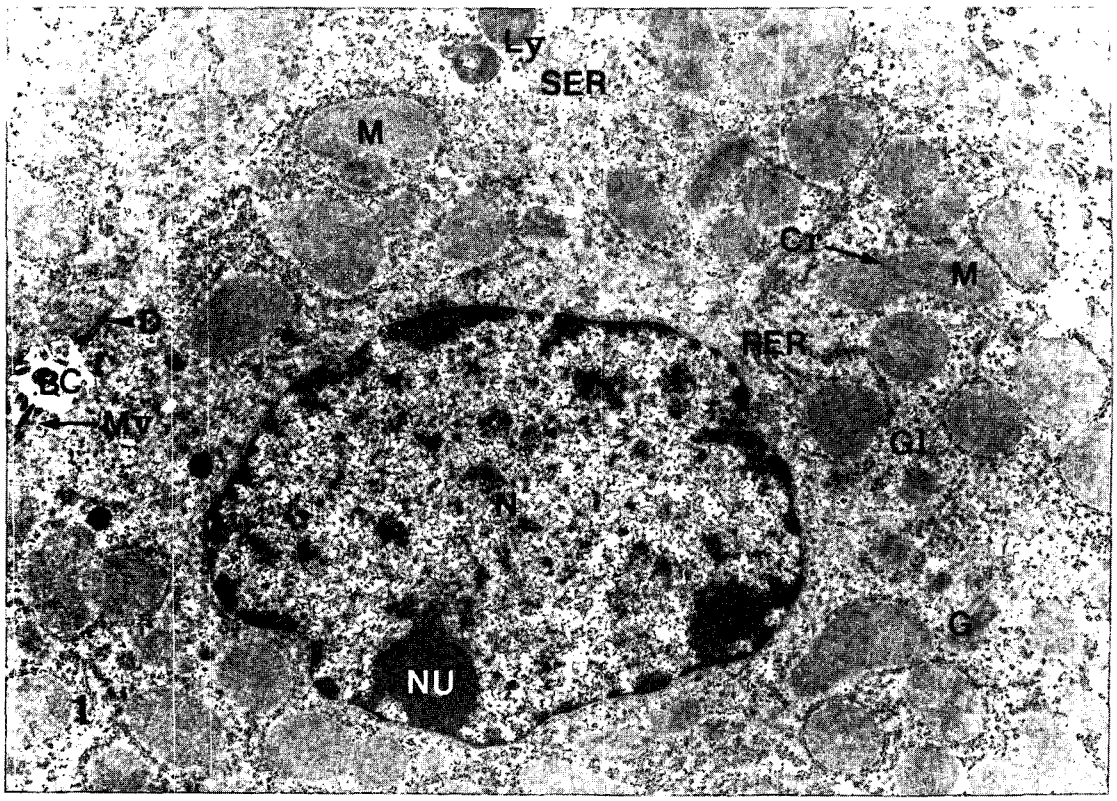
Fig. 2. Electron micrograph of hepatocyte from control mouse. Microvilli(Mv) are projected into the space of Disse(*). ×9,500.

Fig. 3. Electron micrograph of hepatocyte from control mouse. Note the mitochondria(M) with well developed cristae(Cr). Microbody(Mb) is typical structure. ×12,000.

Fig. 4. Electron micrograph of hepatocyte from the mouse treated with methyl mercury. Note the swollen mitochondria(M) with matrix rarefaction and loss of cristae(Cr). The large vacuoles(V) appeared. Nucleus(N) is destructed partially(arrow) and glycogen granules(Gl) are decreased. ×12,000.

Fig. 5. Electron micrograph of hepatocyte from the mouse treated with methyl mercury. There are numerous large vacuoles(V). ×12,000.

Fig. 6. Electron micrograph of hepatocyte from the mouse treated with methyl mercury and red ginseng extract. Nucleus(N), nucleolus(Nu) and mitochondria(M) are simillar to those of the control. Vacuoles(V) are decreased in size and number as compared with the methyl mercury treatment group. RER, Rough endoplasmic reticulum. ×9,000.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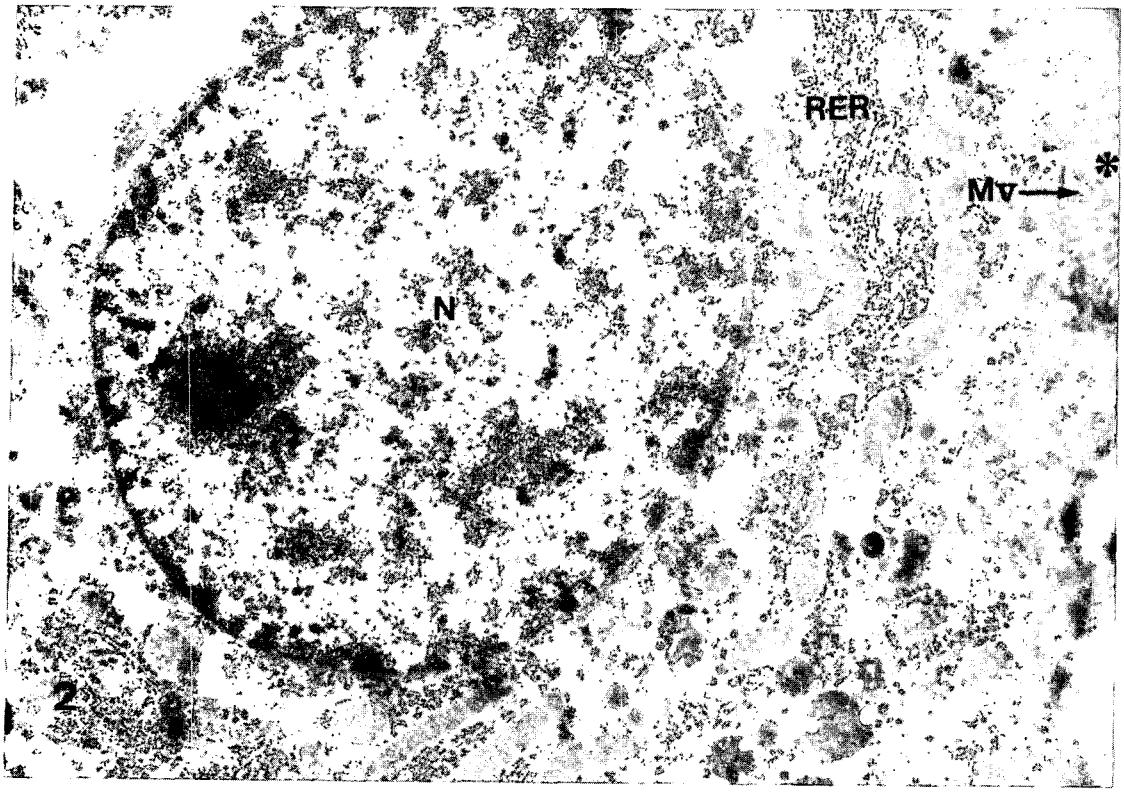
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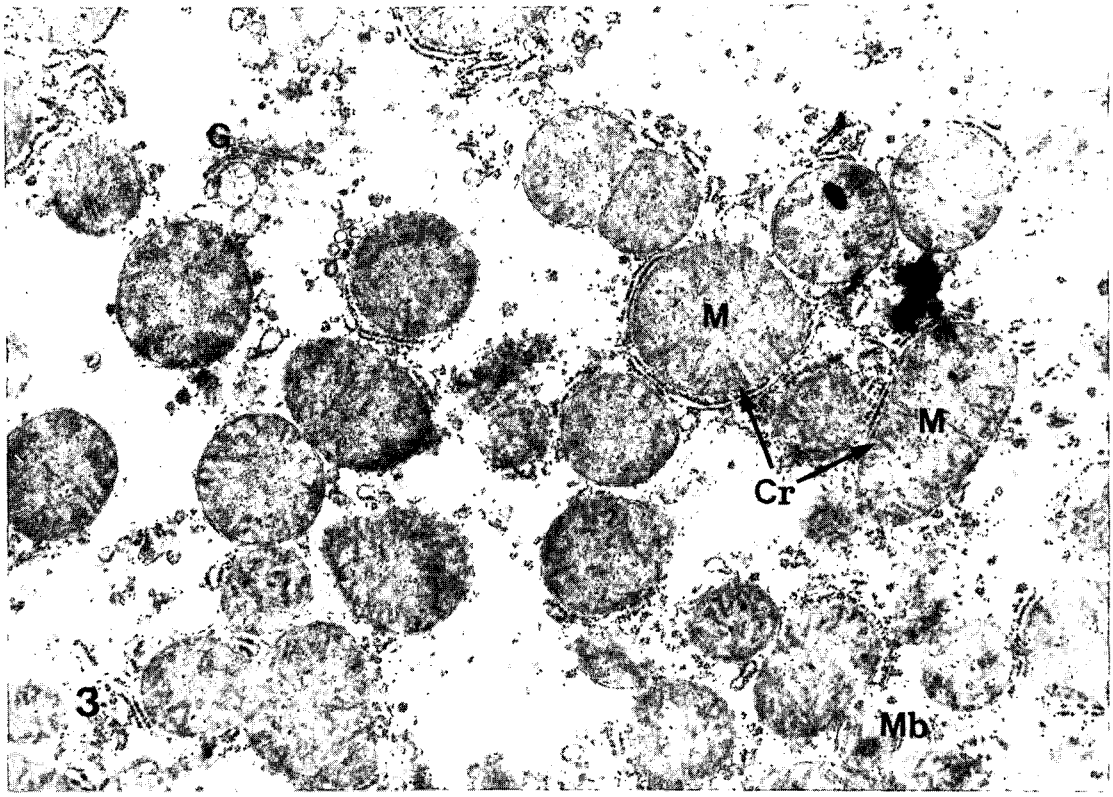
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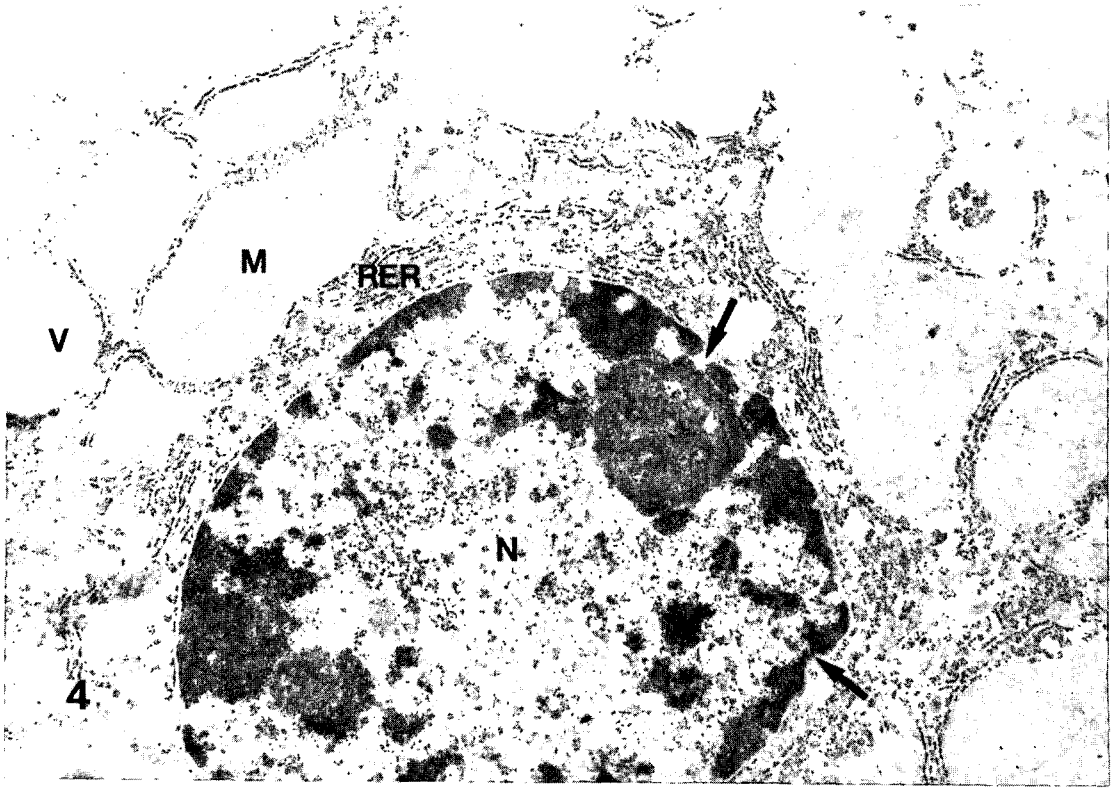
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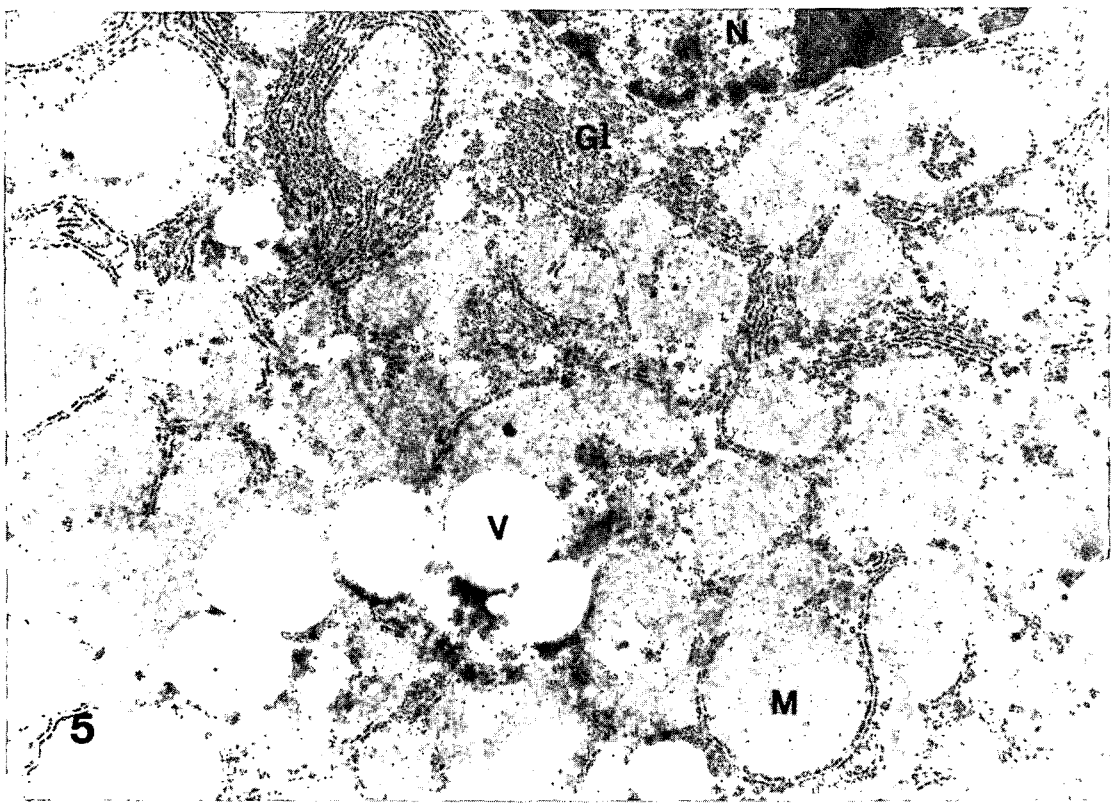
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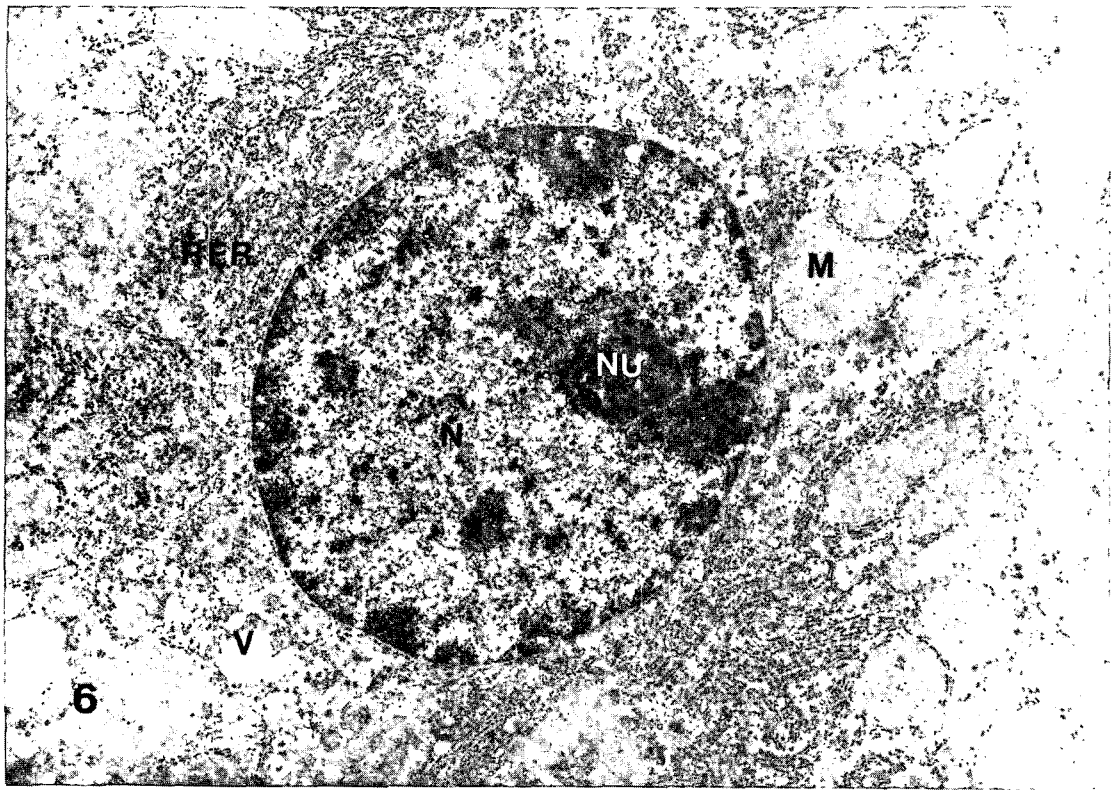
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